Chapter

Role of Antioxidants Supplementation in the Treatment of Male Infertility

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Abstract

Nutritional utilization of antioxidants, such as vitamins C, E, ß-Carotene and micronutrients, such as folate and zinc, have been shown to be critically essential for normal semen quality and reproductive function. However, it is still, a large knowledge gap exists concerning the role of antioxidants on semen parameters and the role in treatment of male subfertility. Therefore, the current review article designed to find out the positive effect of antioxidants on semen quality, alterations in physiological functions of spermatozoa and infertility treatment It is advisable that patients with oxidative DNA disruption should be asked to take a simple course of antioxidants prior to undertaking assisted reproduction treatment (ART). In conclusion, antioxidant may be employed as a potent antioxidant and may improve infertility treatment outcomes with ART.

Keywords: antioxidants, male infertility, semen quality, ART

1. Introduction: The impact of oxidative stress on spermatozoa

ROS include a broad category of species including: a) Oxygen free radicals, such as superoxide anion (O_2), hydroxyl radical (OH) and hyperoxyl radical (HOO). b) Non radical species, such as hypochlorous acid (HOCl) and hydrogen peroxide (H_2O_2). c) Reactive nitrogen species and free nitrogen radicals such as nitroxylion, nitrous oxide, peroxynitrite, etc. [1–3]. These ROS are generated during normal aerobic metabolism and their level increases under stress which reflects a basic health danger. Mitochondrion is the primary cell organelle involved in ROS production. Besides, several endogenous cells and cellular components contribute towards the initiation and propagation of ROS [4, 5].

Overproduction of ROS or the deficiency of antioxidants provokes an imbalance between the per-oxidation and the anti-oxidation in the normal human body. This phenomenon is termed as Oxidative stress [6–8].

Subsequently, it leads to alterationsin peroxidation of lipid membranes of sperm, disrupting the structure of membrane receptors, enzymes, transport proteins, and causes an increase in the level of DNA fragmentation of sperm [9] (**Figure 1**).

ROS have a significant effect on the sperm plasma membrane and subsequent functional integrity of the sperm resulting in a loss of acrosome reaction [11],

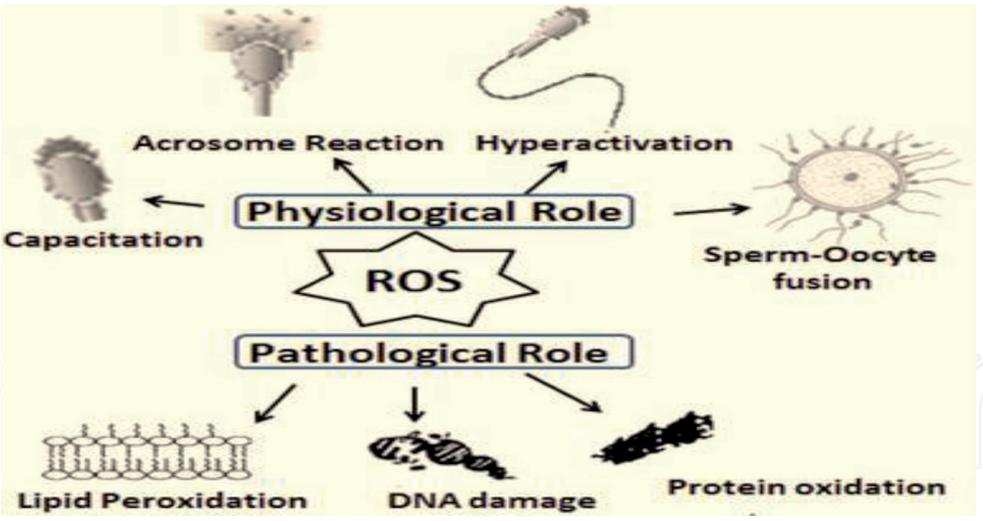

Figure 1.
Role of reactive oxygen species in male reproduction [10].

the sperm potential for fertilization [12], sperm motility and vitality [13, 14] as well as impair the sperm capacitation [15] (**Figure 1**).

Both seminal plasma and spermatozoa contain antioxidant systems able to detoxify the harmful effects of ROS. The imbalance between total antioxidant capacity and ROS generationin seminal fluid presents oxidative stress and is strongly correlated with male infertility [16].

In addition, insufficient penetration of the sperm into the oocyte in oligospermia men with elevated levels of ROS has been identified [7]. Moreover, ROS concentrations are considerably elevated in semen samples from infertile men as compared with those of healthy controls, which has suggests that infertile men may benefit highly from antioxidant supplementation [17].

Several lifestyle, stress, and environmental factors encourage excessive free radical generation and oxidative stress, including: Air pollution, cigarette smoke, alcohol intake, toxins, bacterial, fungal, or viral infections, radiation, including extensive sun bathing, intense and lengthy exercise, which provoke tissue damage, antioxidant deficiency and finally enormous intake of antioxidants, such as vitamins C and E etc.

Hammadeh presented that the oxidative stress and smoking markers, (ROS), malondialdehyde, 8-Hydroxyguanosine (8-OHdG) and cotinine were significantly higher ($P < 0.010$) in smokers as compared to non-smokers [18].

In a second study, both fertile and infertile smokers presented elevated seminal ROS level [19].

Alcohol, known as ethyl alcohol or ethanol; EtOH, promotes ROS. These ROS ultimately interact with macromolecules, among them membrane lipids, generating aldehydes such as 4-Hydroxynonenal (4-HNE) and Malondialdehyde (MDA). It is also known that aldehydes and ROS can directly interact with both proteins and DNA, ultimately leading to transcription-repression of concrete genes. In fact, the impactof ROS and aldehydes seems to serve as key factor for these alterations, partially affirmed by the fact that intake of antioxidants prevents these EtOH-induced cellular alterations [20].

