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REVIEW

A systematic review of the effect of oral antioxidants on male infertility

C Ross [a], A Morriss [a], M Khairy [a], Y Khalaf [a], P Braude [a,b], A Coomarasamy [c], T El-Toukhy [a,*]

[a] *Assisted Conception Unit, Guy's and St. Thomas' Hospital NHS Foundation Trust, 11th Floor Tower Wing, Guy's Hospital, St. Thomas Street, London SE1 9RT, UK;* [b] *Division of Reproduction and Endocrinology, King's College London, London, UK;* [c] *School of Clinical and Experimental Medicine, University of Birmingham, Birmingham, UK*
[*] Corresponding author. *E-mail address:* tarek.el-toukhy@gstt.nhs.uk (T El-Toukhy).

Tarek El-Toukhy is a Consultant in reproductive medicine and surgery and pre-implantation genetic diagnosis (PGD) at the Assisted Conception Unit at Guy's and St Thomas' Hospital in London, UK. His clinical and research interests are in the fields of recurrent IVF implantation failure, prevention of ovarian hyperstimulation syndrome, PGD and minimal access surgery. He has published over 80 original articles, reviews and opinion papers.

Abstract The use of antioxidants in treatment of infertile men has been suggested, although the evidence base for this practice is unclear. A systematic review of randomized studies was conducted to evaluate the effects of oral antioxidants (vitamins C and E, zinc, selenium, folate, carnitine and carotenoids) on sperm quality and pregnancy rate in infertile men. MEDLINE, EMBASE, Cochrane Library and CINAHL were searched for relevant trials published from respective database inception dates to May 2009. Study selection, quality appraisal and data extraction were performed independently and in duplicate. Seventeen randomized trials, including a total of 1665 men, were identified, which differed in the populations studied and type, dosage and duration of antioxidants used. Only two-thirds of the studies (11/17) reported using allocation concealment and three studies (18%) used intention-to-treat analysis. Despite the methodological and clinical heterogeneity, 14 of the 17 (82%) trials showed an improvement in either sperm quality or pregnancy rate after antioxidant therapy. Ten trials examined pregnancy rate and six showed a significant improvement after antioxidant therapy. The use of oral antioxidants in infertile men could improve sperm quality and pregnancy rates. Adequately powered robust trials of individual and combinations of antioxidants are needed to guide clinical practice.

KEYWORDS: antioxidant, male infertility, pregnancy, sperm quality, vitamin

Introduction

Infertility affects about 15% of couples of reproductive age (Sharlip et al., 2002) and impaired semen parameters are implicated in up to 50% of infertile couples (WHO, 1987). The commonest aetiology of male infertility is idiopathic oligoasthenoteratozoospermia (Hirsch, 2003), for which a specific treatment remains elusive. Intra-cytoplasmic sperm injection (ICSI) enables this form of infertility to be circumvented mechanically (Tarlatzis and Bili,

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2000) but does not tackle the fundamental reasons behind male infertility (Georgiou et al., 2006; Johnson, 1998; Pauer et al., 1997; Varghese et al., 2007). As a result, there is growing interest in identifying reversible causes of male factor infertility.

Considerable evidence points towards a significant role of oxidative stress in causing male infertility (Agarwal et al., 2009; Kefer et al., 2009; Lewis and Agbaje, 2007; Tremellen, 2008). Reactive oxygen species (ROS) include hydroxyl radicals, superoxide anions and hydrogen peroxide (Combelles et al., 2009; Sharma and Agarwal, 1996; Tremellen, 2008), the principal sources of which are leukocytes and sperm cytoplasm (Aitken et al., 1998). Mature and morphologically normal spermatozoa produce relatively less ROS compared with immature teratozoospermic forms, which have a greater amount of cytoplasm (Henkel et al., 2005). Spermatozoa and seminal plasma possess an abundance of antioxidant activity, both enzymatic (such as superoxide dismutase, glutathione peroxidase and catalase) and non-enzymatic (vitamins C and E, glutathione, carnitine and carotenoids) (Alvares and Storey, 1992; Tremellen, 2008).

Under normal conditions, the balance between the production of ROS and antioxidant activity is maintained. When ROS are present in excessive amounts either due to increased generation or impaired clearance, they can cause extensive sperm DNA structural damage (Davies, 1987; Lopes et al., 1998), reduced sperm motility (Jones et al., 1979; Kao et al., 2008; MacLeod, 1943) and defective sperm membrane integrity via lipid peroxidation (Agarwal et al., 2003; Aitken et al., 1989), all of which are important mechanisms behind sperm dysfunction (Davies, 1987; Kefer et al., 2009; Lopes et al., 1998; Sharma and Agarwal, 1996; Tremellen, 2008). Furthermore, the antioxidant capacity of semen from infertile men is less effective than that from fertile men (Fraga et al., 1996; Lewis et al., 1997; Saleh et al., 2002; Tremellen, 2008).

Antioxidants (such as vitamins C and E, folate, zinc, selenium, carnitine and carotenoids) are scavengers of ROS and their use has been studied as a treatment to reverse the adverse impact of high ROS concentrations on semen parameters. Observational studies have shown that men with high dietary intake of antioxidants have a lower frequency of sperm aneuploidy and improved semen quality compared with men with lower intake (Silver et al., 2005; Young et al., 2008). Aitken et al. (1989) demonstrated a dose-dependent reduction in the ability of human spermatozoa to fuse with oocytes with increased oxidative stress, which could be reversed by the inclusion of vitamin E. Likewise, Hughes and colleagues (1998) reported a beneficial effect on sperm DNA integrity during Percoll sperm preparation for assisted reproduction by the addition of antioxidants. However, most of these studies were uncontrolled, included fertile men and rarely used pregnancy as an outcome measure (Kefer et al., 2009; Lewis and Agbaje, 2008).

This systematic review sought to rigorously evaluate current evidence from randomized trials on the effect of oral antioxidants on sperm parameters and the likelihood of spontaneous and treatment-related pregnancy in infertile men.

