Antioxidant therapy for male subfertility: myth or evidence-based?

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The authors review the literature investigating the role of common dietary supplements that are being marketed as a remedy for male subfertility. They assess the evidence base supporting their use and their potential role in improving semen parameters alongside overall conception and live-birth rates.

Around 15% of couples experience infertility, with up to 50% of these related to male factors. Oxidative stress caused by reactive oxygen species, including oxygen ions, free radicals and peroxides, has been reported to play a role in around half of all infertile men. These reactive oxygen species can, when present in high concentrations, damage the sperm membrane in addition to directly damaging the sperm DNA (Figure 1).1

Antioxidants are available in a standard diet and, also, as oral supplements, which are sold as single agents or more often as a mixture of different compounds. Multiple nutritional supplements have antioxidant properties and are being used in the treatment of male subfertility. These supplements are understandably popular as they are relatively cheap and widely available over the counter. The misleading term ‘nutraceuticals’ has been coined to confer the idea of a powerful, synergistic effect between natural and pharmaceutical characteristics of these compounds while maintaining only a limited adverse, side-effect profile.

In the UK, certain supplements are considered to be foods and will be regulated by the Food Standards Agency and Department of Health under the general food safety laws. Therefore, these compounds do not require approval by the Medicines and Healthcare Regulatory Agency, which may result in a less stringent licensing process compared to standard pharmaceutical agents. This has allowed certain franchises to market specific products for the treatment of male infertility while potentially targeting this vulnerable patient cohort; where emotive and social factors may play a disproportionate role in the choice of treatment regardless of robust scientific evidence for the efficacy of such products.

There are a number of systematic reviews in the literature on the effect of these supplements on male subfertility. These provide a useful overview of the evidence but are limited by the heterogeneity of the included studies and the quality of the evidence. Despite this, the evidence suggests that there may be a role for antioxidant therapy in certain subgroups of infertile men. For example, men with high levels of oxidative stress may benefit from antioxidant supplementation, as has been demonstrated in animal models.2

In conclusion, antioxidant therapy for male subfertility is not a myth but has the potential to be evidence-based. Further research is needed to identify the optimal agents, dosages and combinations of supplements to achieve the best outcomes for infertile men.

References


agents in the treatment of male subfertility, with variable conclusions.2–4 A recent Cochrane review5 of 34 trials with 2876 couples, of which only three small randomised controlled trials reported on live birth outcomes, concluded that antioxidant supplementation may improve pregnancy and live birth rates for subfertile couples undergoing assisted reproduction techniques. The authors, however, highlighted that the current evidence is inconclusive and that additional well-constructed and sufficiently powered randomised placebo-controlled trials are required. In addition to this, conclusions regarding the effect of antioxidants on sperm parameters were not possible due to the heterogeneity and the low quality of evidence available.

Table 1 summarises the antioxidants commonly used to treat male subfertility and their potential side-effects.

Table 1. Potential side-effects of antioxidants used to treat male infertility

<table>
<thead>
<tr>
<th>Antioxidant</th>
<th>Side-effects</th>
</tr>
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<tbody>
<tr>
<td>Carnitine</td>
<td>Nausea, vomiting, abdominal pain, diarrhoea, body odour, seizures</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>Nausea, vomiting, heartburn, dyspepsia, abdominal discomfort, diarrhoea, headache, kidney stones at higher doses (due to increased oxaluria)</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>Nausea, diarrhoea, asthenia, headache, electrolyte disturbances, alopecia, blurred vision, bruising/bleeding, pruritus, rash</td>
</tr>
<tr>
<td>Selenium</td>
<td>Nausea, vomiting, malodourous breath, hair loss, weakness, hepatic and renal impairment</td>
</tr>
<tr>
<td>Folic acid</td>
<td>Rare, predominantly gastrointestinal disturbances, insomnia</td>
</tr>
<tr>
<td>N-Acetyl cysteine</td>
<td>Gastrointestinal disturbances, fatigue, skin rash, potential liver damage at higher doses</td>
</tr>
<tr>
<td>Zinc</td>
<td>Gastrointestinal disturbances, anorexia, headache, fatigue, blood disorders</td>
</tr>
<tr>
<td>Coenzyme Q10</td>
<td>Gastrointestinal disturbances, headache, insomnia, bleeding, skin rash, elevated liver enzymes at higher doses</td>
</tr>
</tbody>
</table>

CARNITINES
Carnitine is a water-soluble antioxidant, which may have a role in sperm metabolism. It is available in high concentration in red meat and dairy products. L-carnitine (LC) and L-acetyl carnitine (LAC) are the main available forms that are used in the treatment of male subfertility.