The effect of oxidative stress on sperm quality has been studied extensively and estimated to be the problem in 25–87% of male infertility cases [21]. Another major driving hypothesis states that these conditions, by increasing reactive oxygen species (ROS) and nitrogen species (RONS), are capable of altering the balance of the redox status of both the steroidogenic cell population and the germ line cell

populations, causing the disruption of the hypothalamic–pituitary–testicular axis and the impairment of sperm quality [22].

Therefore, the human body has developed a very concise defense system in which antioxidants play a very important role. Antioxidants are capable of decreasing the generation of free radicals, slowing or inhibiting the oxidation and repairing the damage [23]. Mirończuk-Chodakowska also presented that ROS can serveas mediators and regulators of cell metabolism and apoptosis [23].

2. Antioxidants and the sperm quality (count, motility and morphology) and function

Sperms are especially susceptible to oxidative damages due to the presence of excessive amounts of polyunsaturated fatty acids in the plasma membrane, making them highly vulnerable for lipid peroxidation by ROS, causing decreased flexibility of the sperm membrane and decreased tail motion [24]. Besides, the cytoplasm is extracted during the final stages of spermatogenesis, leaving behind the spermatozoa alone without these important enzymes to protect them from ROS altering the sperm DNA [24].

A Significant positive correlation has also been described between levels of reactive oxygen species (ROS) and percentage of spermatozoa with several types of abnormalities such as, abnormal heads, acrosome abnormalities, mid piece anomalies, cytoplasmic droplets and tail defects [25].

In previous studies, it has been demonstrated that the generation of reactive oxygen species has been linked with loss of motility and a reduced capacity for sperm–oocyte fusion [26].

In addition, seminal oxidative stress is rather negatively correlated with sperm count, function, and motility, adversely interfering with fusion required for successful fertilization [27].

Prospective studies have indicated that men with elevated levels of ROS have seven times less chance of fertilizing in comparison with men with low ROS levels. Moreover, ROS results in sperm cell damage and its high values have a negative correlation with sperm number and motility [28].

Other studies reveal that sperm exposed to elevated levels of ROS show reduction in viability and motility as confirmed through both conventional assessment and the utilization of computer-assisted sperm motility analysis [29, 30].

Antioxidant supplements are commercially available to assist treat male infertility, but research on its impact on semen quality and rates of pregnancy and live birth are rather very limited and controversial.

The male reproductive condition can be enhanced by supplementation of beneficial elements such as zinc or selenium which provide positive changes in sperm count and motility [31]. Melatonin, beta-carotene, or luteine also maintain high semen quality [32, 33]. Several studies have affirmed that higher intake of vitamin E, vitamin C, and beta –cartotine, is linked with improved sperm count motility, in both fertile and infertile men [34, 35].

Also, spermatozoa are dependent on antioxidants which already present in seminal plasma as ascorbic acid (vitamin C), α-tocopherol (vitamin E), glutathione (GSH), amino acids (taurine and hypotaurine), albumin, carnitine and carotenoids [36].

A clinical trial on the impact of antioxidants on male factor infertility enrolled a population of 171 couples. The male partner presented at least one abnormal reading concentration, mobility, morphology and DNA quality; the female partners presented normal fertility test results. Men received a placebo or an antioxidant

supplement comprising of vitamins C, E, D, selenium, L-carnitine, zinc, folic acid and lycopene from three to six months [37].

In this study, no statistically significant differences in sperm concentration, mobility, morphologically normal sperm and DNA quality between the placebo and antioxidant groups after three months have been observed. Moreover, live birth rates did not differ at six months between the antioxidant (15%) and placebo (24%) groups.

3. Antioxidants and sperm DNA fragmentation and apoptosis

Spermatozoa chromatin is relatively tightly packed due to the positively charged protamine unlike histone in somatic cells [38]. This highly dense and stable structure decreases the capability of DNA disruption. Unfortunately, as spermatozoa have only a few repair mechanisms, DNA damage is generally encountered in human spermatozoa, even within the fertile donor population [39].

It has been presented that almost 40% of men searching for a fertility treatment are fertile and free of sperm oxidative DNA damage [40].

In fact, this topic was discussed in several studies. Mark et al. described that spermatozoa are especially prone to oxidative stress due to the elevated ROS level can provokea break down of sperm phospholipids and fatty acids [41].

Others have been illustrated that an increase degeneration of ROS by morphologically abnormal spermatozoa and/or decreased antioxidant capacity of seminal plasma are possible reasons of DNA damage in these patients [42, 43].

Hammadeh et al., demonstrated that the ROS level in subfertile patients was significantly higher compared with normal subjects [18].

Besides, 8-hydroxydeoxyguanosine (8OHdG) is considered to be an important sensitive oxidative biomarker for evaluating oxidative sperm DNA damage, and its levels were found be significantly higher in infertile patients compared with normal ones [18, 44].

Defective spermatozoa are also postulated to retain high residual cytoplasm, allowing them to produce excessive reactive oxygen species (ROS), which, provided their incomplete chromatin packaging, causes DNA damage [45]. Therefore, DNA damage in spermatozoa is primarily related to oxidative stress [45].

Sperm DNA damage could be testicular or post-testicular [46]. These damages may include disruptions in spermatogenesis (e.g., genetic or developmental abnormalities) and testicular or post-testicular damage (e.g., gonadotoxins, hyperthermia, oxidants, and endocrine abnormalities). It has been postulated that protamine deficiency (with subsequent aberrant chromatin remodeling), reactive oxygen species and abortive apoptosis may be associated with sperm DNA damage [18, 47].

WalczakJędrzejowska described the damaging impact of oxidative stress on sperm cells including a reduction in activity of anti-oxidative mechanisms, disruption of DNA and accelerated apoptosis [48].