Materials and methods

Identification of oral antioxidants and their mode of action

This study reviewed substances with recognized antioxidant properties. These were: (i) vitamin C (ascorbic acid), a water-soluble potent ROS scavenger that can also influence the expression of genes involved in the intracellular redox pathways (Duarte and Lunex, 2005; Gershoff, 1993); (ii) vitamin E (α-tocopherol), a lipid-soluble antioxidant whose primary role is to protect the integrity of the phospholipid bi-layer of the cell membrane and mitochondrial sheath by interrupting the chain reactions involved in lipid peroxidation and enhancing the production of scavenger antioxidant enzymes (Fukuzawa et al., 1985; Suleiman et al., 1996; Traber and Atkinson, 2007); (iii) folic acid (folate), which acts as an antioxidant by reducing homocysteine concentrations and via its free ROS scavenging properties (Alvares Delfino et al., 2007; Joshi et al., 2001); (iv) zinc, an antioxidant trace element which has a membrane stabilizing activity by inhibiting membrane-bound oxidative enzymes such as NADP oxidase (Powell, 2000; Prasad, 2008) and may also have an immunological function (Omu et al., 2008); (v) selenium, which plays a pivotal role in increasing glutathione peroxidase-1 expression and activity, which in turn destroys hydrogen peroxide molecules (Rotruck et al., 1973; Schnabel et al., 2008); (vi) carnitines, which are involved in transport of long chain fatty acids into the mitochondrial matrix for beta-oxidation and exert antioxidant activity via increasing expression of antioxidant enzymes such as haeme oxygenase-1 and endothelial nitric oxide synthetase (Arduini, 1992; Balercia et al., 2005; Calò et al., 2006; Jeulin and Lewin, 1996) and buffering acetyl-CoA pools (Sigman et al., 2006); (vii) *N*-acetyl cysteine, an amino acid precursor of glutathione and that acts as an antioxidant by increasing the availability of intracellular glutathione and by acting directly as a free oxygen radical scavenger (Circu et al., 2009); and (viii) astaxanthin, a carotenoid extracted from algae *Haematococcus pluvialis* with a high number of conjugated double bonds rendering it a more potent antioxidant than either vitamin E or carnitine (Comhaire et al., 2005; Rao and Agarwal, 2000; Wolf et al., 2009).

Literature search methodology

A systematic review was conducted on randomized controlled studies evaluating the effects of the oral antioxidants (vitamins C and E, folate, zinc, selenium, carnitine, *N*-acetyl cysteine and carotenoids) on semen parameters and spontaneous or treatment-related pregnancy rate in infertile men. MEDLINE, EMBASE, Cochrane Library and CINAHL were searched for relevant trials published from respective database inception dates up to 31 May 2009 with no language restrictions.

Two subsets of search terms were used, one describing the antioxidants (oral antioxidants, antioxidant therapy, folate, folic acid, selenium, zinc, vitamin, vitamin C, vitamin E, ascorbic acid, ascorbate, tocopherol, carnitine, cysteine, *N*-acetyl cysteine, beta carotene, carotenoid and trace

elements) and the other describing spermatozoa, infertility and pregnancy (semen, sperm, spermatozoa, oligozoospermia, athenozoospermia, teratozoospermia, male subfertility, male infertility, in-vitro fertilization, intra-cytoplasmic sperm injection conception, pregnancy, embryo transfer and assisted reproduction techniques, which were combined in the search using the 'OR' operator). The two subsets of terms were then combined using the 'AND' operator.

Study selection

Studies were selected if they had a randomized design, the target population was infertile men and the therapeutic intervention was oral antioxidant(s) compared with placebo or no treatment. Outcome measures were basic semen parameters (sperm concentration, motility and morphology) and/or occurrence of pregnancy. Randomized studies with a cross-over design were excluded to avoid any possible residual effect from an inadequate wash out period (Sigman et al., 2006). Studies involving the use of antimicrobial or hormonal medication were also excluded.

The titles and abstracts retrieved from the electronic searches were scrutinised and full manuscripts of relevant citations that definitely or possibly met the search predefined selection criteria were obtained. Final study inclusion was made on examination of the full manuscripts. The reference lists of these studies and those of related reviews were further searched to ensure all relevant randomized studies were included. Study selection, quality appraisal and data extraction were performed independently and in duplicate by two authors (CR and AM) with a third author (TE) acting as an arbitrator to resolve uncertainty or disagreement about study inclusion.

Data extraction and quality assessment

The selected studies were assessed for methodological quality by using the components of study design that are related to internal validity (Jadad et al., 1996), including method of randomization, allocation concealment, double blinding, intention-to-treat analysis and follow up (or drop-out) rate.

Study characteristics, participant features, study inclusion and exclusion criteria, nature of intervention (type and dose of antioxidant(s) used and duration of treatment) were extracted from each study. Exploration of clinical heterogeneity was conducted using variation in features of the population, intervention and study quality.

Statistical analysis

Data entry and analysis were performed using a Statview software package (Abacus Concepts, Berkeley, California, USA). Analysis of the study outcome measures and associated clinical variables was performed using a two-sample *t*-test (for continuous variables) and chi-squared test (for categorical variables). The odds ratio (OR) and 95% confidence interval (CI) were calculated for the spontaneous pregnancy outcome. A *P*-value <0.05 was considered statistically significant.

Results

The initial search identified 3740 citations, of which 3702 were excluded following screening of the titles and abstracts and 38 manuscripts were retrieved and reviewed in full, including one article published in Polish and translated into the English language (**Figure 1**). Of the 38 manuscripts examined in full, 17 original randomized controlled studies including 1665 men met the inclusion criteria (**Figure 1**). Sixteen studies (including 1605 randomized men) evaluated the effect of oral antioxidants on semen parameters and 10 studies (including 783 randomized men) reported the effect of oral antioxidants on pregnancy rate, including one study reporting the effect of oral antioxidants on pregnancy rate after assisted conception treatment (Tremellen et al., 2007). All 17 studies were published in full in the English language.

Methodological quality of included studies

The methodological quality of the trials included in this review was variable (**Table 1**). Less than half (41%, 7/17) of the studies described a recognized method of randomization, and less than two-thirds (59%, 10/17) reported using allocation concealment during group assignment. The majority of studies were placebo-controlled (88%, 15/17) and had a double-blind design (82%, 14/17), but only three studies (18%) analysed their results according to the principles of intention-to-treat (ITT) analysis. The follow up rate varied from 37% to 100%, but was greater than 75% in 15 of the 17 studies (88%). Only two studies (12%) satisfied all five quality assessment criteria (Ciftci et al., 2009; Galatioto et al., 2008).

