The Importance of Diet, Vitamins, Malnutrition, and Nutrient Deficiencies in Male Fertility

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Landon W. Trost, Ahmet Gudeloglu, Edmund Y. Ko, and Sijo J. Parekattil

Introduction

Male-factor infertility/subfertility is a relatively common condition, affecting up to 1 in 20 men and accounting for an estimated 80 million cases worldwide [1, 2]. Among couples attempting to conceive, 15 % will experience infertility, with a male factor implicated in 50 % of cases [3, 4].

Temporal changes to the prevalence of sub-/infertility remain a controversial topic, with some authors suggesting an increasing prevalence in recent decades [5]. As numerous societal changes occurred concurrent with this time period, including environmental, dietary, and lifestyle alterations, some investigators have sought to find associations among these conditions. Although the true prevalence of infertility and its change over time remain unknown, the possibility of identifying and treating modifiable risk factors for male infertility remains an important subject for ongoing research.

The underlying etiologies of male-factor infertility are numerous and include congenital, hormonal, iatrogenic, and infectious causes, among others. Despite these recognized factors, up to 20–40 % of infertile males are classified as idiopathic [6, 7]. Similarly, among males undergoing infertility evaluation, only 50 % are found to have abnormal semen analyses [8]. This suggests that in addition to known causes, several unidentified factors likely have a significant impact on overall fertility status.

Reactive Oxygen Species

One potential etiology contributing towards male-factor infertility is an elevated level of reactive oxygen species (ROS). ROS are the product of, and are required for, normal spermatogenesis, including capacitation, acrosomal reaction, and fertilization [9]. Excessive production of ROS, however, results in lipid peroxidation of the spermatozoal membrane, DNA damage, reduced sperm motility, disrupted membrane integrity, and impaired fertilization [10–15]. As abnormal sperm are associated with a higher rate of ROS production, this further contributes towards the ROS imbalance and leads to additional spermatic impairment [16, 17].

ROS are normally counterbalanced in seminal plasma and spermatozoa through the natural excretion/production of endogenous (enzymatic)

L.W. Trost, MD
Department of Urology, Mayo Clinic,
200 First Street SW, Rochester, MN 55905, USA
e-mail: trost.landon@mayo.edu

A. Gudeloglu, MD • S.J. Parekattil, MD (✉)
Department of Urology, University of Florida &
Winter Haven Hospital, 199 Avenue B North West
Suite 310, Winter Haven, FL 33881, USA
e-mail: sijo.parekattil@winterhavenhospital.org

E.Y. Ko, MD
Department of Urology, Loma Linda University,
11234 Anderson Street, A560, Loma Linda, CA
92354, USA
e-mail: edko05@gmail.com

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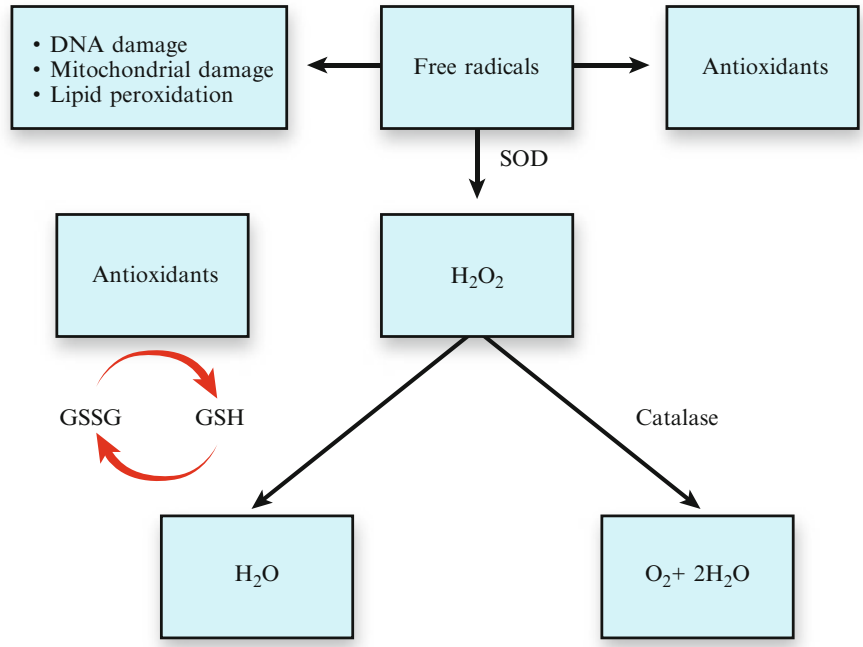

Fig. 5.1 Graphical representation of the interaction between endogenous and exogenous antioxidants in the metabolism of reactive oxygen species. GSH—reduced (active) form of glutathione peroxidase; GSSG—oxidized (inactive) form of glutathione peroxidase; SOD—superoxide dismutase

and exogenous (vitamin) antioxidants, such as Vitamins C and E, superoxide dismutase (SOD), glutathione peroxidase, catalase, and thioredoxin, among others [1, 18]. Antioxidants act as free radical scavengers to reduce oxidative damage and may be supplemented through dietary sources [17]. See Fig. 5.1 for graphical representation of antioxidant conversion of free radical compounds. Increased seminal antioxidant levels have been repeatedly linked with improved semen parameters and fertility outcomes [1, 17, 19–21].

Numerous studies have evaluated the efficacy of dietary supplementation on improving male-factor fertility. Several vitamins, minerals, and fatty acids with antioxidant properties have been studied to date and include alpha-lipoic acid (ALA), anthocyanins, L-arginine, astaxanthin, beta-carotene, biotin, L-carnitine (LC)/L-acetyl carnitine (LAC), cobalamin, co-enzyme Q10 (CoQ10), ethylcysteine, folic acid, glutathione, inositol, lycopene, magnesium, *N*-acetyl cysteine (NAC), pentoxifylline, phosphodiesterase (PDE) 5 inhibitors, polyunsaturated fatty acids (PUFAs), selenium, Vitamins A, C, D, and E, and zinc. In addition to these supplements, several authors have evaluated the impact of dietary patterns and obesity on fertility outcomes.

In reviewing the currently available literature, it is important to recognize several important limitations, which restrict potential conclusions. The majority of studies available are non-randomized by design, lack placebo groups, include small populations with short-term follow-up, lack standardization of dose or defined end points, involve varied numbers of agents studied, lack controls for other infertility-relevant male and/or female pathologies, and have varied baseline nutritional status/dietary intake. Regarding these limitations, a recent Cochrane meta-analysis of randomized, controlled trials (RCTs) concluded that findings were only able to achieve their lowest rating for overall quality of evidence [22]. As such, the astute reader should interpret any outcomes and conclusions with

significant caution and recognize the need for larger RCTs with strict methodology prior to the definitive recognition of efficacy.