Another study demonstrated that oxidative parameters in the semen of infertile men were significantly rather higher than in fertile men, and a very high correlation was observed between oxidative parameters, sperm ROS generation, and DNA fragmentation level [49].

Since sperm DNA disruption can be associated by oxidative and non-oxidative stress (caused by incomplete sperm protamination or aberrant apoptosis), the utilization of DNA fragmentation tests may not be the perfect procedure for identifying individuals with high sperm DNA damage related to oxidative stress.

Therefore, the most ideal parameters to assess DNA damage might not be DNA fragmentation but sperm-oxidation level which indirectly interferes with DNA disruptions [50, 51].

Antioxidants utilized as dietary supplements removed free radicals and decrease the degree of oxidative insult by improving the cellular redox equilibrium [52].

Several studies demonstrated positive results in the therapy of patients with moderate DNA damage exploiting oral antioxidants [42, 53, 54].

Greco and colleagues reportedan enhancement in DNA damage in 76% of patients with moderate DNA damage (≥15%) after oral intake with vitamins [53].

The beneficial impact of raised antioxidant consumption on sperm concentration and motility in the infertile patient population with no effect on sperm DNA integrity has also been observed [55].

The advantages of antioxidant therapy in male infertility are rather inconclusive with both a positive effect [53, 54] and no significant effect reported [56]. Also, Silver analyzed a cohort of fertile men and did not report any relationships between dietary antioxidant consumption (vitamins C, E or ß-carotene) and sperm DNA damage [56].

Treatment with antioxidant supplements is generally related with decreased levels of sperm DNA integrity and/or increased fertility potential [57].

The impactof these antioxidants in prohibiting sperm from endogenous ROS, gentle sperm processing and cryopreservation has yet not been established [58].

It is advisable that patients with oxidative DNA disruption should be asked to take a simple course of antioxidants prior to under taking assisted reproduction treatment. The utilization of antioxidants in decreasing sperm oxidative stress has been the topic of some 20 clinical trials over the last decade for a review [59].

Systematic review involving 29 studies evaluated the impact of oral antioxidant therapy on fertility outcomes and affirmed an overall positive effect of antioxidant supplementation on basic semen parameters, advanced sperm function, results of assisted reproductive therapy (ART), and live birth rate [60].

Oral supplementation of a either a single antioxidant or a combination of antioxidants such as L-carnitine, L-acetyl carnitine, N-acetyl-cysteine, Coenzyme Q10, selenium, vitamin C, vitamin E, and lycopene has been reported to enhance semen parameters and sperm DNA integrity in idiopathic infertile men [61].

4. Types of antioxidants

Seminal plasma comprises of a number of antioxidant enzymes, such as, superoxide dismutase, glutathione peroxidase, glutathione reductase, and catalase, and also include non-enzyme antioxidants, such as vitamin C, vitamin E, beta carotenes, carotenoids, flavonoids and metal binding proteins such as albumin, ferritin, coenzyme Q10 and myoglobin, which serve as antioxidant by inactivating pro-oxidant transition metal ions (**Figure 2**) [62–64].

The non- enzymatic antioxidants may protect the spermatozoa from oxidative DNA and membrane disruption by decreasing the singlet oxygen and the detrimental effect of lipid peroxidation on sperm [65].

4.1 Enzymatic natural antioxidants

The male reproductive system has an endogenous antioxidant for shielding spermatozoa from oxidative insult and these are categorized into enzymatic and non-enzymatic [1]. The enzyme system includes ssuperoxide dismutase (SOD), glutathione peroxidase/reductase and catalase acting as a defense against lipid peroxidation in mammalian sperm. The malfunction of these enzyme activities could lead to a loss of the cell function [66].