Study characteristics

Study population

All studies were performed as single-centre studies, except that of Wong and colleagues (2002), which was conducted across two fertility centres. Nine of the 17 studies (53%) were performed in Europe (Balercia et al., 2005; Cavallini et al., 2004; Comhaire et al., 2005; Galatioto et al., 2008; Greco et al., 2005a; Lenzi et al., 2004; Rolf et al., 1999; Scott et al., 1998; Wong et al., 2002), five (29%) were performed in Asia (Ciftci et al., 2009; Omu et al., 1998, 2008; Safarinejad and Safarinejad, 2009; Suleiman et al., 1996), one (6%) in Africa (Keskes-Ammar et al., 2003), one in the USA (Sigman et al., 2006) and one in Australia (Tremellen et al., 2007).

The number of men randomized per study varied widely and ranged between 26 and 468 (median 60), with 11of the 17 studies (65%) randomizing less than 100 men per study. Only four studies (24%, Rolf et al., 1999; Safarinejad and Safarinejad, 2009; Tremellen et al., 2007; Wong et al., 2002) described an a-priori power calculation for estimation of sample size.

Three studies (18%) recruited men with unclassified infertility (Ciftci et al., 2009; Comhaire et al., 2005); Keskes-Ammar et al., 2003; Wong et al., 2002), eight (47%) recruited infertile men with asthenozoospermia

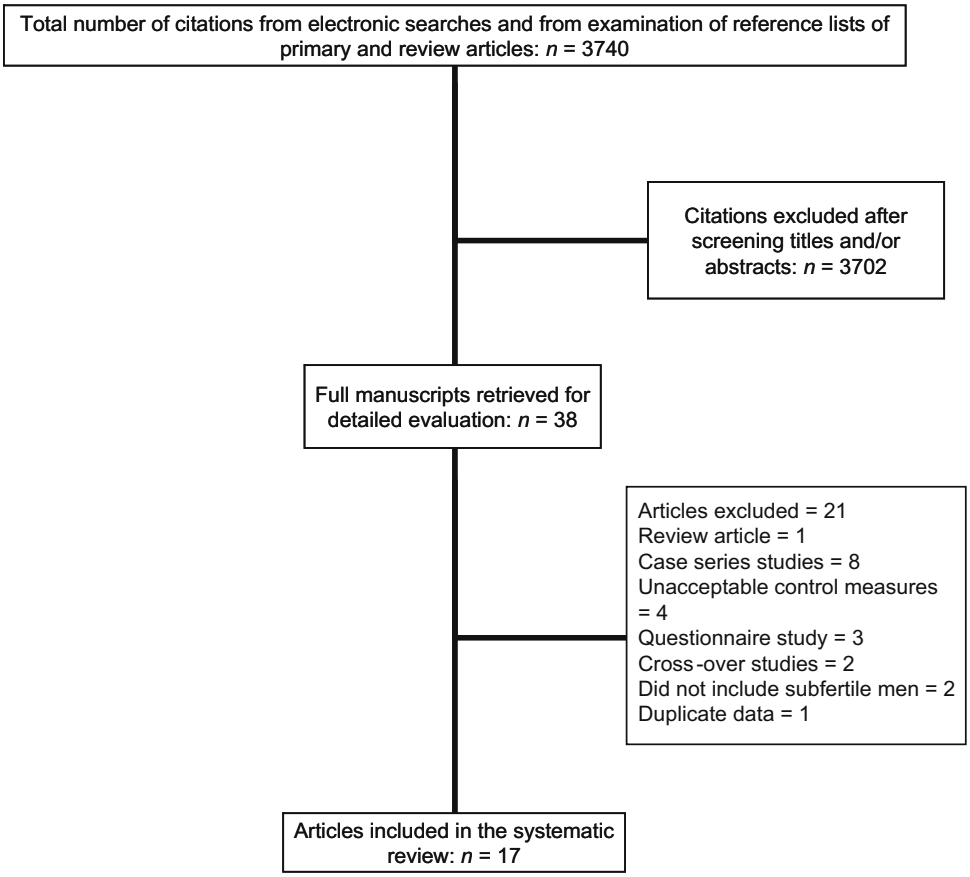

Figure 1 Study selection process.

Table 1 Quality assessment of included trials.

Study	*Randomization method*	*Allocation concealment*	*Blinding*	*ITT analysis*	*Follow-up rate (%)*
Suleiman et al. (1996)	Not clear	Not clear	Double	No	79
Scott et al. (1998)	Random no. tables	Adequate	Double	No	93
Omu et al. (1998)	Not clear	Not clear	No	No	97
Rolf et al. (1999)	Random no. tables	Adequate	Double	No	94
Wong et al. (2002)	Computer generated	Adequate	Double	No	92
Keskes-Ammar et al. (2003)	Random no. tables	Adequate	Double	No	37
Lenzi et al. (2004)	Not clear	Not clear	Double	No	93
Cavallini et al. (2004)	Not clear	Adequate	Double	No	86
Balercia et al. (2005)	Not clear	Not clear	Double	Not clear	98
Comhaire et al. (2005)	Not clear	Not clear	Double	Yes	100
Greco et al. (2005a)	Not clear	Adequate	Double	Not clear	100
Sigman et al. (2006)	Not clear	Not clear	Double	Not clear	76
Tremellen et al. (2007)	Computer generated tables	Adequate	Double	Not clear	87
Galatioto et al. (2008)	Computer generated tables	Adequate	Double	Yes	100
Omu et al. (2008)	Not clear	Not clear	Not clear	Not clear	Not clear
Safarinejad and Safarinejad (2009)	Computer generated tables	Adequate	Double	No	89.7
Ciftci et al. (2009)	Not clear	Adequate	Single	Yes	100

ITT = intention to treat.

(Balercia et al., 2005; Lenzi et al., 2004; Omu et al., 1998, 2008; Rolf et al., 1999; Scott et al., 1998; Sigman et al., 2006; Suleiman et al., 1996), one (6%) recruited infertile men with oligozoospermia (Galatioto et al., 2008; Wong et al., 2002); Safarinejad and Safarinejad, 2009) and two studies (12%) recruited infertile men with oligoasthenozoospermia (Cavallini et al., 2004; Safarinejad and Safarinejad, 2009). Only two studies (Greco et al., 2005a; Tremellen et al., 2007) recruited infertile men based on actual sperm DNA damage level as measured by the TdT (terminal deoxynucleotidyl transferase)-mediated dUDP nick-end labelling (TUNEL) assay using more than 15% and 25% sperm DNA fragmentation as their inclusion threshold, respectively (**Table 2**). For the study of Wong et al. (2002), the group comprised of fertile men was excluded from this review.