The current review is structured to identify the role of diet in general on male infertility and compare outcomes among several published dietary programs. The impact of obesity and weight loss will be briefly discussed, followed by a more detailed review of available literature on the various individual and combined supplements for the treatment of male infertility. The proposed mechanisms and classifications of the supplements as well as available data reporting outcomes on male-factor infertility are also discussed. To perform the review, a PubMed search was performed of all English-language publications from 1970 to present using the search items male fertility, subfertility, infertility, supplement, diet, vitamin, nutrition, and antioxidant. Preference was given towards more recent publications, meta-analyses, and RCTs, when available.

Role of Nutrition in Male Fertility

Despite an abundance of research on the role of nutrition, exercise, and body composition with female fecundity, there is limited data available regarding male fertility [23, 24]. Although the impact of lifestyle modifications, including exercise and exogenous substance use (tobacco, alcohol, testosterone supplementation, etc.), is beyond the scope of this chapter, the impact of diet on obesity and maintaining appropriate nutritional status is relevant and will be reviewed.

Diet and Obesity

Several studies have identified associations between impaired male-factor fertility and dietary patterns. Among 701 young Danish men undergoing routine screening prior to entry into military service, those with higher intakes of saturated fat were found to have lower sperm counts, with the highest quartile experiencing a 41 % lower count than those in the lowest quartile [25]. This is supported by Gaskin and colleagues who reported on 188 men aged 18–22 from the University of Rochester [26]. In comparing semen analysis (SA) outcomes of patients with a "Western" pattern diet (high intake of red meat, refined grains, pizza, snacks, high-energy drinks, sweets) to those with a "prudent" diet (high intake of fish, chicken, fruit, vegetables, legumes, whole grains), the authors noted a positive association with progressively motile sperm among those eating a "prudent" diet. Further studies have confirmed lower risks of asthenospermia with higher intakes of fruits, vegetables, poultry, skim milk, and seafoods and increased risks among those consuming the highest levels of processed meats and sweets (OR 2.0, CI 1.7–2.4 and OR 2.1, CI 1.1–2.3, respectively) [27, 28].

Outcomes of assisted reproductive techniques (ART) have similarly demonstrated a nonsignificant trend towards higher rates of pregnancy among couples adhering to a Mediterranean diet compared to a "health conscious-low processed" diet (OR 1.4, CI 1.0–1.9 versus OR 0.8, CI 0.6–1.0, respectively) [29].

In addition to specific diets, semen quality has been associated with overall body mass index (BMI). In reviewing the outcomes of 250 couples undergoing intracytoplasmic sperm injection (ICSI), sperm motility and concentration were negatively influenced by BMI, while those undergoing a weight-loss diet experienced improved sperm counts [30]. Semen parameters were positively influenced by the higher consumption of cereals and legumes.

Animal studies have repeatedly confirmed the role of diet on maintaining optimal semen characteristics and fertility. Rato and colleagues evaluated fertility parameters in rats fed with high-energy (high fat) diets and demonstrated increases in abnormal sperm morphology and elevated markers of oxidative stress [31]. Similar reductions in sperm quality, motility, capacitation, and acrosomal reaction have been demonstrated in obese and hypercholesterolemic animal models [32–34]. Impairments in fertility may be improved through a combination of diet and/or exercise. One study of obese animals showed significant improvements in sperm motility (1.2-fold), morphology (1.1-fold), reduced sperm

DNA damage (1.5-fold), ROS (1.1-fold), and sperm binding (1.4-fold) following treatment with a standard diet [35].

Obesity may impair host defenses against toxic exposures in addition to its direct effects on fertility. Obese mice treated with acrylamide (reproductive toxin) experienced fewer successful pregnancies compared to lean mice receiving acrylamide [34]. Although this may be due, in part, to the observed higher rate of DNA damage sustained with obesity, underlying mechanisms remain unknown [36].

Fullston and colleagues recently reported perhaps the most intriguing findings on the impact of obesity on male fertility [37]. In their analysis of rats fed high-fat diets, they noted that two subsequent generations of both sexes experienced impaired fertility rates, despite being fed a standard diet. These findings suggest that obesity may impair fertility in future progeny, as well as in the obese animal itself. Potential mechanisms to account for this result include alteration of epigenetic profiles, which are influenced by environmental factors and can negatively affect implantation, placentation, and fetal growth [38]. Certainly these results have several potential significant implications regarding the importance of diet and obesity on both current and future generational fertility.

Malnutrition/Nutrient Deficiencies

Appropriate nutrition likely has a significant role in maintaining optimal fertility. Although little data is currently available, several observational studies and animal models have identified associations between sub-/infertility and reduced vitamin/mineral concentrations [39–44]. As many vitamins and minerals have potent indirect or direct antioxidant activity, reduced levels may result in an altered ROS to antioxidant ratio with subsequent reduction in total antioxidant capacity (TAC) [39, 45].

Several studies have identified an optimal range for select vitamin/mineral administration, with impairments in fertility noted with both under- and over-supplementation. Two studies evaluating the role of selenium in mice demonstrated reduced fertility among animals receiving either under- or over-supplementation, with resultant oxidative stress causing germ cell apoptosis [43, 46]. Other studies have reported similar optimal ranges for Vitamin D, with impairments in fertility at high and low serum levels [44, 47].

Although data regarding the toxicity of over-supplementation is limited, all nutrients likely have a certain threshold above which their impact is negated or detrimental. This is particularly relevant given that many studies use varying doses and/or combinations of vitamins/minerals in their patient cohorts. Similarly, as individual populations likely experience varying degrees of nutrient deficiencies, select groups may benefit more from supplementation than others. This may also account (in part) for contradictory findings of studies examining the effect of individual nutrients.