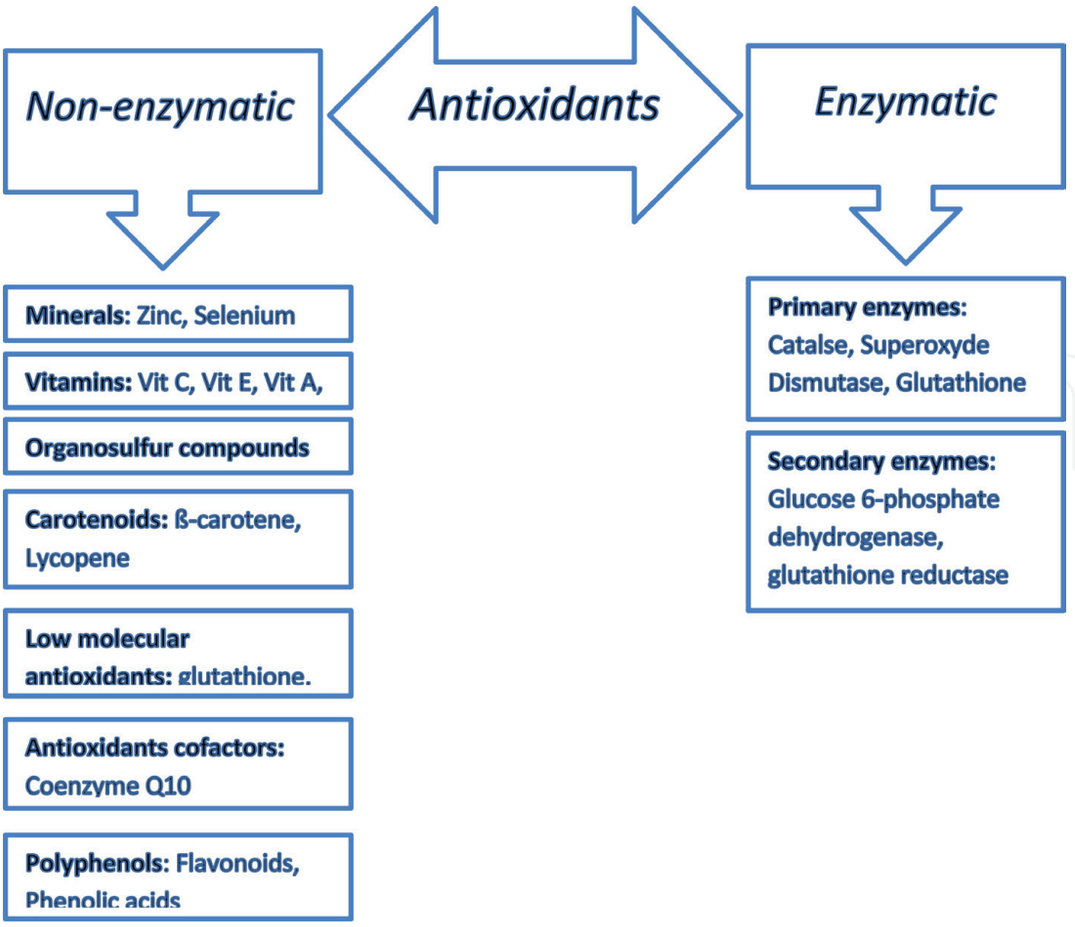

Figure 2.
Enzymatic and non-enzymatic antioxidants.

These enzymes catalytically eliminate reactive oxygen species from biological systems. Sperm themselves predominantly channel this enzymatic antioxidant [66]. Spermatozoa comprising of low intracellular antioxidant activity include superoxide dismutase (SOD), nuclear glutathione peroxidase (GPx), peroxiredoxin (PRDX), thioredoxin (TRX), and thioredoxin reductase (TRD) [67].

4.1.1 N-acetyl-cystein (NAC)

NAC serves as a precursor of glutathione (GSH) and has thus been employed as an antioxidant in many studies and trials.

The most important studies which demonstrate positive impact with varying doses in vitro are those reported by Baker et al., Oedaet al., and Lopes et al., [68–70].

Baker et al., witnessed decreased ROS generation and enhanced motility with doses ranging from 1 to 10 mmol [68]. Oeda et al. reported preserved motility with doses from 0.1 to 15 mg/ml [69] and Lopes et al. reported decreased DNA damage with 0.1 mmol of NAC [70]. The three most relevant in vivo studies provided conflicting outcomes: Comhaire et al., did not find any enhancement in sperm parameters after 3 months' administration of NAC (600 mg for 21 days) [71]; whilst the same dosage seemed to enhance sperm motility, volume, viscosity and oxidative status according to Ciftci et al., [72]. In another trial conducted on 468 patients with idiopathic OAT treated for 6 months with 600 mg/day of NAC or 200 mg/day of selenium or both, an enhancement in sperm count, motility and morphology was observed, with additive beneficial impact when both therapies were dispensed [73].

4.1.2 Glutathione peroxidase (GPx4)

Like peroxidase, Gpx4 is a member of the large family of peroxide reducing enzymes that employs glutathione as electron donor [74].

GPX4 exploits thiols in nuclear protein to reduce glutathione and may function as a redox regulation of sperm motility activation [75].

Sakai et al., found a correlation between undue production of glutathione peroxidase (GPx4) and infertility due to inhibition of ROS generation. GPx4 is not only an essential antioxidant enzyme required for sperm motility; it also plays a key role prior to capacitation [76]. However, while GPx4 shields sperm apoptosis, it decreases ROS which is an important factor for capacitation. Naturally occurring mutations in the human GPX4 gene have been related to the pathogenesis of oligoasthenozoospermia [77].

4.1.3 L-Glutathion (GSH)

GSH is one of the most essential endogenous antioxidant, implicated in sustaining the antioxidant balance in human tissues and directly engaged in the removal of ROS [78].

Due to its widely recognized antioxidant characteristics, GSH has been explored as a possible therapy for infertile patients. In vitro studies performed by Griveau and Le Lannou and by Lopes et al. have demonstrated a protective impact of 10 mmol of GSH against DNA damage caused by ROS [70, 79].

In vivo studies, two trials performed by (Lenzi et al.; Lenzi et al.,) revealed that GSH exerted a positive, statistically significant impact on sperm parameters [80, 81]. The first, Lenzi et al., discovered that 600 mg of GSH dispensed every other day for 2 months increased the motility and morphology in 21 men treated with varicocele or male accessory gland infection [82]. The second, performed the following year on 10 infertile men with the same attributes, led to similar outcomes, although a significant upsurge in sperm concentration was also observed [80, 81].

Tail-beat frequency and the shielding effect of 5–10 and 1–10 mmol GSH in vitro against the disruption of sperm motility by activated polymorphonuclear leukocytes has been reported [68, 83].

Parinaud et al., investigated the impact of Sperm-Fit, an antioxidant solution comprising of glucose and GSH, on sperm motility and showed that motility was well-preserved in leukocyte spermic samples, thus enhancing the possibility of recovering motile sperm after liquefaction and centrifugation [84].

Raijmakers et al., reported the presence of substantial amounts of GSH in the seminal plasma of both fertile and subfertile men. However, the median GSH concentrations were remarkable higher in fertile men in comparison with subfertile men. Furthermore, the concentrations of GSH in seminal plasma were directly correlated with sperm motility and inversely correlated with sperm morphology [85].

4.1.4 Superoxide dismutase (SOD)

There is some consensus on the helpful impacts of SOD on lipid peroxidation in vitro. A plethora of studies, published in last two decades of 20th century i.e. between 1991 and 1998, (for a review Lombardo et al.,) described enhanced motility and decreased lipid peroxidation in samples dosed with between 87.7 and 500 IU ml of SOD [86]. Similarly, Kovalski et al. reportedthat the incorporation of 1 mg of SOD conserved motility [87].

