Reductive Stress and Male Infertility

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Abstract

Male infertility research and clinical advances had vast progress in the last few decades. Strong research evidence underpinned the concepts of oxidative stress (OS)-mediated male reproductive disruptions, which bear answers to several cases of idiopathic male infertility. Antioxidant treatment held the prime solution for OS-mediated male infertility. But excess use of antioxidants is challenged by the research breakthrough that reductive stress also predisposes to male infertility, resolutely instituting that any biological extremes of the redox spectrum are deleterious to male fertility. Superfluity of reducing agents may hinder essential oxidation mechanisms, affecting physiological homeostasis. These mechanisms need to be explicated and updated time and again to identify the fine thread between OS-mediated male infertility treatment and induction of reductive stress. This chapter thus presents the evidence-based concepts pertaining to the antioxidants actions to combat OS-induced male infertility, the mechanism of induction of reductive stress and its impact on male reproduction.

Keywords

Antioxidants · Male infertility · Oxidative stress · Reductive stress

17.1 Introduction

Human infertility has been identified as a disease by the World Health Organization (WHO), and it afflicts about of 7–15% of the world population (Louis et al. 2013; Datta et al. 2016). Male factor reportedly is the only or the prime cause of infertility overall 20–30% of infertility cases (Agarwal et al. 2015; Vander Borght and Wyns 2018). This justifies the strengthened research focus on male infertility over the past few decades, surfacing new concepts regarding the causes, mechanisms, treatment, and management of male infertility (Sheweita et al. 2005; Dutta et al. 2019). However, there are still

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gaps in knowing the exact etiology of male infertility and a huge proportion of male infertility cases remain idiopathic (Aktan et al. 2013). Substantial research has underpinned the concepts of oxidative stress (OS) being the underlying mechanism for most of the idiopathic male infertility cases (Alahmar et al. 2021a; Alahmar and Sengupta 2021). Excess reactive oxygen species (ROS) can override the endogenous antioxidant capacities, approaching pathological levels and disrupting normal male reproductive functions. While excess ROS levels have deleterious impact on male fertility (Agarwal and Sengupta 2020), physiological level of ROS are required for the sperm to execute natural functions. For example, nitric oxide and hydrogen peroxide are required molecules for capacitation, which allows for the acrosome process to take place, which is regulated by ROS (Dutta et al. 2020; Dutta and Sengupta 2021). Furthermore, ROS mediates sperm hyperactivation or contact with the oocyte, making it necessary for them to fertilize the egg (Dutta et al. 2020). Normal sperm activity necessitates a proper balance of oxidant and antioxidant systems.

Antioxidant treatment is the prime regimen for OS-mediated male infertility (Sheweita et al. 2005; Poljsak and Šuput 2013). Nevertheless, excessive use of antioxidants faced a major blow by the revelation that such overuse of antioxidants, "over-the-counter" consumption of antioxidant supplements, or even paucities in antioxidant efficacies are assumed to cause adverse consequences by shifting the endogenous redox balance to the reductive end of the spectrum (Pérez-Torres et al. 2017). Thus, any biological extremes of the redox spectrum, reductive stress, and OS are both deleterious to male fertility. These concepts are yet to be fully revealed and are subjected to be updated time and again to facilitate rapid advances in understanding of reductive stress-mediated male infertility. The present chapter aims to elucidate the concepts of antioxidants used to combat OS-induced male infertility, the evidence-based mechanism of reductive stress induction, and its effects on male reproduction.