In the eight studies which recruited athenozoospermic men, the threshold for study inclusion also varied. Whereas asthenozoospermia was defined as sperm motility below 50% in five studies (Balercia et al., 2005; Lenzi et al., 2004; Rolf et al., 1999; Sigman et al., 2006; Suleiman et al., 1996), it was defined as the presence of 40% or more immotile spermatozoa in the studies of Omu et al. (1998) and Omu et al. (2008) and was undefined in the study of Scott et al. (1998).

Study exclusion criteria

Nine studies (53%) excluded infertile men with oligozoospermia, defined as sperm concentration of: less than 20×10^6 spermatozoa/ml in the studies of Balercia et al. (2005), Galatioto et al. (2008) and Omu et al. (2008); less than 10×10^6 spermatozoa/ml in the study of Lenzi et al. (2004); less than 7×10^6 spermatozoa/ml in the study of Rolf et al. (1999); and less than 5×10^6 sperm/ml in the studies of Wong et al. (2002), Cavallini et al. (2004), Sigman et al. (2006) and Safarinejad and Safarinejad (2009).

Only eight studies (47%) excluded men with genital infection (Balercia et al., 2005; Cavallini et al., 2004; Galatioto et al., 2008; Greco et al., 2005a; Lenzi et al., 2004; Rolf et al., 1999; Safarinejad and Safarinejad, 2009; Sigman et al., 2006), six studies (35%) excluded smokers (Balercia et al., 2005; Cavallini et al., 2004; Galatioto et al., 2008; Greco et al., 2005a; Safarinejad and Safarinejad, 2009; Sigman et al., 2006) and five studies (29%) excluded men with varicocele (Balercia et al., 2005; Greco et al., 2005a; Lenzi et al., 2004; Safarinejad and Safarinejad, 2009; Sigman et al., 2006).

Intervention

The studies varied considerably in the nature and dose of antioxidant(s) used and duration of treatment (**Table 2**). Eight studies (47%) used a single antioxidant, while nine studies (53%) used a combination of antioxidants. The antioxidants used were vitamins C and E, zinc, selenium, folic acid, carnitine, *N*-acetyl cysteine, astaxanthin (a strong natural antioxidant) and Menevit (a commercially available combination of vitamins E and C, zinc, selenium, folate, lycopene and garlic). The antioxidant studied most as a single agent was carnitine (four studies: Balercia et al., 2005; Cavallini et al., 2004; Lenzi et al., 2004; Sigman et al., 2006), while vitamin C is the only antioxidant that has not been studied as a single agent among the studies selected for this review. Treatment duration lasted on average 18 weeks and ranged from 8 to 26 weeks (median = 13 weeks).

Outcomes

All studies reported outcome in terms of semen variables, pregnancy outcome or both (**Tables 2 and 3**). Overall, 14 of the 17 studies (82%) showed improvement in at least one of these outcome measures in the treatment group. In two of the three studies in which no improvement occurred in the treatment group (Greco et al., 2005a; Rolf et al., 1999), the antioxidants were used for only 8 and 9 weeks, respectively.

In addition, seven studies used a measure of sperm oxidative stress to assess treatment effect. This was performed either directly by measuring ROS concentration (Comhaire et al., 2005) or indirectly by measuring the degree of sperm lipid peroxidation (via determining the malondialdehyde concentration), DNA fragmentation (using the TUNEL assay) or total antioxidant capacity (Balercia et al., 2005; Ciftci et al., 2009; Greco et al., 2005a; Keskes-Ammar et al., 2003; Omu et al., 2008; Suleiman et al., 1996). All seven studies reported evidence of reduction in oxidative stress in the treatment group compared with controls.

Effect of antioxidants on semen variables

Of the 16 trials examining the effect of oral antioxidants on semen variables, 12 (75%) showed an improvement in at least one sperm parameter compared with placebo or no treatment (**Table 3** and **Figure 2**).

Ten out of the 16 studies (63%) showed a significant improvement in sperm motility compared with placebo (Balercia et al., 2005; Cavallini et al., 2004; Ciftci et al., 2009; Keskes-Ammar et al., 2003; Lenzi et al., 2004; Omu et al., 1998, 2008; Safarinejad and Safarinejad, 2009; Scott et al., 1998; Suleiman et al., 1996), five out of 15 studies (33%) showed an improvement in sperm concentration (Cavallini et al., 2004; Galatioto et al., 2008; Omu et al., 1998; Safarinejad and Safarinejad, 2009; Wong et al., 2002) and two out of 12 studies (17%) showed an improvement in sperm morphology (Cavallini et al., 2004; Safarinejad and Safarinejad, 2009).

Effect of antioxidants on pregnancy rate

Ten studies including 783 randomized men examined the effect of oral antioxidants on pregnancy rate and reported data on 745 men who completed the studies (**Table 4**). Nine of the 10 studies reported results for spontaneous conception (Balercia et al., 2005; Cavallini et al., 2004; Comhaire et al., 2005; Galatioto et al., 2008; Lenzi et al., 2004; Omu et al., 1998; Rolf et al., 1999; Scott et al., 1998; Suleiman et al., 1996) and, overall, showed a significantly higher pregnancy rate in the treatment group compared with controls (19% (69/368) versus 3% (9/317), OR = 7.9, 95% CI 3.9–16.1, $P < 0.0001$). One study (Tremellen et al., 2007) reported results after ICSI treatment and reported a significantly higher viable pregnancy rate per embryo

Table 2 Study characteristics in all included studies.