In the contrary, other found no decreasein lipid peroxidation after the in corporation of between 100 and 500 IU ml SOD [88]. Besides, some investigators could

not affirm the impact of SOD on semen quality and sperm fertilizing potential [89]. However, the amount of endogenous antioxidants such as superoxide dismutase and catalase were also observed to be lower in smokers in comparison with fertile non-smokers [19, 90].

A direct correlation between the SOD activity and sperm damage and sperm motility was endorsed by several researchers [7].

4.1.5 Catalase (CAT)

Catalase represents one of the most effective natural enzymes which catalyzes the conversion of H_2O_2 into H_2O and O_2. CAT could play an essential role in male fertility and could turn out to be an excellent target for male infertility diagnosis.

In vitro investigations confirmed the positive impact of catalase in doses ranging from 0.008 to 0.1 mg/ml or from 50 to 2000 IU/ml on motility, peroxidation and DNA disruption [70, 79, 87].

However, Twigg et al., reported that the addition of 250, 500 or 2000 IU/ml of catalase to sperm samples did not provide any effect on lipid peroxidation. In the same study, no enhancement was observed in lipid peroxidation after the supplementation of albumin at a dose of 0.3–10% [88].

Other researcherse valuated the effects produced by different types of antioxidant therapies on semen quality in infertile males via measurement of CAT activity on seminal plasma through exogenous H_2O_2 degeneration. The results revealed that catalase activity improved after the administration of antioxidant treatments when compared to control samples without antioxidant administration [91–93].

Moreover, Catalase has also been shown to play a crucial role in male fertility. Its antioxidant activity prevents the upsurge of oxidative stress which can cause damage in sperm level, and accordingly, to reduced fertility. However, even though consensus prevails among most authors on the correlation of this scavenger with male fertility, it has only been investigated to a very limited degree of extent in this field [94].

4.1.6 Coenzyme Q10

A very strong relationship has been described to occur between sperm count, motility, and ubiquinol concentration in seminal fluid [95].

Lewin and Lavon employed coenzyme Q10 both in vivo and in vitro and revealed that the incorporation of 50 mmol in vitro caused a significant improvement in motility, whilst in vivo incorporation of 60 mg/day for almost four months resulted in an enhancement in the fertilization rate but did not provide any impact on motility, morphology or concentration in 17 patients with decreased fertilization rates after in vitro fertilization with intracytoplasmic sperm injection for male factor infertility [96].

A stimulating in vivo study conducted on 60 patients with idiopathic asthenozoospermia who were provided with either placebo or 200 mg daily of coenzyme Q10 showed a remarkable advancement in motility after 6 months of treatment; this enhancement was markedly decreased after a period of 3 months in treated subjects [97].

The exogenous incorporation of CoQ10 enhances both ubiquinone and ubiquinol levels in semen and can be useful in increasing sperm kinetic features in patients suffering from idiopathic asthenozoospermia [97].

CoQ10 biosynthesis is highly active in the testes and elevated levels of ubiquinol are found in sperm [98, 99]. Gvozdjakova et al., revealed a direct relationship between seminal plasma CoQ10 total concentration and sperm motility [100].

4.2 Non-enzymatic/synthetic/dietary supplements

4.2.1 Vitamin A

The name vitamin A includes both preformed vitamin A (retinol and its esters) and pro vitamin A carotenoids (mostly α-carotene, β-carotene, and β-cryptoxanthin). Retinol can be oxidized to retinal in a reversible manner, which provides all of the biological implications of retinol; or even further oxidized to retinoic acid, which represents the primary active metabolite of vitamin A [101].

Pro-vitamin A carotenoids can be endogenously transformed into retinoic acid whilst other carotenoids, including lutein, zeaxanthin, and lycopene, are not precursors of vitamin A but contain antioxidant capacity. Carotenoids are naturally found in fruits and vegetable. They impart the yellow, red and orange pigment in plant [102].

The most important phytochemical in the carotenoid family is lycopene which among the carotenoids, ranks as one of the most highest quencher of singlet oxygen. A combination of carotenoids, however, is highly potent as compared to individual compounds [103].

In in vitro models, β-carotene may act both as an antioxidant or a prooxidant, depending upon the redox status of the biological environment in which it acts [104].

Comhaire et al., investigated the impact of a combination therapy of 180 mg day vitamin E and 30 mg day b-carotene on a group of 27 infertile men: ROS generation decreased significantly, although no significant enhancement in semen parameters was observed [71].

Zareba et al., evaluated the effect of regular carotenoid intake on the enhancement of sperm quality in 189 young, healthy men. They evaluated semen volume, total sperm count, motility, and morphology after a period on antioxidant rich diet and observed that beta-carotene and lutein utilization elevated sperm motility [33]. However, high exploitation of some vitamins, such as vitamin A, may cause adverse reproductive outcomes [105].

4.2.1.1 Lycopene

Lycopene is one of the several constituents of the carotenoid family. Provitamin A carotenoids can be endogenously transformed into retinoic acid whilst other carotenoids, including lutein, zeaxanthin, and lycopene, which are not the precursors of vitamin A but contain antioxidant capacity. Lycopene exhibits good antioxidant activity includes singlet oxygen and free radical scavenging [106].

The presence of lycopene in human semen has been confirmed and its quantity can be enhanced after dietary intake with a natural source of Lycopene [107]. Also, very little is known about the efficacy of carotenoids, particularly that of lycopene—a potent antioxidant and singlet oxygen quencher [108].

Zini et al., preincubated washed sperm suspensions with and without lycopene followed by incubation with H_2O_2 and presented a significant decrease in sperm mobility and significant elevation of sperm DNA defragmentation in lycopne untreated samples whilst the treated samples showed a significant decrease in sperm DNA defragmentation [109].