Nutrient Supplementation in Male Infertility

A comprehensive listing of every study performed on nutrients associated with infertility is beyond the scope of any one publication. As the quality of evidence for each study varies, this chapter attempts to highlight studies with the highest level of evidence available. In the absence of human RCTs, all available literature is reviewed, with an emphasis on higher quality studies. However, findings from non-RCT should not be interpreted as equivalent to the higher quality trials. The current listing also does not represent a complete listing of every nutrient or supplement, but rather those with the most abundant literature available. Each nutrient will be presented in alphabetic order, with a brief description as to its classification and mechanism if available. Data will be provided supporting or contradicting its use with male fertility as well as the authors' interpretation as to a consensus of evidence. See Table 5.1 for a brief summary of nutrient supplements with data

Table 5.1 Summary of effects of nutrient supplementation on male-factor infertility

Agent	Class/function	Proposed mechanism of action	Summary of effect on infertility
Antioxidants (in general)	Reduction of reactive oxygen species	Improved sperm function, DNA integrity, fertilization capacity	May improve live birth and pregnancy rates, DNA fragmentation, sperm motility
L-Arginine	Amino acid; precursor to NO	Precursor for putrescine, spermidine, spermine, and required for sperm capacitation	Conflicting and inadequate data
L-Carnitine, L-acetyl carnitine	Quaternary ammonium compounds; transport fatty acids to mitochondria	Antioxidant; assists with sperm maturation and motility	May result in improved pregnancy rates
Cobalamin	Form of vitamin B12	Functions in combination with folic acid in DNA synthesis	Deficiency associated with infertility; insufficient data to suggest benefit to supplementation
Coenzyme Q10	Vitamin-like substance in mitochondria; produces adenosine triphosphate through electron transport chain	Lipid-soluble antioxidant; may enhance motility through mitochondrial activity	Improves markers of oxidative stress; insufficient data to suggest benefit to supplementation
Folic acid	Vitamin B9	Functions in DNA synthesis, repair, methylation; required for normal spermatogenesis; indirectly functions as antioxidant	Deficiency associated with infertility; insufficient data to suggest benefit to supplementation
Glutathione	Endogenous antioxidant	Endogenous antioxidant; replenishes other antioxidants; detoxifies foreign compounds and carcinogens	May improve sperm motility, morphology, DNA integrity; may be used adjunctively in ART sperm media
Lycopene	Nonessential carotenoid pigment	Antioxidant; functions in genetic expression, cell regulation, immunomodulation	Conflicting results on antioxidant capacity; no RCTs available
N-Acetyl cysteine	Derivative of the amino acid cysteine	Antioxidant; helps regenerate glutathione	Conflicting and inadequate data
Pentoxifylline	Methylated xanthine derivative; nonselective PDEI	Possibly reduces inflammation through PDEI	May improve sperm motility; may be used adjunctively in ART sperm media
Polyunsaturated fatty acids	Triglyceride compounds (omega-3, -6, -9)	Modulates inflammation; required for sperm capacitation and spermatogenesis	Conflicting and inadequate data
Selenium	Chemical element required for cellular function	Essential for normal function of endogenous antioxidants	Deficiency associated with infertility; combination therapy with vitamin E may improve motility
Vitamin C	Vitamin cofactor for enzymatic reactions (e.g., collagen synthesis)	Indirect and direct antioxidant	Deficiency associated with infertility; may improve DNA fragmentation; conflicting data on impact on sperm
Vitamin E	Fat-soluble vitamin, which encompasses tocopherols and tocotrienols	Antioxidant; restores other antioxidants to reduced state	May improve pregnancy and live birth rates
Zinc	Metallic element; cofactor in enzymatic processes	Required for reproductive gland growth and spermatic function	Deficiency associated with infertility; may benefit semen parameters, pregnancy, and birth rates

ART assisted reproductive techniques, *NO* nitric oxide, *PDEI* phosphodiesterase inhibitor, *RCT* randomized, controlled trial

available, including class of agent, proposed mechanisms of action, and efficacy with improving infertility.

L-Arginine

Class and Mechanism

L-Arginine is an amino acid, which serves as a precursor of nitric oxide (NO) via nitric oxide synthase. NO subsequently functions as an endogenous ROS and is required for routine signal transduction during sperm capacitation [48]. L-Arginine is also utilized in the synthesis of putrescine, spermidine, and spermine, which regulate various cellular processes and are thought to function in sperm motility [49].

Data Supporting Use

Very limited data is available on the use of L-arginine for male fertility. Initial human studies of supplemental L-arginine administered up to 4 g/day reported improved sperm concentration and motility [50–52]. A placebo-controlled, crossover, RCT comparing the supplement Prelox (combination of L-arginine and the antioxidant pycnogenol) in 50 men similarly reported improved sperm concentration, volume, and motility in the treatment group [53, 54]. However, as the study used combination therapy, it is unclear which agent accounted for the improvements noted.

Data Contradicting Use

Several human and animal studies have reported no improvements or impaired fertility with L-arginine directly, or through its downstream product, NO. Two early human studies failed to identify any improvements in SA parameters or pregnancy rates, with more recent studies demonstrating impaired fertility, decreased sperm motility, spermatic toxicity, and reduced sperm-zona binding following L-arginine administration [55–59].

Consensus of Opinion

Inadequate and contradictory data exist regarding the effect of L-arginine on SA parameters and pregnancy rates.

L-Carnitine and L-Acetyl Carnitine

Class and Mechanism

L-Carnitine (LC) and its acetylated form (LAC) are quaternary ammonium compounds, which serve to transport fatty acids to the mitochondria. From a fertility standpoint, carnitine assists with sperm maturation and motility and functions as an antioxidant [60].

Data Supporting Use

LC and LAC are among the most studied nutrients in male fertility, with deficiencies in seminal carnitine previously associated with reduced sperm concentration, motility, and DNA integrity among infertile males [61]. Similarly, compared to normal controls, infertile males have been shown to have lower levels of seminal free LC [62]. Multiple RCTs are available comparing its efficacy to placebo and other agents, with a recent Cochrane meta-analysis performed to summarize results [22, 63–71]. Combined outcomes of the RCTs demonstrated significantly improved pregnancy outcomes with LC or combination of LC + LAC versus placebo (OR 4.5 and 5.1; CI 1.5–17.1 and 1.8–11.4, respectively) [63–66, 68]. Comparing LC + LAC to Vitamin E + C supplementation, the carnitine group demonstrated improved motility and concentration at 3 months compared to vitamins (OR 23.1; CI 20.2–25.9 and OR 15.5; CI 12.5–18.5, respectively) [67].

A less-stringent meta-analysis performed in 2007 of clinical and RCTs comparing LC and/or LAC to placebo, reported improved pregnancy rates (OR 4.1; CI 2.1–8.1), motility (weighted mean difference [WMD] 7.43; CI 1.7–13.1), and morphology (WMD 5.7; CI 3.6–7.9) [72]. No significant differences were noted on sperm concentration or semen volume.