Study	No. of randomized participants and characteristics	Intervention (daily dose)	Control group	Duration (weeks)
Suleiman et al. (1996)	110; asthenozoospermia	Vit E 300 mg	Placebo	26
Omu et al. (1998)	100; asthenozoospermia	$ZnSO_4$ 500 mg	None	13
Scott et al. (1998)	69; asthenozoospermia	Vit A 1 mg, vit C 10 mg, vit E 15 mg and selenium 100 μg	Placebo	13
Rolf et al. (1999)	33; asthenozoospermia	Vit C 1000 mg and vit E 800 mg	Placebo	8
Wong et al. (2002)	103; unclassified subfertility	Folic acid 5 mg and/or $ZnSO_4$ 66 mg and folic acid 5 mg/$ZnSO_4$ 66 mg	Placebo	26
Keskes-Ammar et al. (2003)	54; unclassified subfertility	Vit E 400 mg and selenium 225 μg	Vit B	13
Lenzi et al. (2004)	60; asthenozoospermia	Carnitine 2 g and acetyl L-carnitine 1 g	Placebo	26
Cavallini et al. (2004)	219; oligoasthenozoospermia ± varicocele	Carnitine 2 g and acetyl L-carnitine 1 g/carnitine 2 g plus acetyl L-carnitine 1 g	Placebo	26
Balercia et al. (2005)	60; asthenozoospermia	L-Carnitine 3 g or acetyl L-carnitine 3 g or L-carnitine 2 g and acetyl L-carnitine 1 g	Placebo	26
Comhaire et al. (2005)	30; unclassified subfertility	Astaxanthin 16 mg	Placebo	13
Galatioto et al. (2008)	64; >15% of sperm fragmented DNA	Vit C 1 g and vit E 1 g	Placebo	9
Sigman et al. (2006)	26; asthenozoospermia	L-Carnitine 1 g and acetyl L-carnitine 500 mg	Placebo	16
Galatioto et al. (2008)	42; oligospermia 6/12 after varicocele embolisation	Vit A 0.06 IU/kg, vit C 3 mg/kg, vit E 0.2 mg/kg, *N*-acetyl cysteine 10 mg/kg, zinc 0.01 mg/kg and others[a]	Placebo	13
Tremellen et al. (2007)	60; poor morphology, motility or membrane integrity and >25% of spermatozoa showing significant DNA fragmentation[b]	Menevit one capsule[c]	Placebo	13
Omu et al. (2008)	45; asthenozoospermia	Vit C 10 mg, vit E 20 mg with $ZnSO_4$ 400 mg, vit E 20 mg with $ZnSO_4$ 400 mg or $ZnSO_4$ 400 mg	None	13
Safarinejad and Safarinejad (2009)	468; idiopathic oligoasthenoteratozoospermia	*N*-acetyl cysteine 600 mg/*N*-acetyl cysteine 600 mg and selenium 200 μg/selenium 200 μg	Placebo	26
Ciftci et al. (2009)	120; idiopathic infertility	*N*-acetyl cysteine 600 mg	Placebo	13

[a]Thiamine 0.4 mg/kg/day, riboXavin 0.1 mg/kg/day, piridoxin 0.2 mg/kg/day, nicotinamide 1 mg/kg/day, pantothenate 0.2 mg/kg/day, biotin 0.04 mg/kg/day, cyanocobalamin 0.1 mg/kg/day, ergocalciferol 8 IU/kg/day, calcium 1 mg/kg/day, magnesium 0.35 mg/kg/day, phosphate 0.45 mg/kg/day, iron 0.2 mg/kg/day, manganese 0.01 mg/kg/day, copper 0.02 mg/kg/day.

[b]>25% of spermatozoa were microscopic TdT (terminal deoxynucleotidyl transferase)-mediated dUDP nick-end labelling assay positive.

[c]Menevit, a commercially available supplement, contains vitamin C 100 mg, vitamin E 400 IU, folate 500 μg, garlic 1000 mg, lycopene 6 mg, selenium 26 μg and zinc 25 mg.vt = vitamin.

Table 3 Effect of antioxidants on sperm parameters in the included studies.

Study	*Antioxidant used*	*Study outcome measures*	*Improvements*
Suleiman et al. (1996)	Vitamin E	Motility, level of lipid peroxidation	Motility in asthenozoospermic men and reduction in level of lipid peroxidation
Omu et al. (1998)	$ZnSO_4$	Concentration, motility, membrane integrity, serum zinc, cadmium, antisperm antibodies, FSH, LH, prolactin, TNFα and IL-4 concentrations	Count, progressive motility, membrane integrity and reduction in antisperm antibodies
Scott et al. (1998)	Vitamins A, C, E, selenium	Concentration, motility, serum selenium	Motility
Rolf et al. (1999)	Vitamins C and E	Concentration, motility, morphology, volume, 24-h sperm survival	No improvement
Wong et al. (2002)	Folic acid and/or $ZnSO_4$	Concentration, motility, morphology, seminal plasma, blood plasma and erythrocyte folate and zinc	Concentration and total normal sperm count
Keskes-Ammar et al. (2003)	Vitamin E and selenium	Concentration, motility, morphology, volume, viability, semen MDA, serum vitamin E and cholesterol	Motility
Lenzi et al. (2004)	Carnitine	Concentration, motility, morphology, volume	Number of total motile sperm and forward motile sperm
Cavallini et al. (2004)	Carnitine	Concentration, motility, morphology, testicular volume	Concentration, motility and morphology, except for grade IV and V varicocoeles, where there was no difference.
Balercia et al. (2005)	Carnitine	Concentration, motility, morphology, volume, semen total oxyradical scavenging capacity	Straight progressive velocity and total oxyradicalscavenging capacity
Comhaire et al. (2005)	Astaxanthin	Concentration, motility, morphology, volume, pregnancy rate, serum FSH, LH, testosterone, inhibin B, ROS generation,[a] a, zona-free hamster oocyte test, seminal γ-glutamyltransferase, gluconidase.	No improvement.
Greco et al. (2005a)	Vitamins C and E	Concentration, motility, morphology, sperm DNA fragmentation[b]	DNA fragmentation.
Sigman et al. (2006)	Carnitine	Volume, motility, concentration, forward progression, seminal plasma and semen carnitine	No improvement.
Galatioto et al. (2008)	Vitamins A, C, E, *N*-acetyl cysteine and zinc	Concentration, motility, morphology, volume	Odds of having a normal sperm count.
Omu et al. (2008)	Vitamins C and E with $ZnSO_4$, vitamin E with $ZnSO_4$ or $ZnSO_4$ alone	Concentration, motility, morphology, volume, sperm chromatin integrity and apoptosis,[c] sperm membrane integrity, serum and seminal plasma antioxidants, cadmium, magnesium, selenium, zinc, cytokines, oxidants, antisperm antibodies antibody levels and *Bcl2* and *Bax* expression	Sperm motility, fertilizing capacity (*in vivo*) and DNA fragmentation index (*in vitro*).
Safarinejad and Safarinejad (2009)	*N*-acetyl cysteine, *N*-acetyl cysteine and/or selenium	Concentration, motility, morphology, serum testosterone, inhibin B, LH, FSH	Count, motility, morphology.
Ciftci et al. (2009)	*N*-acetyl cysteine	Concentration, motility, morphology, volume, viscosity, liquefaction time, oxidative status	Motility, volume, viscosity, liquefaction time, oxidative status (total antioxidant capacity, total peroxide and oxidative stress index).