Ghyasvand et al., performed another study to evaluate the levels of lycopene, beta-carotene and retinol in serum and their relationship with sperm DNA disruption and lipid peroxidation in infertile and normo-spermic males, and concluded that lycopene, beta-carotene, and retinol can decrease sperm DNA fragmentation and lipid peroxidation via their antioxidant effect [110].

4.2.1.2 Lutein and zeaxanthin

Comhaire et al., conducted a study involving 30 subfertile men which were treated with 16 mg a day of astaxanthin, a carotenoid which is not transformed into vitamin A in humans, for 3 months and did not find any, significant enhancement in concentration, motility, morphology or volume in the 11 treated men compared with the 19 control patients [111].

Intriguingly, Ming-Chieh Li, et al., found an unusual and unexpected inverse relationship of β-carotene administration from foods and of lutein and zeaxanthin administration with live birth rates. Within the observed administration ranges, total consumption of vitamins A, C and E before initiating infertility treatment with ART was not correlated with live birth rates [112].

4.2.2 Vitamin B (folic acid)

Folic Acid is a B-vitamin which is necessary for the synthesis of DNA and transfer of RNA.

The micronutrients folate and zinc are closely related with semen quality. Blood plasma and seminal plasma levels of folate are positively related with sperm concentration and count [113, 114].

Moreover, folic acid, the synthetic form of folate, effectively scavenges oxidizing free radicals and inhibits lipid peroxidation [115].

The evidence on the impact of folate on fertility is unclear. Wong et al., reported that in vivo utilization of folic acid, alone or combined with zinc sulphate (5 and 66 mg day respectively), enhanced sperm concentration and count in their formerly discussed controlled trial. The results were, however, significant only for the 103 infertile men involved in the study, whilst the 107 fertile men did not present any enhancement in sperm parameters [116].

Also, nutritional utilization of antioxidants, such as vitamins C and E, and β-carotene, and micronutrients, such as folate and zinc, have been shown to be critically essential for normal semen quality and reproductive function as confirmed by a large number of studies in both animals and humans [55].

Ebisch et al., showed that administration of zinc and folic acid enhancesnot only sperm quality but also the outcome of varicocelectomy [117].

Other researchers have revealed that a low folate concentration in seminal plasma is correlated with increased sperm DNA damage in infertile men [118].

Schisterman et al., designed and conducted a trial involving 2,370 couples planning infertility treatments. The men were assigned arbitrarily and received either a placebo or a daily supplement comprising of 5 milligrams of folic acid and 30 milligrams of zinc. The results indicated that the live births did not alter significantly among the two groups: 404 (34%) in the supplement group and 416 (35%) in the placebo group. Similarly, the groups did not show difference among various semen parameters, such as sperm motility, morphology and total count. However, the fraction of sperm DNA fragmentation was elevated in the supplement group (29.7%), when compared to the placebo group (27.2%). Men in the supplement group also presented an elevated proportion of gastrointestinal symptoms, when compared to the placebo group: abdominal discomfort (6% vs. 3%), nausea (4% vs. 2%) and vomiting (3% vs. 1%). The authors reported that these dietary supplements have a very little to no impact on fertility and may even provoke mild gastrointestinal symptoms [119].

4.2.3 Vitamin C

Ascorbic acid represents the main natural water-soluble antioxidant and is an essential dietary nutriment. This water-soluble antioxidant is an essential dietary nutrient.

Beside, its high potency for scavenging ROS [120], Vit-C serves as an excellent source of electrons, providing an electron to free radicals such as superoxides and hydroxyls radicals, which decreases their reactivity and damages [121].

Ascorbic acid exhibits both antioxidant and prooxidant properties depending upon the amount [122]. As an exogenous antioxidant in semen, ascorbic acid played a very crucial role in controlling the oxidative stress [47].

In an extensive study comprising of 30 infertile but healthy men, daily intake of 200 mg and 100 mg vitamin C enhanced sperm count by 112 and 140 percent respectively. Intriguingly the concentration of ascorbic acid in the seminal plasma is 10-fold higher than the serum [123].

The concentration of ascorbic acid in the seminal plasma was observed to be negatively correlated with reactive oxygen species activity in sperm of infertile men, and the reduced ascorbic acid intake was correlated with an elevated oxidative damage in the sperm of healthy men [124].

Hughes et al., reported the significance of vitamins C (300-600 mmol/l) and E (30–60 mmol/l) in protecting sperm DNA integrity in Percoll preparations; however, this combination has generated contrasting outcomes with excessive DNA damage, which is likely associated to pro-oxidant effects [125].

Donnelly et al. reported decreased H_2O_2-induced ROS production and DNA damage after the utilization of both vitamin C (300 and 600 mmol/l) and vitamin E (40 and 60 mmol/l) to normozoospermic and asthenozoospermic samples [126].

Vitamin C acts as a cofactor for various key enzymes. It assists in the metabolic processes of folic acid, tyrosine and tryptophan [127]. In cells, ascorbic acid interacts with glutathione in maintaining the reduced form of tocopherol [128].

Kini et al., reported a significant increase in the testicular GSH and SOD level but a reduction in testicular MDA level after pre-treatment of rats with Vitamin-C before cadmium chloride exposure [129]. Similar outcomes were observed by Behairy et al. [130]. In addition, vitamin C is vital in protecting from oxidative damage to the sperm and steroid cells of the Leydig cells and in the sperm chamber [131].

A potent and combined action of vitamin C and E has been observed to protect the spermatozoa against peroxidative insult and DNA fragmentation [132].

Vitamin C reduces the inter chain disulphide bridges in protamines opening the cysteine net and subsequently causing DNA decondensation in spermatozoa [54, 133, 134].