17.2 Oxidative Stress: Oxidants and Reductants (Antioxidants)

Oxidative stress (OS) acts as a central key mechanism in the etiopathogenesis of most the human diseases, including aging, cancer, and even reproductive disorders. Antioxidant treatment has attracted a lot of interest in research lately, owing to a rapidly aging population. Antioxidants are appealing because they are regarded "natural" and hence "good" compounds, and they are linked to a healthy diet. Aggressive marketing initiatives of antioxidative products in a multibillion-dollar business support this widespread notion. Reduction of OS is projected as the way to avoid ailments including cancer and cardiovascular pathologies (Halliwell 1999; Willcox et al. 2004). Given that these components may without a prescription be bought over the counter and even foods are supplemented by these supplements, it is essential to have no hazardous effects. While the original antioxidant supplement research revealed that these supplements are effective in disease prevention, more current clinical trials and the usefulness of such therapies were questioned. Antioxidant supplementation for any physiological ailment is not under the regulation of the US Food and Drug Administration (FDA). Moreover, current consensus guideline by the European Society for Human Reproduction and Embryology (ESHRE) has put forth that there is presently insufficient report to underpin the use of antioxidants supplementation (Barratt et al. 2017). Excessive supplementation has been shown in several trials to be harmful (Bjelakovic et al. 2007, 2004; Stanner et al. 2004). Later studies have backed up these concerns (Bjelakovic et al. 2012). Antioxidant therapies have also been shown to boost male fertility in numerous research (Alahmar and Sengupta 2021; Busetto et al. 2018; Sengupta et al. 2018; Torres-Arce et al. 2021; Izuka et al. 2020). Furthermore, excessively high amounts of antioxidants have been shown to be teratogenic to embryos (Wang and Rogers 2007). Current attention of reproductive medicine research has thus been intensive on the use of antioxidants for male infertility therapy.

Reactive oxygen species (ROS) are produced by oxygen metabolism and include the superoxide anion ($\cdot O_2^-$), hydrogen peroxide (H_2O_2), and the hydroxyl ($\cdot OH$) radical whose outer orbit has unpaired electrons, rendering them unstable and highly reactive (Greabu et al. 2008). During aerobic metabolism, ROS are produced via mitochondrial oxidative phosphorylation, and about 1–5% of it leaks out during the process (Dutta et al. 2020; Bioveris and Chance 1973; Halliwell 2011; Hayyan et al. 2016; Turrens 2003) and secreted as cytotoxic cellular metabolic by-products (Agarwal and Sengupta 2020; Raha and Robinson 2000). ROS may damage numerous biological components, including proteins, lipids, and nucleic acids, resulting in oxidations of lipids and proteins, and DNA damage, due to their super-reactivity and half-life periods in the nanosecond range (Halliwell and Gutteridge 2015). Different pathologies like aging, neurological disorders, cancer, and infertility can be caused due the overproduction of ROS (Agarwal et al. 2003; John Aitken et al. 1989; Li et al. 2013). Although ROS have a negative impact on cellular processes at greater concentrations, they are also required for cellular growth, signalling, immunological response to infections and inflammation, maturation of sperm, capacitation, and embryonic morphogenesis (Dutta et al. 2020; Dröge 2002; Hampton et al. 1998; O'Flaherty 2015; Sengupta et al. 2020). Superoxide scavenging through superoxide dismutase (SOD) catalysis, however, blocked capacitation-related tyrosine phosphorylation in human sperm, which basically makes them incapable of fertilizing oocytes (Cn et al. 2005). Cells normally use multiple intrinsic and extrinsic antioxidant pathways to scavenge excessive ROS. Enzymatic antioxidants like SOD, catalase, and thiol peroxidases, as well as nonenzymatic antioxidants like glutathione, are endogenous antioxidants. Exogenous antioxidants, on the other hand, are micronutrients like vitamins A, C, and E, coenzyme Q10, L-carnitine, and trace elements like selenium and zinc (Halliwell and Gutteridge 2015), which must be supplemented to the body exogenously to preserve a healthy redox balance in every living cell (Valko et al. 2007). Obesity, caused by unhealthy lifestyle may result in a systemic inflammation and increased generation of inflammatory cytokines and result in OS (Kahn and Brannigan 2017; Tsatsanis et al. 2015; Bhattacharya et al. 2020). Furthermore, OS is caused by alcohol consumption, smoking, exposure to radiation, and occupational or environmental toxins (Mathur et al. 2011; Sharma et al. 2013).