Individual studies demonstrated varied improvements in semen characteristics. Among men with no, small, or moderate-sized varicoceles, Cavallini and colleagues noted that supplementation with carnitine and cinnoxicam (nonsteroidal anti-inflammatory) resulted in improved sperm concentration, motility, and morphology, although

similar improvements were not found among patients with higher-grade varicoceles [64]. A similar combination study of carnitine and cinnoxicam in men with prostato-vesiculo-epididymitis demonstrated selective improvements in motility in men with <1 million/mL seminal WBCs [69]. Among patients with oligo-asthenoteratospermia (OAT) undergoing ICSI, combination of LC + LAC and cinnoxicam resulted in improved sperm morphology and reduced aneuploidy with resultant higher rates of pregnancies and live births [73].

One study by de Rosa and colleagues supplemented oligospermic infertile males with motilities <50 % and demonstrated improvements in motility, live sperm count, and cervical penetration capacity compared to baseline [61]. In comparing LC + Vitamin E versus Vitamin E alone, patients receiving combination therapy demonstrated improved motility (45 % versus 28 % pretreatment, $p < 0.01$) and higher pregnancy rates (31.1 % [combination] versus 3.8 % [Vitamin E alone], $p < 0.01$), with otherwise unchanged sperm concentrations and morphology [71].

Data Contradicting Use

The effect of LC and/or LAC on sperm motility is inconclusive, with 3- and 6-month data from a Cochrane meta-analysis demonstrating conflicting findings [22]. Data ≥9 months on LC versus placebo demonstrated significant improvements with a wide confidence interval for motility (OR 11.5; CI 1.7–21.4) and no significant improvements with LC or combination of LC + LAC [63]. The Cochrane meta-analysis similarly demonstrated no significant differences in sperm concentration among two included studies at 6- and ≥9-month time points with LC, LAC, or combination therapy [63, 66]. When comparing LC + LAC to Vitamin E + C supplementation, no significant differences were noted with pregnancies achieved (OR 2.9; CI 0.9–9.5) [67]. A separate RCT not included in the Cochrane review similarly demonstrated a lack of improvement with LC on seminal volume, sperm concentration, motility, or morphology [70].

Consensus of Opinion

Although the data remain inconclusive as to the benefits of LC ± LAC on individual semen characteristics, significant improvements in pregnancy rates have been reported. However, as the confidence intervals remain very wide, this finding requires larger, well-controlled trials to validate.

Cobalamin (Vitamin B12)

Class and Mechanism

Cobalamin represents one of several forms of B12 and may be considered equivalent physiologically to B12. The underlying mechanism for the role of B12 in fertility is unclear, although it may relate to its role in DNA synthesis, activation of antioxidant enzymes, or methyl group donation.

Data Supporting Use

Cobalamin deficiency has long been recognized to be associated with sub-/infertility, with observational studies confirming reduced levels in cases of non-obstructive azoospermia [40, 74, 75]. Among men undergoing in vitro fertilization (IVF)/ICSI, sperm concentrations have been shown to positively correlate with cobalamin levels [76].

Multiple early Japanese studies were performed to evaluate the efficacy of methylcobalamin supplementation on fertility and SA parameters [77–80]. The majority of trials reported significant improvements in sperm concentration and motility; however, the criteria for success were loosely defined. Since these early reports, no additional human trials have been reported.

Data Contradicting Use

Although the majority of studies demonstrate an association between cobalamin deficiency and sub-/infertility, cobalamin concentrations beyond a minimal threshold may not result in improvement in semen parameters [41]. One multi-institution, double-blinded, placebo-controlled, RCT performed on cobalamin supplementation demonstrated no overall impact of therapy on sperm motility or concentration [77].

Consensus of Opinion

Cobalamin deficiency (resulting in clinical pernicious anemia) is linked with infertility. The impact of cobalamin supplementation beyond minimum requirements lacks sufficient data to suggest a proven benefit on male fertility.

Co-enzyme Q10

Class and Mechanism

CoQ10 is a vitamin-like substance present in the mitochondria, which functions to produce adenosine triphosphate through the electron transport chain. From a fertility standpoint, CoQ10 may provide benefit through lipid-soluble antioxidant properties and/or through enhancing motility via mitochondrial activity [81].

Data Supporting Use

CoQ10 concentrations in seminal plasma have been correlated with total sperm counts and motility, while ratios of its reduced (ubiquinol) to oxidized (ubiquinone) state are associated with alterations in the percentage of abnormal sperm morphology [82].

A Cochrane meta-analysis and review of RCTs summarized outcomes from two RCTs utilizing CoQ10 [22, 81, 83]. It is noteworthy that one of the trials reported results of questionable validity and reliability, suggesting that conclusions must be interpreted with caution [83]. Combined results demonstrated significant improvements in motility at 6 months (OR 4.5; CI 3.9–5.1), which was not sustained at ≥9 months (OR −0.0; CI −1.1 to 6.2). Two additional RCTs, which were not included in the Cochrane review, demonstrate significant improvements in markers of oxidative stress with CoQ10 supplementation among patients with OAT, including elevated catalase, SOD, and TAC [84, 85]. Additionally, one trial identified a positive correlation between CoQ10 concentrations and normal sperm morphology [85].

Data Contradicting Use

The previously cited Cochrane review demonstrated no significant improvements with CoQ10 supplementation on the rate of pregnancies (OR 2.2; CI 0.5–8.8) or sperm concentration at 6 or ≥9 months (OR 3.9; CI −2.1 to 9.8 and OR 1.5; CI 0.5–2.6, respectively). Similarly, two RCTs performed on CoQ10 administration among OAT patients failed to demonstrate improvements in sperm concentration or motility despite demonstrably higher levels of TAC [84, 85].

Consensus of Opinion

CoQ10 supplementation appears to improve markers of oxidative stress and improve TAC. However, its impact on semen parameters and pregnancy rates remains unproven.

Folic Acid (Vitamin B9)

Class and Mechanism

Folic acid is a water-soluble B Vitamin, which has roles in DNA synthesis, repair, and methylation and is an essential cofactor. Folic acid is required for normal spermatogenesis and may function as an indirect antioxidant through creation of methyl donor compounds [86, 87].

Data Supporting Use

Folic acid levels have been associated with sperm characteristics, including chromosomal aneuploidy and total sperm concentration, with low levels correlating with infertility [20, 41, 88]. Several RCTs have demonstrated beneficial effects of folic acid on semen parameters and pregnancy rates. The earliest RCT was performed by Wong and colleagues, who compared folic acid, zinc, folic acid + zinc, or placebo among 211 men of mixed fertility [89]. Results demonstrated a 74 % increase in total sperm count and improved morphology in the combination group. Subsequent RCTs by Ebisch and colleagues confirmed improved sperm concentrations among patients (regardless of fertility status) supplemented with folic acid and zinc compared to controls [90, 91].