4.2.4 Vitamin D

Vitamin D plays a significant role in regulating both male and female fertility [135]. The quality of spermatozoa strongly dependen upon vitamin D [136]. Vitamin D is also responsible for retaining sperm motility [137]. Low fertility rates have also been credited to low vitamin D levels in the serum of males [138]. Vitamin D deficiency, in contrast, decreases the probabilities of success when undergoing assisted reproductive technology [139–141]. Islamian et al. investigated the combined effect of vitamin D complement and docosahexaenoic acid on oxidative stress indices of semen in asthenospermic men. The outcome showed that the combined treatment with fatty acid complement, docosahexaenoic acid and vitamin E in asthenospermic men decreased oxidative stress in seminal plasma [142].

4.2.5 Vitamin E (α-tocopherol)

Vitamin E represents a fat-soluble antioxidant molecule which serves as a major chain breaking antioxidant in membranes.

Numerous studies were conducted on vitamin E. The first clinical trials showed that vitamin E supplementation increase the fertilization rates by decreasing lipid peroxidation potential [143]. Kodama et al., reported elevated sperm concentration and decreased DNA damage in 36 infertile patients who underwent 2 months of therapy with vitamin E (200 mg day), vitamin C (200 mg day) and glutathione (GSH; 400 mg day), with no significant enhancement in motility or morphology [144].

Similarly, in another study in which a co-administration of vitamin E and selenium for six months resulted in a significant increase in sperm motility and a decreased percentage of defective spermatozoa in comparison with pre-supplementation period [145, 146]. Besides, it has also been reported that vitamin E decreases the oxvidative damage resulting an increase in the fertilization rate [71].

A plethora of research has been dedicated to show that antioxidants such as vitamins E and C and carnitines assist in reducing oxidative stress by quenching free radicals [42]. Greco et al. showed a significant enhancement in 38 men with increased sperm DNA fragmentation after 2 months of combined therapy comprising of 1 g of vitamin C and 1 g of vitamin E daily: clinical pregnancy and implantation rates presented a remarkable increase after the second attempt in comparison with an initial failed intracytoplasmic sperm injection attempt before treatment [53].

Also, vitamin E as an antioxidant may directly quench free radicals and together with CoQ10 shield lipid membranes from peroxidative insult [147].

Moslemi et al., showed that supplementation of vitamin E can significantly decrease lipid peroxidation in seminal plasma, enhance sperm motility and boost pregnancy occurrence [148].

However, in a Meta-analysis, four studies confirmed that it had a very little or no impact on semen parameters, whilst beneficial effects were shown in the remaining 18 studies [86].

4.2.6 Carnitine

The antioxidant activity of carnitines shields against lipid peroxidation of cells membrane, stimulating a large number of in vivo trials examining their impact on sperm parameters.

In the trial conducted by Costa and colleagues [149], 3 g day of L-carnitine was provided to 100 asthenozoospermic men. After 4 months of treatment, a significant enhancement in sperm concentration, motility and morphology was observed.

Balercia et al., evaluated various doses of L-carnitine and acetyl-L-carnitine (3 g of L-carnitine, 3 g of acetyl-L-carnitine, or 2 g L carnitine and 1 g acetyl-L-carnitine daily), showing enhanced sperm motility in asthenozoospermic subjects [150].

Also, Carnitine supplementation has been reported to enhance sperm concentration, mobility, viability, morphology, and total antioxidative capacity [151].

In contrast, Sigman et al., did not find any enhancements in semen parameters in 26 men diagnosed with asthenozoospermia and underwent 6 months of therapy with L-carnitine and L-acetyl-carnitine with 2 and 1 g day, respectively [152].

Gvozdjáková et al., demonstrated a beneficial impact of treatment with a combination of different antioxidants (carnitine with ubiquinol, vitamins E and C) on sperm density, which improved by 39.8% after 3 months of therapy and by 78.0% after 6 months of therapy and sperm motility was enhanced. Sperm pathology reduced in 25.8% after 3-month treatment [153].

4.2.7 Flavonoids

Flavonoids comprise of a very large heterogeneous group of benzopyran derivatives found naturally in fruits, vegetables, and herbs. This group of plant antioxidants has many beneficial health effects.

Flavonoids exhibit a positive health effect in cancer and neurodegenerative diseases, owing to their innate free radical-scavenging activities [154].

These flavonoid glycosides serve as extremely potent free radical scavengers [155, 156]. Some of the most popular and well-known antioxidant flavonoids also serve as prooxidants even when a transition metal is available [157].

Quercetinis one of the most abundant natural flavonoids found in a large variety of fruits and vegetables [158, 159].

The pentahydroxy flavone protect from oxidative injury and cell death by scavenging free radicals, donating hydrogen compound, quenching singlet oxygen, and shielding lipid peroxidation or chelating metal ions [160].

4.2.8 Selenium

Selenium is a very important trace mineral which is highly essential for the human body, including the immune system, cognitive function, and male and female fertility.

According to the United States Office of Dietary Supplements, it participates in the metabolism of thyroid hormone and DNA synthesis, and protects against infection and oxidative damage.

Selenium significantly contributes in the construction of the mitochondrial protective shield in sperm cells and interferes with the condition and function of sperm, and is potent in the treatment of impaired fertility [161].

Searching for a concrete proof of the advantages of in vivo selenium therapy is problematic, since various studies with a range of dosages have resulted in conflicting results.

Iwanier and Zachara did not find any positive impact after 3 months of therapy with selenium at a dose of 200 mg day in 33 subfertile men [162].

In 1998, Scott and his colleagues executed a trial on a group of 64 men (comprising of 46 men diagnosed with OAT and 16 classified as subfertile), dispensing selenium alone or in combination with vitamins A, C and E at daily doses of 100 mg, 1 mg, 10 mg and 15 mg respectively. No significant enhancement was observed in sperm concentration even after 3 months, although motility was increased in treated subjects [163].