17.3 Antioxidant Overdose and Male Fertility: The Concept of "AntioxidantParadox"

Antioxidant supplements are commonly prescribed by physicians to treat OS-related disorders, including male infertility. Antioxidants, by definition, are substances that prevent oxidation by donating electron, in contrast to oxidants which are electron acceptors (Atta et al. 2017). The body's redox potential must be balanced in order for homeostasis to be maintained. ROS, free radical productions, and antioxidant levels must be kept in balance. To accomplish this, human system must absorb antioxidants through nutrition, or antioxidant supplements should be given if this is not adequate owing to exposure to high amounts of oxidants. One must thus differentiate dietary antioxidants from antioxidant supplements, which are strongly publicized and widely accessible over the counter. In several cases patients consume excessive over-the-counter antioxidants since these substances are frequently marketed as health boosters since they may "fight" pathological ailments. Exogenous supplements generally have high quantities of a few refined antioxidants including vitamins A, C, and E, as well as lycopene. In addition, many typical food products already contain several vitamins and antioxidants. As a result, it is likely that patients will unintentionally take very high dosages, or perhaps excessively high dosages, of antioxidants (Poljsak and Šuput 2013). Antioxidant supplementation, on the other hand, has not consistently had positive results. Furthermore, some research have even

documented the negative consequences of antioxidant use. For instance, vitamin E has been demonstrated to increase all-cause mortality in individuals at larger dosages (Miller III et al. 2005). Additionally, it is well-known that the vitamin A supplementation has no anticancer impact, while the opposite impact has been noticed in smokers (Alpha-Tocopherol Beta Carotene Cancer Prevention Study Group 1994). Increased OS and DNA fragmentation have linked to low vitamin C consumption (Fraga et al. 1991). High ascorbate concentrations, on the other hand, are reported to cause the same effects by producing OS (Aruoma et al. 1991). Halliwell (2000) coined this unorthodox impacts of antioxidants as the "antioxidant paradox" (Halliwell 2000). In essence, these paradoxical results underscore the need of ROS formation and the scavenging actions of antioxidants for proper physiological functions, including regulation of male fertility. Therefore, to maintain this "homeostasis," a delicate cellular redox balance is required (De Lamirande and Gagnon 1995; Kothari et al. 2010).

17.4 Antioxidant Paradox: Generation of "Reductive Stress"

Following the above discussion, it can be perceived that uncontrolled, unphysiologically high exposure to antioxidants disturbs the cellular redox balance resulting in "reductive stress" (RS). The term "reductive stress" refers to a shift in the body's redox balance in the reductive direction (Wendel 1987), a situation that has been compared to OS in terms of its harmful effects (Castagne et al. 1999).

The GSH/GSSG and/or NAD/NADH+ ratio elevation or overexpression of antioxidant enzymes can reduce all ROS in the presence of excessive reducing equivalents, and that may cause a H_2O_2 surplus and spillage from mitochondria, propelling the cells to RS (Bjørklund and Chirumbolo 2017) (Fig. 17.1).

Pathological mitochondrial oxidation, breakdown of mitochondrial homeostasis, and protein misfolding in the endoplasmic reticulum may result from NADH excess (Zhang et al. 2012). Mitochondrial ROS and their RS-mediated diminution have crucial role in proper protein folding process as well as in the disulfide bonds formation, which are among the key determinants of normal proteins structure and functions (Murphy 2009). When mitochondrial oxidant generation is suppressed, the amounts of cellular disulfide bonds drop dramatically in many cells (Yang et al. 2007). RS disrupts disulfide bond formation and triggers the "unfolded protein response of the endoplasmic reticulum" (UPR^{ER}) (Walter and Ron 2011). To recover proteostasis in this compartment, the proper folding of proteins must be restored (Walter and Ron 2011). Chronic RS can also cause OS, which, in turn, drives RS via a feedback regulation. When electron acceptors are predicted to be largely reduced during RS, for example, several redox proteins can transfer electrons to O_2, boosting ROS generation (Korge et al. 2015). Thus, this is double-edged sword. Excessive reducing equivalents hinders cellular growth responses, diminish mitochondrial function, affect disulfide bonds formation in proteins, and reduce cellular metabolism (Pérez-Torres et al. 2017). The notion of RS has previously been accepted in various other medical fields to explain pathological ailments including carcinogenesis, cardiomyopathy, cerebral microvasculature, malfunction of blood-brain barrier, and neurodegenerative diseases (Klein et al. 2011; Brewer et al. 2013; Mentor and Fisher 2017). Male fertility is also not an exception.