IL = interleukin; ROS = reactive oxygen species; TNF = tumour necrosis factor.
[a]Measured by chemoluminescence in high quality spermatozoa after centrifugation.
[b]Measured using the TUNEL test.
[c]Chromatin integrity determined by acid denaturation by acridine orange and apoptosis by light and electron microscopy.

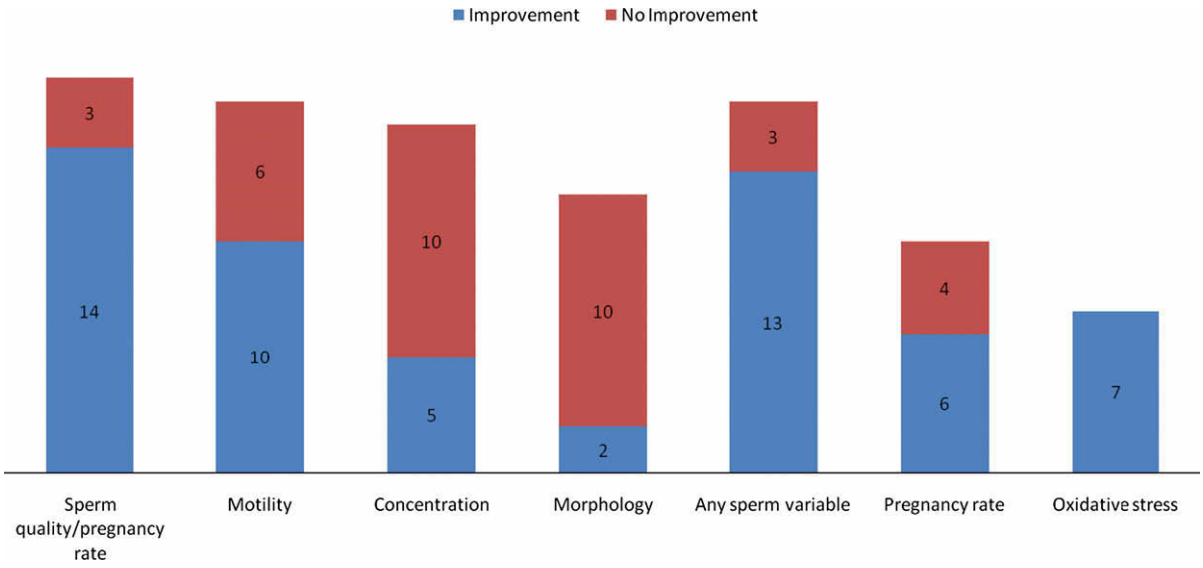

Figure 2 Effect of antioxidants on sperm parameters, pregnancy rate and oxidative stress. Numbers indicate the number of studies that showed an improvement or no improvement.

Table 4 Effect of antioxidants on spontaneous or treatment-related pregnancy rate in the included studies.

Study	*Antioxidant*	*Effect of antioxidants on pregnancy rate*
Suleiman et al. (1996)	Vitamin E	21% (11/52) versus 0% (0/35), $P = 0.003$[a]
		Live birth rate 17% (9/52) versus 0% (0/35), $P = 0.009$
Omu et al. (1998)	Zinc sulphate	22% (11/49) versus 4% (2/48), $P < 0.03$[a]
Scott et al. (1998)	Selenium, vitamins A, C and E	11% (5/46) versus 0% (0/18), $P = 0.15$[a]
Rolf et al. (1999)	Vitamins C and E	0% (0/15) versus 0% (0/16), $P = 1$[a]
Lenzi et al. (2004)	Carnitine	13% (4/30) versus 0% (0/26), $P = 0.04$[a]
Cavallini et al. (2004)	Acetyl L-carnitine and carnitine	22% (22/101) versus 2% (2/118), $P < 0.01$[a]
Balercia et al. (2005)	L-Carnitine and acetyl L-carnitine	20% (9/44) versus 20% (3/15), $P = 1$[a]
Comhaire et al. (2005)	Astaxanthin	55% (6/11) versus 11% (2/19), $P = 0.028$[a]
Galatioto et al. (2008)	Vitamins A, C and E, *N*-acetyl cysteine and zinc	5% (1/20) versus 0% (0/22), $P = 0.95$[a]
Tremellen et al. (2007)[b]	Menevit	64% (23/36) versus 38% (6/16), $P = 0.077$
		Viable pregnancy rate at 13 weeks (38% (20/52) versus 16% (4/25), $P = 0.046$

[a]Pregnancy rates are for spontaneous pregnancies that occurred during the study period.
[b]Pregnancy rates per embryo transfer.

transfer in the treatment group (39% (20/52) versus 16% (4/25), $P = 0.046$).

Individually, six of the 10 studies reported a significantly higher pregnancy rate following antioxidant treatment compared with controls (**Figure 2**). In two of the four remaining studies (Galatioto et al., 2008; Scott et al., 1998), there was a higher pregnancy rate in the treatment group but the difference did not reach statistical significance. Interestingly, four of the nine studies reporting data on spontaneous pregnancy rates during the study period failed to provide sufficient confirmation that female partners of the randomized men had been objectively investigated for female

causes of infertility (Comhaire et al., 2005; Galatioto et al., 2008; Omu et al., 1998; Rolf et al., 1999).

As shown in **Table 3**, in five of the six studies in which there was a significantly higher pregnancy rate in the treatment group, sperm quality was also examined and four of these showed an improvement in at least one sperm parameter, with all four studies demonstrating improved motility (Cavallini et al., 2004; Lenzi et al., 2004; Omu et al., 1998; Suleiman et al., 1996).

Effect of individual antioxidants on semen variables and pregnancy rate

In this review, the different antioxidants studied have been used either as a single therapy or in combination with other antioxidants. Overall, all the antioxidants encountered in this review, except vitamin C, have been studied individually and resulted in improvement in at least one semen variable in one or more studies. However, these results were not consistent and varied considerably between the studies (**Tables 2–4**).