However, most of the other studies have claimed enhancements in several parameters after several months of combined therapy with selenium and other antioxidants.

Vezina et al., reported that combination of selenium and vitamin E enhanced the sperm motility, morphology and viability, and the concentration, however, did not alter significantly, as previously discussed [145].

Keskes-Ammar et al., and Safarinejad employed a combination therapy—selenium and vitamin E in the former and selenium and NAC in the latter—which enhanced the motility in both studies and improved the sperm count and morphology in the latter [73, 164].

Besides, it has been demonstrated that Selenium is a fundamental element for semen quality and plays an important function in keeping reproductive condition [31, 48].

4.2.9 Zinc

Zinc is an important micronutrient which also serves as a multifunctional co-factor for more than 80 metallo enzymes involved in DNA transcription and protein synthesis [117]. Moreover, zinc finger proteins are extensively involved in the genetic expression of steroid hormone receptors [165], and it also has antioxidant [166] and anti-apoptotic properties [167].

An interesting correlation was also revealed between the level of Zn in serum and semen in oligozoospermic infertile men, with significantly decreased levels of Zn in serum and semen of men with fertility problems [168]. Zinc levels in seminal plasma have also been positively correlated with sperm concentration and motility in the literature [169, 170].

The Zinc in seminal plasma also serves as antioxidant and anti-bacterial agent which shields the semen from heavy metals accumulation [171].

Two studies were published in which $ZnSO_4$ was utilized: in the first, 500 mg/day of Zinc sulphate ($ZnSO_4$) was dispensed to 100 asthenozoospermic men for 3 months, and a significant upturn in sperm count, motility and membrane integrity was observed [172]. The second study, published 10 years later, employed a combination of drugs and Zinc sulphate ($ZnSO_4$) has been postulated as an infertility treatment, although only a few studies have shown its impact on semen parameters when applied in vivo [173].

Wong et al. observed an elevated sperm count and enhanced semen concentration in a controlled trial with 103 infertile and 107 fertile men who had consumed 5 mg folic acid and 66 mg $ZnSO_4$ per day for 6 months, either alone or in combination but no enhancements in any of the sperm parameters, most notably concentration were observed [116].

Due to its key role in the processes of DNA compaction, administration of this micronutrient i.e. Zn was very successful in enhancing sperm morphology and DNA integrity in patients suffering from prostate abnormalities [174, 175].

High seminal Zn levels may provide harmful effect on the spermatozoa-zona pellucida-induced acrosome reaction in normozoospermic men [176].

Zinc, is extensively involved in processes of reproduction, not only in the hormone metabolism but also in sperm formation and in the regulation of sperm viability and motility [31].

4.2.10 Manganese (Mn)

Manganese (Mn^{2+}) is one of the most abundant element which is widely distributed in soil, air, water, and food [177].

It is known by its ability to quench the superoxide anions and hydroxyl radicals and due to its chain breaking capacity [178].

The structural flexibility induced by Mn^{2+} is also vital for enzyme dynamics, since Mn is crucial for RNA polymerization [179].

Therefore, Manganese is an essential metal which serves as a co-factor for several enzymes and plays several important biological functions [180]. Mn^{2+} may stimulate the enzymes of glutathione cycle and interact with the total thiols (TSH), glutathione reduced (GSH), glutathione oxidized (GSSG) contents in human spermatozoa. It reduces the generation of thiobarbituric acid reactive substances. In several organisms, elevated intracellular manganese shields against oxidative damage via unknown pathways [181].

Few studies have evaluated the impact of Mn exposure on male reproductive health. Lafond et al., showed that decreased Mn levels in seminal plasma from men with diminished sperm densities [182]. Besides, Mn^{2+} is a very potent stimulator of sperm motility through the stimulation of adenylate cyclase activity [183].

In addition, Manganese accelerates the progressive motility of human washed sperm in a time and dose dependent manner [184]. Moreover, it is proposed that anti-oxidative activity of Mn2+ stabilizes the plasma membrane, thereby sustaining the membrane integrity and viability [185].

Also, Mn is a necessary element for humans, but although it has several crucial functions for normal reproductive health, overexposure to Mn2 may be harmful to reproductive health [176, 186].

4.2.11 Pentoxyfilline (PTX)

PTX is a derivative of xanthine and a methylxanthine, it is a vasodilating compound which increases red blood cell deformability, prevents from inflammatory reactions and decreases blood viscosity by avoiding platelet aggregation [187].

Pentoxifylline serves as a phosphodiesterase inhibitor and shields the cells from lipid peroxidation by H_2O_2, therefore it may be helpful to minimize H_2O_2 induced embryo damage and improve IVF outcome [188].

Controversial outcomes were presented in various studies. Some studies reported that the pentoxifylline exerts a beneficial effect on sperm parameters, by decreasing the superoxide anion generation [189–191] or by minimizing lipid peroxidation [191, 192]. However, Twigg et al., did not find any enhancement in lipid peroxidation after the utilization of 3.6 mmol/l of pentoxifylline [88].

Safarinejad carried out a randomized controlled trial on a population comprising of men with idiopathic Oligoasthenoteratozoospermie (OAT). He investigated the response of semen parameters to supplementation with 400 mg of PTX twice daily for a 24-week therapy phase followed by a 12-week therapy-free period. The outcomes of that study revealed a significant enhancement in seminal parameters such as concentration, motility, and morphology [91].

It was also reported that PTX exhibited a positive impact on ICSI outcomes, including fertilization, embryo quality, and pregnancy rates, in asthenozoospermic patients [187].

In conclusion, antioxidants intake separately or combined for at least three monthes is advisable for patients who planning to undergoing ART therapy.

Conflict of interest

The authors declare that they have no conflict of interest.

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