17.5 Reductive Stress, Antioxidant Paradox, and Male Fertility

To reduce the consequences of OS, physicians have begun treating patients with an excessive antioxidants for various causes for infertility (Greco et al. 2005; Abad et al. 2013). In this context many groups have demonstrated a considerable good impact on sperm parameters, with sperm DNA damage, chromatin packing, with additional intakes of antioxidants including

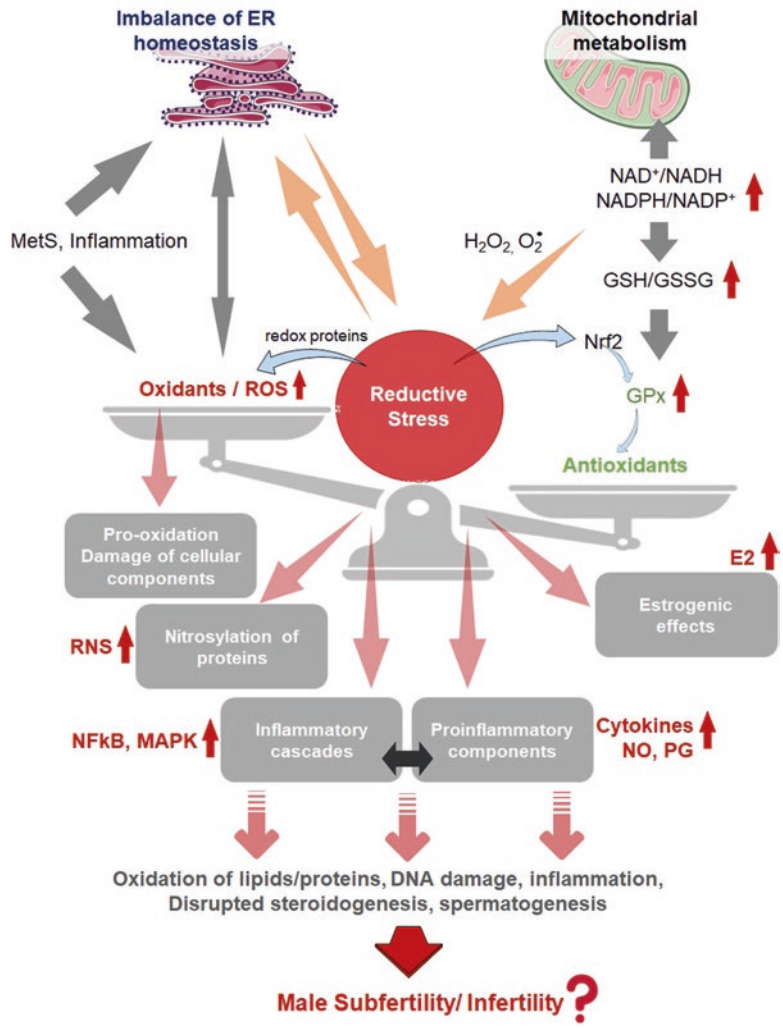

Fig. 17.1 The mechanism of generation of reductive stress and its consequences on male fertility. ER endoplasmic reticulum, MetS metabolic syndrome, GSH reduced glutathione, GSSG oxidized glutathione, ROS reactive oxygen species, RNS reactive nitrogen species, GPx glutathione peroxidase, Nrf2 nuclear factor-erythroid factor-2-related factor-2, E2 estradiol, NFkB nuclear factor kappa B, MAPK mitogen-activating factor kinase, NO nitric oxide, PG prostaglandin