Data Contradicting Use

With limited data available, the previously cited RCT by Wong and colleagues failed to demonstrate significant improvements in concentration,

motility, or morphology with folic acid or zinc supplementation alone, despite significant improvements noted in the combination group [89]. This may be a reflection of limited statistical power or indicate a need for combined mechanisms of action to achieve improved semen characteristics. A prior study similarly demonstrated no significant improvements on sperm counts, motility, or DNA content among normo- and oligospermic men treated with folic acid [92].

Consensus of Opinion

Currently available data is insufficient to demonstrate improved outcomes with folic acid supplementation in regard to SA characteristics or pregnancy rates.

Glutathione

Class and Mechanism

Glutathione is an endogenous antioxidant and is one of the most abundant found in the body. It has important roles in maintaining supplementary antioxidants (i.e., Vitamins C and E) in their reduced (active) states and in detoxifying carcinogens and foreign compounds [93, 94].

Data Supporting Use

Multiple studies have associated reduced glutathione levels and infertility, including decreased sperm motility and morphology [95]. However, as glutathione is an endogenous antioxidant, lower levels signify a higher rate of oxidative stress and may not relate to inadequate production. When provided as an adjunctive compound in ART sperm media, sperm motility, plasma membrane integrity, overall viability, fertility success, and DNA integrity are all improved [96, 97].

An initial pilot study examining intramuscular supplementation with glutathione in 11 men resulted in improved sperm motility [98]. A subsequent placebo-controlled, blinded, crossover study of 20 infertile patients with varicoceles ($n=10$) or non-bacterial genitourinary inflammatory conditions ($n=10$) confirmed improved sperm motility, progression, and morphology [99]. An additional pilot study of infertile males identified a higher rate of sperm DNA damage, which was significantly improved with intramuscular glutathione administration [100]. No studies have evaluated the impact of glutathione supplementation on pregnancy outcomes in non-ART settings.

Data Contradicting Use

None.

Consensus of Opinion

Limited data suggests a potential benefit of glutathione supplementation on improving sperm motility, morphology, and DNA integrity as well as its adjunctive use in sperm media with ART. Due, in part, to the need for intramuscular administration, the widespread adoption of glutathione has been limited [101].

Lycopene

Class and Mechanism

Lycopene is a nonessential, carotenoid pigment with no Vitamin A activity. It has received increasing attention as a potential anticarcinogenic agent due to its antioxidant properties and role in genetic expression, cell regulation, and immunomodulation [102].

Data Supporting Use

Lycopene is highly concentrated in reproductive organs, including the testes and seminal fluid, with decreased levels associated with male sub-/infertility [103]. In a placebo-controlled, crossover trial evaluating the effect of lycopene on advanced glycation end products in seminal fluid (marker of oxidative stress), Oborna and colleagues noted significant improvements in lycopene-supplemented men [104].

Currently, no human placebo-controlled RCTs have evaluated the efficacy of lycopene supplementation on fertility and SA parameters. A pilot study of infertile males with OAT undergoing lycopene supplementation demonstrated significant improvements in sperm count (66 % with median 22 million increase) and motility (53 % with median 25 % improvement) [103].

Minimal changes were noted in men with severe oligospermia (<5 million/mL). Among men presenting for IVF, higher arachidonic acid (AA) to docosahexaenoic acid (DHA) ratios (indicating oxidative stress) have been reported when compared to control subjects [105]. When patients were treated with lycopene, these levels returned to baseline in patients without SA abnormalities, while nonsignificant improvements were noted among those with SA abnormalities. Results further demonstrated an observed increased rate of spontaneous pregnancies (16 %) and successful IVF outcomes (42 %) in treatment patients (control results not reported).

Data Contradicting Use

One trial found no increase in TAC among men undergoing supplementation, despite elevated blood and semen levels of lycopene [45].

Consensus of Opinion

Currently available data are insufficient to suggest any potential benefits with lycopene supplementation on SA parameters or pregnancy rates.

N-Acetyl Cysteine

Class and Mechanism

NAC is a derivative of cysteine and is commonly utilized as a mucolytic agent and in the management of acetaminophen overdose. Its role in infertility is likely due to antioxidative properties through regeneration of endogenous glutathione levels [106].

Data Supporting Use

A Cochrane review of two RCTs performed demonstrated significant improvements with NAC administration on sperm motility at 3- and 6-month time points (OR 11.0; CI 7.9–14.0 and OR 1.9; CI 1.0–2.8, respectively) [22, 107, 108]. Sperm concentration was unchanged at 3 months (OR −0.5; CI −6.7 to 5.8) and increased at 6 months (OR 3.3; CI 1.2–5.4); however, as previously noted, the reliability of results from the study author involved at the 6-month time period has previously been called into question [22, 107–110].

One non-RCT performed by Comhaire and colleagues supplemented 27 men with AA/DHA and either NAC or Vitamins E+C [111]. Following treatment, men with oligospermia were found to have increased sperm counts from 7.4 to 12.5 million, with additional improvements in ROS noted. The overall pregnancy rate was 4.5 % at 134 months follow-up. However, these findings are of limited benefit due to the lack of a control group and an undefined number of patients receiving NAC compared to Vitamins E+C.

One animal study of diabetic rats demonstrated an upregulation of endogenous antioxidants and attenuation of diabetes-induced testicular cell death among NAC-treated animals [112]. Similarly, an in vitro study of semen samples incubated with or without supplementary NAC demonstrated a dose-dependent decrease in ROS [113]. Sperm additionally had improved motility, without changes in acrosome reaction.

Data Contradicting Use

In the RCT previously described by Ciftci and colleagues, among the 120 patients randomly divided to receive NAC or placebo, no changes were noted in total sperm counts or morphology [107]. Similarly, in their prospective trial of 27 men treated with NAC or Vitamins E+C and AA/DHA, Comhaire and colleagues demonstrated no effect on sperm motility, morphology, WBC, or round cells in semen among all patients, and no improvement in sperm counts in non-oligospermic men [111].

Consensus of Opinion

Limited data suggests a possible benefit of NAC supplementation on sperm motility, without improvements in other SA parameters. No information is available regarding its impact on pregnancy rates or live deliveries.