Vitamin C

Sole use: Vitamin C has not been evaluated as the sole antioxidant in any of the 17 studies included in this review and therefore its effectiveness as a single oral antioxidant agent in infertile men is unknown.

Combined use: Vitamin C has been used in conjunction with other antioxidants in six studies (Galatioto et al., 2008; Greco et al., 2005a; Rolf et al., 1999; Scott et al., 1998; Tremellen et al., 2007; and one arm of Omu et al., 2008). Sperm motility improved in two out of five studies (40%, Omu et al., 2008; Scott et al., 1998), sperm concentration improved in one out of five studies (20%, Galatioto et al., 2008) and sperm DNA fragmentation index was reduced in two out of two studies (Greco et al., 2005a; Omu et al., 2008). However, sperm morphology did not improve in any of the five studies. Pregnancy rate after ICSI was significantly improved in the treatment group in one study ($P = 0.046$; Tremellen et al., 2007).

Vitamin E

Sole use: Vitamin E has been studied as the sole antioxidant supplement in one study (Suleiman et al., 1996) at a daily dose of 300 mg for 26 weeks. This study reported improvement in both sperm motility and pregnancy rate.

Combined use: Vitamin E has been used in conjunction with other antioxidants in seven studies (Greco et al., 2005a; Galatioto et al., 2008; Keskes-Ammar et al., 2003; Omu et al., 2008; Rolf et al., 1999; Scott et al., 1998; Tremellen et al., 2007). Sperm motility improved in three out of six studies (50%, Keskes-Ammar et al., 2003; Omu et al., 2008; Scott et al., 1998), sperm concentration improved in one out of six studies (17%, Galatioto et al., 2008) and sperm DNA fragmentation index in two out of two studies (Greco et al., 2005a; Omu et al., 2008). However, sperm morphology did not improve in any of the five studies where this parameter was evaluated (Galatioto et al., 2008; Greco et al., 2005a; Keskes-Ammar et al., 2003; Scott et al., 1998; Rolf et al., 1999). Pregnancy rate was higher in only one out of four studies where this was considered (Tremellen et al., 2007).

Zinc

Sole use: Zinc sulphate has been studied as a sole antioxidant in three studies (Omu et al., 1998, 2008; Wong et al., 2002). Motility improved in two studies (Omu et al., 1998, 2008), concentration improved in two studies (Omu et al., 1998; Wong et al., 2002), but morphology did not improve in any of the three studies. Pregnancy rate improved in the only study where this was examined (Omu et al., 1998).

Combined use: Zinc sulphate has been used in conjunction with other antioxidants in four studies (Galatioto et al., 2008; Omu et al., 2008; Tremellen et al., 2007; Wong et al., 2002). Motility improved in one out of three studies (Omu et al., 2008), concentration improved in two out of three studies (Galatioto et al., 2008; Wong et al., 2002), but morphology did not improve in any of the three studies. Pregnancy rate improved in one out of two studies (Tremellen et al., 2007).

Selenium

Sole use: Selenium has been used as the sole antioxidant in only one study (Safarinejad and Safarinejad, 2009), which reported an improvement in all three sperm variables; concentration, motility and morphology.

Combined use: Selenium has been used in conjunction with other antioxidants in four studies (Keskes-Ammar et al., 2003; Safarinejad and Safarinejad, 2009; Scott et al., 1998; Tremellen et al., 2007). Sperm motility was improved in three out of three studies (Keskes-Ammar et al., 2003; Safarinejad and Safarinejad, 2009; Scott et al., 1998), concentration improved in one out of three studies (Safarinejad and Safarinejad, 2009), morphology improved in one out of two studies (Safarinejad and Safarinejad, 2009) and pregnancy rate improved in one out of two studies (Tremellen et al., 2007).

Folic acid

Sole use: Folic acid has been studied as the sole antioxidant in one study (Wong et al., 2002, at a daily dose of 5 mg for 26 weeks). This study reported improvement in sperm concentration, but not in sperm motility or morphology.

Combined use: Folic acid has been used in conjunction with other antioxidants in two studies (Tremellen et al., 2007; Wong et al., 2002). Only sperm concentration, but not motility or morphology, was improved in the study of Wong et al. (2002) and pregnancy rate after assisted conception treatment was significantly improved in the study of Tremellen et al. (2007).

Carnitine

Sole use: L-Carnitine and acetyl L-carnitine have been used as sole antioxidants in four studies (Balercia et al., 2005; Cavallini et al., 2004; Lenzi et al., 2004; Sigman et al., 2006). Sperm motility improved in three out of four studies (Balercia et al., 2005; Cavallini et al., 2004; Lenzi et al., 2004), concentration improved in one out of four studies one (Cavallini et al., 2004) and morphology in one out of three studies (Cavallini et al., 2004). Pregnancy rate

improved in two out of three studies (Cavallini et al., 2004; Lenzi et al., 2004).

Astaxanthin

Astaxanthin has only been used in one study as the sole antioxidant in the study of Comhaire et al. (2005). Only the pregnancy rate was improved in this study, but none of the three main semen variables showed an improvement after treatment.

N-acetyl cysteine

Sole use: *N*-Acetyl cysteine has been used in two studies as a sole antioxidant: Safarinejad and Safarinejad (2009) (600 mg daily for 26 weeks) and Ciftci et al. (2009) (600 mg daily for 13 weeks). Sperm motility improved in both studies, but concentration and morphology improved only in the study of Safarinejad and Safarinejad (2009).

Combined use: *N*-acetyl cysteine has been used in combination with other antioxidants in two studies (Galatioto et al., 2008; Safarinejad and Safarinejad, 2009). Sperm motility improved in one study (Safarinejad and Safarinejad, 2009), whereas concentration improved in both studies and morphology improved in only one study (Safarinejad and Safarinejad, 2009). Pregnancy rate did not show an improvement in the study of Galatioto et al. (2008).

Discussion

High ROS level and oxidative stress have been implicated in the pathophysiology of male infertility (Tremellen, 2008) and correlated with sperm DNA damage, impaired fertilization and embryo development, low rates of implantation and occurrence of miscarriage (Agarwal et al., 2006; Aitken et al., 1989; Aitken and Baker, 2002; Carrell et al., 2003; Lewis and Aitken, 2005; Loft et al., 2003; Morris et al., 2002; Seli et al., 2004). This systematic review of a large number of randomized trials shows that treatment of infertile men with oral antioxidants reduces seminal oxidative stress and could improve sperm motility, but has a less predictable impact on sperm concentration and morphology (**Figure 2**).