L-carnitine, vitamins C and E, and/or coenzyme Q10 (Alahmar et al. 2021a, b, c; Abad et al. 2013; Ahmadi et al. 2016). According to a Showell et al., supplementation of antioxidants to infertile males may enhance pregnancy outcomes and minimize live birth rates (Showell et al. 2014). Huang and colleagues have established that seminal OS caused by lower levels of antioxidants is linked to male infertility. Specifically, antioxidant therapy has been found to be more beneficial for idiopathic male infertility and varicocele (Garg and Kumar 2016; Alahmar 2018). Although it is obvious and the findings are distinct that antioxidants are essential for the mitigation of OS, some scientists find little or even harmful effects of such therapies on particular sperm parameters (Silver et al. 2005; Stenqvist et al. 2018). Thus, the truth remains that while ROS play a significant part in the etiology of various human diseases, higher dosages of antioxidant supplements have had contradictory outcomes, leading to the coining of the phrase "antioxidant paradox" by researchers (Halliwell 2000). This underlines the limited understanding of the mechanisms of antioxidant treatment. Current antioxidant treatments to treat male infertility seems to be founded on an erroneous premise

that antioxidants are generally good, resulting in the misuse of the antioxidant composition.

As overproduction of ROS and thus OS is a primary cause of male infertility (Agarwal et al. 2008), unphysiologically high antioxidant concentrations, on the other hand, have considerable negative impacts on cells, and male fertility appears to be no exception (Fig. 17.1). Preserving the redox balance is important not only for the body but also for sperm since vital sperm activities such as capacitation, acrosome reaction requires modest amount of ROS, and excessive antioxidants will quench these effects and prevent sperm from fertilizing oocytes (De Lamirande and Gagnon 1995; Kothari et al. 2010). Mammalian embryos have been found to require strict regulation of the cellular redox system (Ufer et al. 2010); in this context, high antioxidant levels have been linked to the development of teratogenicity (Wang and Rogers 2007). As these early embryos are normally exposed to a relatively hypoxic environment in utero, glycolytic energy production appears to be favorable for cellular compaction and blastulation (Harvey et al. 2002). According to the scientists, alterations in redox-sensitive transcription factors and gene expression may occur as a result of this metabolic shift. This can disrupt critical embryonic development processes such as the process of fertilization, genome activation, or cellular differentiation. In general, an examination of the current literature reveals that reproductive redox biology is poorly understood. There are substantial evidences on OS in respect to male infertility, and despite the evidence that excessively high levels of antioxidants or the improper composition of antioxidant mixtures may have harmful effects on male infertility, its counterpart, RS, is often misunderstood and underappreciated (Bouayed and Bohn 2010). In this respect, Menezo and coworkers investigated daily supplementation with a mixture containing vitamins C and E, β-carotene, zinc, and selenium improved sperm DNA fragmentation (Ménézo et al. 2007). Sperm nuclear decondensation, on the other hand, increased, probably as a result of vitamin C reducing the disulfide bonds in the protamines (Giustarini et al. 2008), due to which, the chromatin becomes destabilized, resulting in unsuccessful fertilization.

Indeed, as discussed earlier, several of the normally suggested antioxidants like selenium and vitamins C and E have documented certain unfavorable effects (Ménézo et al. 2014). In the case of selenium, no deficit has yet been shown in the scientific literature. As reported in some studies, higher levels of seminal plasma selenium (≥80 ng/ml) is related with reduced sperm motility, asthenozoospermia, and high rates of abortion, whereas 40 and 70 ng/ml of selenium are found to be ideal in reproductive efficiency (higher pregnancy outcomes and lower rates of abortion) (Bleau et al. 1984). Moreover, there are growing evidences that antioxidant or prooxidant actions of antioxidants ultimately depend on their concentration even if they come from natural sources (Bouayed and Bohn 2010). Furthermore, given the synergistic action of many antioxidant plant chemicals, antioxidant therapy will not only fail but can also be harmful when the synergistic molecule is lacking. This may be demonstrated in smokers with 20 mg/day β-carotene or 30 mg β-carotene and 25,000 IU retinyl palmitate per day, since in 29,133 and 18,314 participants, the risk of pulmonary carcinoma was considerably raised in a placebo efficacy study (Alpha-Tocopherol Beta Carotene Cancer Prevention Study Group 1994; Omenn et al. 1996). Furthermore, many animal research and human findings have shown that high antioxidant levels might have clinical consequences. These effects might be the consequence of redox imbalances in the body, which can result in OS (by the "antioxidant paradox") or also "reductive stress" (RS) (Wendel 1987), which, by causing cellular dysfunction, can be just as harmful to cells or the body as OS (Castagne et al. 1999).