Pentoxifylline

Class and Mechanism

Pentoxifylline is a methylated xanthine derivative and is a nonselective PDE inhibitor. Its mechanism

for improving fertility has not been defined, although it may be secondary to downstream effects of PDE inhibition, including reduced inflammation [114].

Data Supporting Use

Two placebo-controlled RCTs have evaluated the efficacy of pentoxifylline on improving semen parameters [22, 115, 116]. Compared to no treatment, pentoxifylline resulted in significant improvements in sperm motility (OR 12.8; CI 9.2–16.3) and morphology at 3 months [22, 115].

One in vitro study comparing pentoxifylline to the hypoosmotic swelling test for selection of appropriate sperm for ART demonstrated improved fertilization (62.1 % versus 41.1 %) and pregnancy rates (32 % versus 16 %) with pentoxifylline [117]. These data are consistent with other studies, which suggest a potential role for pentoxifylline as an adjunctive therapy with ART [118–120].

Data Contradicting Use

In the previously described RCT by Wang and colleagues, the authors found no significant improvements in sperm concentration at the 3- and 6-month time points (OR 4.3; CI −0.7 to 9.3 and OR 2.8; CI −2.6 to 8.2) [116].

Consensus of Opinion

With limited data available, pentoxifylline supplementation may result in improved sperm motility and may have benefits as an in vitro adjunct in couples undergoing ART.

Polyunsaturated Fatty Acids

Class and Mechanism

PUFAs (alternatively named highly unsaturated fatty acids) represent a class of triglyceride compounds, which include the omega-3, -6, and -9 fatty acids. Two commonly reported PUFAs are AA (omega-6) and DHA (omega-3), which have been shown to have pro- and anti-inflammatory effects, respectively [121]. AA is required for normal sperm capacitation, and the ratio of AA:DHA is hypothesized to affect the functional capacity of spermatozoa [122–124]. DHA additionally functions as an indirect antioxidant through regeneration of glutathione levels [125].

Data Supporting Use

The proposed benefit of PUFA supplementation is based on the association between reduced omega-3 levels and an altered omega-6:omega-3 ratio and impaired fertility [105, 124]. One double-blinded, placebo-controlled, RCT performed by Safarinejad and colleagues reported significant improvements in sperm count (38.7–61.7 million, $p=0.001$), with positive associations noted between DHA concentrations and seminal SOD and catalase activities (markers of oxidative stress) [126]. However, as previously mentioned, the validity and reliability of results from this author are suspect, with a prior retracted article and several authors noting discrepancies in reported findings [22, 109, 110].

Data Contradicting Use

One double-blind, placebo-controlled, RCT of 28 men with asthenospermia evaluated the efficacy of varying dosages of DHA on semen characteristics [127]. Results demonstrated elevated levels of DHA and DHA:AA ratio, without evidence for DHA incorporation into the spermatic membrane. Additionally, no significant differences were noted on sperm motility (OR −15.2; CI −34.3 to 3.9) or concentration (OR 1.5; CI −35.2 to 38.2) at 3 months (higher dosage ORs listed).

The impact of DHA supplementation on pregnancy outcomes is unknown. Among infertile men undergoing IVF therapy, altered DHA:AA ratios were identified. Following supplementation with lycopene, this ratio returned to control levels among normospermic infertile males; however, when comparing successful versus unsuccessful pregnancies achieved, no differences were noted with DHA:AA ratios between groups [105].

Consensus of Opinion

Available data on the efficacy of DHA supplementation on sperm characteristics is contradictory and inconclusive.

Selenium

Class and Mechanism

Selenium is a chemical element required for normal cellular function. It is an essential component of the endogenous antioxidants glutathione peroxidase and thioredoxin reductase and thus functions indirectly to enhance intrinsic antioxidant capacity [128].

Data Supporting Use

Selenium deficiency is associated with decreased sperm motility, altered midpiece stability, and abnormal sperm morphology [129, 130]. Two placebo-controlled RCTs have evaluated the efficacy of selenium supplementation on improving sperm characteristics. Scott and colleagues reported on 69 patients randomized to placebo, selenium, or combination of selenium with Vitamins A, C, and E [131]. Although individual groupings failed to achieve significant results, when both treatment groups were combined, significantly improved motility was noted without any benefits on sperm concentration. An 11 % rate of paternity was observed in the treatment group versus 0 % in the placebo arm. Safarinejad and colleagues reported significant improvements in sperm motility (OR 3.2; CI 2.3–4.1) and concentration (OR 4.1; CI 1.9–6.3) at 6 months. However, as previously indicated, these results are suspect (given the uniquely narrow confidence intervals when compared to all other available antioxidant RCTs, the author's prior inconsistencies, and redacted manuscript) and are therefore of questionable validity and reliability [22, 109, 110].

A head-to-head randomized comparison of 20 patients receiving Vitamin E and selenium versus Vitamin B demonstrated improved sperm motility and oxidative stress markers among those receiving selenium and Vitamin E [132]. Similarly, in comparing selenium to selenium + Vitamins A, C, and E, no difference in sperm motility was noted [131].

Two studies evaluated the efficacy of combination of selenium and Vitamin E compared to baseline SA levels [133, 134]. Moslemi and colleagues reported on a large series of 690 infertile males with asthenoteratospermia [134]. Following 100 days of supplementation, 43 % experienced improved motility, 9 % improved morphology, and 10.8 % achieved spontaneous pregnancies. A second, smaller study evaluated nine men with OAT treated with combination of selenium and Vitamin E [133]. Compared to baseline values, results demonstrated significant improvements in motility (19 %), morphology (28.6 %), and sperm viability (27.9 %), which returned to baseline levels following therapy discontinuation.

Data Contradicting Use

One trial of 33 subfertile men treated patients with selenium alone over a period of 3 months [135]. Results demonstrated no significant improvements in sperm count, motility, or morphology, with weak correlations between selenium seminal levels and glutathione peroxidase activity noted.

Of interest, two animal studies evaluating variable dosages of selenium noted impaired fertility, increased ROS, and germ cell apoptosis, among animals receiving either too high or too low levels of selenium [43, 46]. These findings suggest a specific range of selenium required for optimal function.

Consensus of Opinion

Data is lacking on solitary administration of selenium; however, combination therapy of selenium with other vitamins (particularly Vitamin E) may result in improved motility. The effect of combined selenium and vitamins on pregnancy remains unclear.

Vitamin C

Class and Mechanism

Vitamin C (L-ascorbic acid) is a nutrient cofactor in several enzymatic reactions, including those involved with collagen synthesis. In the reproductive tract, Vitamin C is highly concentrated in seminal fluid and is required for normal reproductive function [136]. It exerts antioxidant effects both directly and indirectly through reduction of oxidized Vitamin E [137, 138].