These results concord with evidence from other randomized studies not included in this review. Two randomized studies examined the effect of oral antioxidants on sperm parameters in fertile men. Dawson et al. (1987) treated 30 men with sperm agglutination of over 25% with vitamin C 200 mg or 1000 mg daily or placebo. The authors found that motility, viability and morphology significantly increased after 4 weeks of treatment compared with baseline and more so in the 1000 mg group than the 200 mg group, but not in the placebo group. The same group (Dawson et al., 1992) supplemented 75 heavy smokers with normal reproductive function with vitamin C at a daily dose of either 200 mg or 1000 mg of vitamin C or placebo. The authors reported that the control group showed no improvement in sperm quality whereas the vitamin C supplemented groups had improved sperm agglutination, 24-h viability and morphology.

This review also excluded two randomized studies with a cross-over design. Kessopoulou et al. (1995) studied the effect of daily administration of 600 mg of vitamin E versus placebo for 3 months followed by a 1-month washout period and then cross-over to the other treatment on 30 infertile men with high levels of ROS generation. The authors reported a significant improvement in the zona-binding test for both orders of treatment, but that a significant carry-over effect was only evident in the group receiving vitamin E first.

Likewise, Lenzi et al. (2003) treated 100 infertile men with L-carnitine 2 g/day or placebo for 2 months followed by a 2-month washout period and then 2 months of the other treatment. They found that total motile spermatozoa and forward motile spermatozoa were significantly improved following carnitine treatment and that all the eight pregnancies which occurred during the trial were in the carnitine therapy period. Furthermore, the greatest increases in the number of motile spermatozoa were found in those subjects with the lowest baseline number of motile spermatozoa/ml of ejaculate.

More relevant clinically, this review shows that oral antioxidant therapy was associated with a significant improvement in spontaneous and assisted conception pregnancy rates in six of the 10 randomized studies identified in the database search (Cavallini et al., 2004; Comhaire et al., 2005; Lenzi et al., 2004; Omu et al., 1998; Suleiman et al., 1996; Tremellen et al., 2007). This finding could possibly be explained, at least in part, by the antioxidant-related improvement in either sperm motility and total motile sperm count, both of which have been reported to predict male fertility (Ibérico et al., 2004; Shulman et al., 1998; Zhao et al., 2004) or sperm DNA integrity (Hughes et al., 1998; Lopes et al., 1998). It also concurs with the results of several observational studies involving assisted conception treatment. Geva and colleagues (1996) treated 15 men with low IVF fertilization rates with 200 mg of vitamin E daily for 3 months and reported a significant reduction in sperm lipid peroxidation and increase in oocyte fertilization rate after 1 month of treatment. Likewise, Greco et al. (2005b) treated 38 men with elevated sperm DNA fragmentation with 1 g of vitamin C and 1 g of vitamin E daily after one failed ICSI attempt for 2 months and reported marked improvement in clinical pregnancy (48.3% versus 6.9%, $P < 0.05$) and implantation rates (19.6% versus 2.2%, $P < 0.01$) in the second ICSI attempt compared with the first cycle.

The clinical heterogeneity of the included randomized studies meant a meta-analysis of their results could not be performed and therefore bar charts were used as a visual alternative (**Figure 2**). Although the use of oral antioxidants may seem beneficial in ameliorating male infertility, the evidence in favour of their use is by no means compelling. Three randomized studies included in this review failed to show any benefit from using antioxidants on sperm parameters or the likelihood of pregnancy (**Figure 2**). Such discrepancy in the effect of oral antioxidants encountered in this review could be attributed to the wide variation in nature, dose and duration of the antioxidants used (**Table 2**) or to the heterogeneity in the characteristics of the study populations included in these studies. Menezo and colleagues (2007) treated 58 men with a sperm DNA fragmentation index of greater than 15% with oral antioxidant therapy (vitamins C and E, beta carotene, zinc and selenium) for 13 weeks and reported a significant improvement in the

DNA fragmentation index in the participants following treatment. However, the degree of high DNA stainability (i.e. sperm decondensation) increased significantly following treatment, which might lead to asynchronous chromosome condensation and cytoplasmic fragments in the embryo, thereby potentially reducing the success rate of IVF. The authors suggested that this finding may explain some of the discrepancies in the results of available studies and that care should be taken when using antioxidant treatment in men with a high degree of sperm decondensation (Menezo et al., 2007). Likewise, in an in-vitro study, Donnelly and colleagues (1999) also suggested the addition of combinations of antioxidants could somehow have damaging effects on sperm DNA due to alteration in the optimum balance that exists between the antioxidants used. Furthermore, antioxidant therapy could be ineffective if given to males whose subfertility is not caused by oxidative stress (Bolle et al., 2002; Lewis and Agbaje, 2008).

Other factors which warrant interpretation of the results of this review with caution are the inherent tendency of biological fluctuation in consecutive semen samples from the same individual, the regional variation in semen quality (Lewis, 2007), the lack of standardization in carrying out the tests used in assessing semen variables and the intra-observer and inter-observer semen assessment bias (Tielemans et al., 1997; Wong et al., 2002), factors many of which have not been accounted for in the included studies.

Furthermore, the inconsistency in methodological quality and study results encountered in the available literature precludes making any firm recommendations related to which antioxidant or group of antioxidants should be prescribed, the specific dose for each antioxidant or the optimum duration of treatment. Equally important, it does not guide clinicians in selecting the specific group of infertile men who are most likely to benefit from this treatment, although it may seem reasonable to speculate that this group could be men whose predominant semen analysis defects comprise asthenozoospermia associated with reduced seminal antioxidant capacity and that the duration of antioxidant therapy should extend beyond 9 weeks.

In conclusion, systematic review of evidence from randomized controlled trials suggests that oral antioxidant supplementation may ameliorate male infertility by improving some sperm parameters and the likelihood of pregnancy. This improvement, however, is not consistent and there is a wide variation in the treatment regimens used. It is therefore incumbent upon clinicians to undergo further large randomized controlled studies to examine the effect of standardized doses of specific oral antioxidants on spontaneous as well as assisted conception pregnancy rates to elucidate whether and how they should prescribe oral antioxidants to infertile men. It is imperative these studies employ strict inclusion and exclusion criteria and standardized methodology to help understand whether a specific group of infertile men is more likely to benefit from antioxidant therapy.

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