In reproductive biology research, it has been documented that the appropriate balance between oxidation and reduction is essential for normal embryogenesis hence the cellular redox system must be strictly maintained (Ufer et al. 2010). As a result, excessive antioxidant levels

may cause teratogenic alterations (Wang and Rogers 2007), such as alterations in the redox-sensitive transcription factor activities and, therefore, alterations in gene expression (Harvey et al. 2002). Subsequently, deformities and developmental retardation may ensue. The growing embryo, on the other hand, is exposed to an essentially anoxic environment during implantation, showing the limited range of this redox balance for live cells (Leese 1995). Particularly, in andrology, Menezo et al. (2007) had analyzed antioxidants treatment given orally in daily basis to men whose female partners failed to become pregnant after IVF/ICSI, and the antioxidants consisted of vitamins C and E (400 mg each), zinc (500 μmol), β-carotene (18 mg), and selenium (1 μmol) (Ménézo et al. 2007). Sperm DNA damage decreased following the treatment, while sperm DNA decondensation increased, perhaps leading to asynchronous chromosomal condensation. In IVF and ICSI patients, DNA decondensation rates of more than 28% have been demonstrated to induce unsuccessful pregnancy outcomes. The capability of vitamin C to break disulfide bonds in protamines in sperm DNA may be causing greater DNA decondensation (Giustarini et al. 2008; Donnelly et al. 1999). Reduced protamine levels will eventually lead to nuclear decondensation issues and diminished fertility (Ménézo et al. 2007). Vitamin C has been shown to have both positive and negative dose-related effects on sperm membrane lipid peroxidation (LPO) and motility in an earlier in vitro study. Vitamin C at concentrations below 1000 μM resulted in increased sperm motility and decreased LPO, while concentrations above 1000 μM have shown the complete opposite effect with complete sperm immobility at concentrations above 4000 μM (Verma and Kanwar 1998). These studies reveal contradictory antioxidants impacts in the therapy of male infertility, which is dose-dependent [Table 17.1; (Greco et al. 2005; Ménézo et al. 2007; Gual-Frau et al. 2015; Tunc et al. 2009; Schisterman et al. 2020; Moilanen et al. 1993; Kessopoulou et al. 1995; Rolf et al. 1999; Sigman et al. 2006)]. However, one can believe that the makeup of a certain antioxidant combination, such as that found in various antioxidant supplements, has an impact. Finally, physicians would need to know the exact unique cellular redox level of each patient in order to treat them properly. The problem is that (a) no universally accepted method is available to test the bodily or seminal redox status of individuals, (b) one does not know what the normal redox level is, and (c), therefore, no generally accepted cut-off values are available. As a consequence, patients are not only taking antioxidants because they have been persuaded that antioxidants are excellent for antiaging and the body in general, but they are also being treated by physicians for male infertility since OS is regarded to be harmful in this case. Because of our contemporary lifestyle, which includes a lack of vitamin consumption, exposure to environmental toxins, and/or smoking, the body is subjected to OS. As a result, antioxidant therapy can assist to change this situation. Uncontrolled antioxidant consumption, on the other hand, may cause RS and infertility by implosion of antioxidant therapy. As a result, greater research into redox state, its impact on the fertilization process in general, and sperm functional capability in particular is required. Meanwhile, in the absence of recommendations, physicians should do a more comprehensive examination and interview of patients before prescribing more antioxidants to prevent the risk of RS caused by overdose of antioxidants.