Data Supporting Use

Several placebo-controlled RCTs have evaluated the efficacy of Vitamin C alone or in combination with other antioxidants on male fertility. Dawson and colleagues administered Vitamin C at 200 mg/day versus 1,000 mg/day and demonstrated significant improvements in sperm motility only at the higher dosage (OR 45.0; CI 15.3–74.8) [139]. In comparing Vitamin C (1,000 mg) + Vitamin E to placebo, Greco and colleagues noted significant decreases in DNA fragmentation (22.1 % versus 9.1 %; OR −13.8; CI −17.5 to −10.1) in the treatment group [140]. One trial comparing zinc, zinc + Vitamin E, zinc + Vitamins C and E, or placebo in 45 men with asthenospermia demonstrated no significant differences among treatment groups and improved motility in the combined group (OR 26.0; CI 8.9–43.2) [18]. As with any combination therapy trial, it is difficult to elucidate if findings are due to any single agent or the synergistic effect of multiple therapies.

Vitamin C supplementation may reduce the extent of damage sustained from environmental gonadotoxins. An observational study of 120 men exposed to lead from a battery-manufacturing industry experienced improvements in sperm concentration, motility, and morphology following prophylactic Vitamin C administration [141]. A similar animal study evaluating the effect of electromagnetic radiation on rat testes showed a protective effect and reduced oxidative stress in animals treated with combination of Vitamins C + E [142].

Data Contradicting Use

Two placebo-controlled RCTs of combined Vitamin C (1,000 mg) + Vitamin E have demonstrated no improvements in sperm motility, morphology, or concentration [140, 143]. A meta-analysis of the two studies resulted in no significant difference in sperm concentration in the treatment group compared to placebo (OR 1.4; CI −10.0 to 12.7) [22, 140, 143].

Consensus of Opinion

Available RCTs demonstrate conflicting results on sperm motility and no benefits on concentration or morphology with Vitamin C alone, or in combination with Vitamin E. Vitamin C 1,000 mg daily may improve sperm DNA fragmentation.

Vitamin E

Class and Mechanism

Vitamin E encompasses several fat-soluble compounds, including tocopherols and tocotrienols. As a potent antioxidant, it functions to reduce seminal ROS and restore other antioxidants to their functional (reduced) state [144, 145].

Data Supporting Use

Several placebo-controlled RCTs have been performed evaluating the efficacy of Vitamin E alone or in combination with other antioxidants on improving male-factor infertility. A meta-analysis of two RCTs comparing Vitamin E alone to placebo demonstrate significant improvements with live birth rates (OR 6.4; CI 1.7–24.0), pregnancy rate (OR 6.6; CI 1.8–23.9), and sperm motility (OR 13.0; CI 7.0–19.0), without significant differences noted in the rate of miscarriage (OR 5.4; CI 0.3–93.3) [22, 145, 146].

Similar to other vitamins, several studies evaluate Vitamin E in combination with other antioxidants. Greco and colleagues demonstrated significant improvements in DNA fragmentation indices (OR −13.8; CI −17.5 to −10.1) following supplementation with Vitamins C + E [140]. One study comparing Vitamin E + zinc (OR 26.0; CI 9.0–43.0) or Vitamins C + E + zinc (OR 26.0; CI 8.9–43.2) showed improvements in sperm motility at 3 months compared to no treatment [18].

Two uncontrolled studies of selenium and Vitamin E demonstrated improvements in motility, morphology, sperm viability, and pregnancy rate, when compared to baseline SA levels [133, 134].

As with other antioxidants, low Vitamin E levels have been associated with sub-/infertility [39, 147]. Similarly, animal studies of Vitamin E suggest a possible role for prevention of damage in conditions of high oxidative stress (e.g., radiation, cryptorchidism) [142, 148].

Data Contradicting Use

The previously cited study by Greco and colleagues demonstrated no significant change in sperm motility (OR 2.9; CI −7.8 to 13.6) with Vitamins C + E, despite the observed improvement in DNA fragmentation indices [140]. Similarly, in combining the two available RCTs of Vitamins C + E, no significant improvements on sperm concentration were observed (OR 1.4; CI −10.0 to 12.7) [22, 140, 143].

Several RCTs have also compared Vitamin E alone or in combination with other antioxidants to other agents. Akiyama and colleagues reported no differences in sperm motility (OR −1.9; CI −42.0 to 38.2) or concentration (OR 2.2; CI −16.7 to 21.1) between patients supplemented with Vitamin E or ethylcysteine [149]. In comparing Vitamins C + E to LC and LC + LAC, Li and colleagues noted superiority of LC with or without LAC in regard to motility (OR 23.1; CI 20.2–25.9) and concentration (OR 15.5; CI 12.5–18.5) at 3 months [67]. No differences were noted on sperm motility with Vitamin E + selenium versus Vitamin B (OR 0.0; CI −10.7 to 10.7), Vitamin E + zinc versus zinc (OR 1.0; CI −13.0 to 15.0), or Vitamins E + C + zinc versus zinc (OR 1.0; CI −17.7 to 19.7) [18, 22, 132].

Consensus of Opinion

Although Vitamin E has not been shown to consistently improve semen parameters, limited results suggest a potential benefit on improving overall pregnancy and live birth rates.

Zinc

Class and Mechanism

Zinc is a metallic element and essential cofactor in multiple enzymatic processes. Zinc is highly concentrated in the semen and is essential for normal reproductive gland growth and spermatic function [87].

Data Supporting Use

One placebo-controlled RCT evaluating the efficacy of zinc on markers of oxidative stress and fertility has been reported [18]. A combined 45 men with asthenospermia (defined as ≥40 % immotile sperm) were randomized to zinc, zinc + Vitamin E, zinc + Vitamins C + E, or placebo over a treatment period of 3 months. Results demonstrated significant improvements in live birth (OR 3.7; CI 1.0–13.5, $p = 0.05$) and pregnancy rates (OR 4.8; CI 1.5–15.2), with no change in miscarriage rates (OR 7.2; CI 0.1–364.9). Zinc administered alone or in combination with Vitamins E ± C resulted in improved motility at 3 months and further reduced the DNA fragmentation index and markers of oxidative stress. No difference in sperm parameters was noted among the three zinc treatment groups.

Animal models of zinc deficiency have demonstrated impaired spermatogenesis, semen parameters, and a higher sensitivity towards oxidative damage and testicular cell death [112, 150]. Zinc is commonly included in multi-supplement trials and will be discussed in this context later in the chapter [151–153].

Data Contradicting Use

An observational study by Young and colleagues evaluated sperm samples from 89 healthy men who completed questionnaires on food intake [20]. Data was extrapolated from questionnaires to estimate levels of zinc, folate, Vitamins C and E, and beta-carotene intake. In comparing estimated nutrient levels against sperm aneuploidy testing, no association was noted among high or low levels of zinc and overall sperm aneuploidy. Given the nature of the study design and methodology, limited conclusions may be drawn from the results presented.

Consensus of Opinion

Limited data suggests a potential benefit for zinc supplementation on semen parameters, pregnancy, and birth rates.

Other Supplements

Several additional nutritional supplements have been evaluated for their potential beneficial effects on male fertility, including but not limited to ALA, anthocyanins, astaxanthin, beta-carotene,

biotin, ethylcysteine, inositol, magnesium, PDE5 inhibitors, and Vitamins A and D, among others. Given the relatively limited amount of data currently available on the efficacy of these compounds in male fertility, only brief mention will be made of selected studies for each nutrient. The proposed mechanisms for the compounds vary, with several purported to function via antioxidant pathways either directly or indirectly through reduced inflammation or restoration of endogenous antioxidant levels.

Individual RCTs are available on five of the above listed compounds [70, 149, 154–156]. Pawlowicz and colleagues performed a placebo-controlled RCT of anthocyanins and demonstrated significant improvements in seminal fructose levels and markers of oxidative stress [154]. Similarly, a double-blinded, placebo-controlled, RCT of the carotenoid astaxanthin noted improvements in ROS, inhibin B levels, sperm linear velocity, and pregnancy rates (54.5 % versus 10.5 %, $p=0.03$) compared to placebo [155]. In comparing the efficacy of Vitamin E to ethylcysteine, Akiyama and colleagues found no difference in regard to sperm density and motility, although ROS were significantly lower among ethylcysteine-treated patients [149]. In a placebo-controlled RCT of two PDE5 inhibitors (vardenafil, sildenafil), Dimitriadis and colleagues reported improved sperm concentration, motility, and morphology following treatment with either agent compared to pretreatment levels [70]. An additional placebo-controlled RCT evaluating magnesium orotate demonstrated no significant improvements in sperm concentration, motility, or pregnancy rates compared to placebo [156].

Vitamin A is commonly utilized in combination supplement trials due to its antioxidant activity [131, 151]. Given the combined use with other agents, individual efficacy on semen parameters and fertility cannot be determined. Vitamin D has also been associated with infertility; however, similar to selenium, both high and low levels have been associated with decreased SA parameters, with one study suggesting an optimal level of 20–50 ng/mL [44, 47, 157, 158]. ALA has been shown in animal models to both attenuate oxidative stress and improve sperm concentration, motility, and testicular histologic features [159, 160]. Low levels of beta-carotene, as an inactive precursor to Vitamin A, have also been associated with infertility [161]. Biotin (Vitamin B7), inositol, and ALA have shown efficacy in improving semen parameters when used as adjunctive agents to sperm media [120, 162, 163].

Each of the above agents has demonstrated some potential for improving SA parameters and overall male-factor fertility. However, due to the limited data available, further studies are required to assess their individual efficacy.

Combined Supplementation and Overall Antioxidant Efficacy

Several studies have evaluated the efficacy of combination supplements on male-factor fertility. Two RCTs compared multiple supplements to placebo: Galatioto and colleagues (NAC, Vitamins C, E, and A, thiamine, riboflavin, pyridoxine, nicotinamide, pantothenate, biotin, cyanocobalamin, ergocalciferol, calcium, magnesium, phosphate, iron, manganese, copper, and zinc) and Tremellen and colleagues (Vitamins C and E, zinc, folic acid, lycopene, garlic oil, selenium) [151, 152]. Combined results from these studies demonstrated a significant improvement in pregnancy rate (OR 4.0; CI 1.4–11.3) and unchanged risk of miscarriage (OR 0.48; CI 0.1–4.0). Numerous additional studies, which evaluate the efficacy of combined nutrients, are reviewed in the individual nutrient sections previously listed.

To evaluate the overall effect of nutrient supplementation on male-factor fertility, a Cochrane meta-analysis was performed of all RCTs meeting strict inclusion criteria [22]. Combined results demonstrated a significantly increased rate of live births (OR 4.9; CI 1.9–12.2) and pregnancy rate (OR 4.2; CI 2.7–6.6), with no impact on miscarriage rates (OR 1.5; CI 0.3–7.3). Antioxidants further improved DNA fragmentation rates (OR −13.8; CI −17.5 to −10.1) and sperm motility (6 months—OR 5.5; CI 3.8–7.2; ≥9-month time point not significant). No significant difference was noted among combined trials on sperm

concentration (3 months—OR 6.0; CI −5.4 to 17.5). In reviewing results, the authors concluded that antioxidants might be recommended for subfertile men whose partners are undergoing ART. They additionally note that the current data is inconclusive and assigned a very low grade to the quality of evidence included.

Summary

Male-factor infertility has long been recognized to be associated with markers of oxidative stress and elevated ROS. Numerous studies have demonstrated reduced levels of both exogenous and endogenous antioxidants among infertile patients. Given these associations, several investigators have sought to evaluate the efficacy of antioxidant and nutrient supplementation on improving direct (pregnancy, live births) and indirect (SA parameters, DNA damage) measures of male fertility. Nutrients demonstrating some beneficial effects on male fertility parameters include ALA, anthocyanins, L-arginine, astaxanthin, beta-carotene, biotin, LC/LAC, cobalamin, CoQ10, ethylcysteine, folic acid, glutathione, inositol, lycopene, magnesium, NAC, pentoxifylline, PDE5 inhibitors, PUFAs, selenium, Vitamins A, C, D, and E, and zinc.

Numerous studies, including RCTs, have been performed evaluating the efficacy of nutrients alone or in combination on improving male sub-/infertility. Although individual studies report varying efficacies, antioxidant supplementation as a whole likely results in improvements in pregnancy rate, live births, DNA fragmentation indices, and sperm motility. Antioxidant supplementation does not likely impact sperm concentration, and the optimal combination of supplements is unknown, with insufficient data available to suggest superiority of any single nutrient. Despite the lack of definitive data, given the relative minimal risks of nutrient supplementation and potential benefits herein discussed, routine use of supplementation in infertile males is reasonable. Further well-designed, placebo-controlled trials reporting main outcome measures of pregnancy and live birth are mandated.

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