17.6 Conclusion

The perception of antioxidants usage to treat OS-induced male infertility seems vastly oversimplified, while the excessive use of widely available antioxidants remains under-discussed. Few studies have emphasized upon the so-called antioxidant paradox phenomenon, and it is perceived that the nonprescription antioxidants supplements and over-the-counter antioxidants consumption might constitute the major reason for antioxidants overuse. Recently, male infertility research has laid

Table 17.1 Individual or combination antioxidant treatments with no significant effects on semen quality

Study	Antioxidant regime	Study population	Sperm parameters
Moilanen et al. (1993)	100 mg of vitamin E daily for 3 months	Unexplained infertility IUI	No change in SC, S_{mot} and S_{morph}
Kessopoulou et al. (1995)	600 mg of vitamin E daily for 3 months	Infertility with high ROS	No change in SC, S_{mot} and S_{morph}
Rolf et al. (1999)	1 g vitamin C and 800 mg vitamin E daily for 56 days	Asthenozoospermia	No change in SC, S_{mot} and S_{morph}
Greco et al. (2005)	1 g of vitamins C and E daily for 2 months	Idiopathic infertility (38 infertile men with previous IVF/ICSI)	No change in SC, S_{mot} and S_{morph}
Sigman et al. (2006)	1 g carnitine and 500 mg L-acetyl carnitine daily for 24 weeks	Asthenozoospermia	No improvement in S_{mot}
Menezo et al. (2007)	400 mg each vitamins C and E, 18 mg β-carotene, 500 µmol zinc, 1 µmol selenium daily for 90 days	38 infertile men with at least two failed IVF or ICSI	Increased chromatin decondensation
Tremellen et al. (2007)	Menevit (lycopene, vitamins E and C, zinc, selenium, folate, garlic oil) daily for 3 months	60 infertile men	No significant difference in DNA fragmentation index (DFI)
Hawkes et al. (2009)	300 mg selenium daily for 48 weeks	Normozoospermia	No improvement in S_{mot} and S_{morph}
Tunc et al. (2009)	Menevit (lycopene, vitamins E and C, zinc, selenium, folate, garlic oil) daily for 3 months	50 infertile men with elevated OS	No change in SC, S_{mot} and S_{morph}
Gual-Frau et al. (2015)	500 mg L-carnitine, 60 mg vitamin C, 20 mg coenzyme Q10, 10 mg vitamin E, 200 µg vitamin B, 91 µg vitamin B12, 10 mg zinc, 50 µg selenium once daily for 3 months	Prospective observational study with 20 men with grade 1 varicocele and primary or secondary infertility	No change in SC, S_{mot} and S_{morph}
Stenqvist et al. (2018)	30 mg vitamin C, 5 mg vitamin E, 0.5 µg vitamin B12, 750 mg L-carnitine, 10 mg coenzyme Q10, 100 µg folic acid, 5 mg zinc, 25 µg selenium twice daily for 6 months	7 infertile men with sperm DNA fragmentation index (DFI) >25% treatment group (37 patients) placebo group (40 patients)	No change in SC, DFI
Schisterman et al. (2020)	5 mg folic acid, 30 mg zinc once daily for 6 months	1773 men planning to undergo infertility treatment with spouse	No improvement in S_{mot} and S_{morph}

SC sperm concentration, S_{mot} sperm motility, S_{morph} sperm morphology

attention to the biochemical characteristics of the oxidant vs. antioxidant equilibrium. Excessive antioxidants exposure drives the endogenous system toward RS, which is as harmful to sperm health as OS. Thus, pre-treatment redox status should be assessed and may be recommended before providing any antioxidants therapy. The exact mechanism by which RS mediate male reproductive disruptions remains largely unexplained; however, it is presumed that either the RS curb sperm functions by reducing ROS below physiological level that is essential for normal sperm functions, or it hinders normal oxidation mechanisms facilitating oxidative damage. There are great scopes for future interventions to reveal the underlying deep-rooted mechanisms of RS-mediated male infertility.

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