Lenzi et al.6 demonstrated in their double-blind placebo-controlled study that combination therapy with LC and LAC led to a significant improvement in sperm motility in patients with oligoasthenoteratospermia (OATS). They had four spontaneous pregnancies in 30 couples during the study period, all of which were in the treatment group (13%).6

Cavallini et al.7 showed that treatment with LC and LAC in patients with low-grade varicocele and idiopathic OATS resulted in a significant improvement in all semen characteristics (sperm concentration, motility, morphology) and was associated with a higher spontaneous pregnancy rate when compared to placebo (1.7% and 21.8% in the placebo and carnitine groups, respectively).7

Balercia et al.8 reported that patients with lower baseline values of motility have a higher probability of responding to treatment. However, no significant difference in pregnancy rate was noted between the treatment and placebo arms.8

De Rosa et al.,9 in an open prospective study, showed that carnitine therapy was associated with an increase in sperm motility and total sperm count but no change in morphology. They also noted a significant improvement in the functional seminal parameters in the form of membrane integrity, linearity of spermatic movement and the capacity for cervical mucus penetration.9

In contrast, Sigm an’s randomised double-blinded, placebo-controlled study did not find any clinically or statistically significant improvement in sperm motility in patients with asthenospermia treated with oral carnitine supplements (2g LC and 1g LAC per day) for 24 weeks.10

VITAMIN C (ASCORBIC ACID)
Vitamin C is an important physiological antioxidant, recorded at high concentrations in the seminal fluid.11 It is available at significant levels in fruit and vegetables; particularly citrus fruits, tomatoes/tomato juice and potatoes.

Although vitamin C supplements have been shown to improve the quality of sperm in men who are heavy smokers,12 there is a lack of studies comparing the effect of vitamin C (as a single agent) with placebo in the treatment of subfertility. Vitamin C has also been used in combination with other supplements. Omu et al.13 demonstrated that combination treatment with zinc, vitamin E and vitamin C led to significant improvement in sperm motility and a lower DNA fragmentation index.
Another study by Galatioto et al.\textsuperscript{14} showed improvement in sperm concentration only, with no change in motility or morphology. Tremellen et al.\textsuperscript{15} studied the effect of combination treatment with antioxidants, including vitamin C, using Menevit (lycopene 6mg, vitamin E 400UI, vitamin C 100mg, zinc 25mg, selenium 26 micrograms, folate 0.5mg, garlic 1g) in couples undergoing assisted fertilisation with in-vitro fertilisation–intracytoplasmic sperm injection (IVF–ICSI). They found a significant improvement in viable pregnancy rate from 16% in the placebo arm to 38.5% in the antioxidant arm.\textsuperscript{15}

Other studies also failed to show any significant change in sperm quality. A randomised study by Rolf\textsuperscript{16} compared the effect of combination treatment of vitamin C (1g) and vitamin E (800m g) with placebo and found no change in the semen parameters after 56 days of treatment. No pregnancies occurred during the study period. Similarly, Greco et al.\textsuperscript{17} showed that combination oral antioxidant therapy with vitamin C and vitamin E (1g each daily for 2 months) did not improve sperm motility, count or morphology when compared with placebo. However, they reported a significant reduction in the percentage of DNA-fragmented sperm with antioxidant treatment.\textsuperscript{17}

\section*{VITAMIN E (ALPHA-TOCOPHEROL)}

Vitamin E is a fat-soluble potent antioxidant, which inhibits the production of reactive oxygen species and may protect spermatozoa from oxidative damage. Numerous foods are rich in vitamin E, particularly nuts, vegetable oils, leafy vegetables and fortified cereals.

Suleim an et al.\textsuperscript{18} compared the effect of vitamin E as a sole treatment with placebo in infertile men. They showed that men with asthenospermia treated with a daily dose of 300mg vitamin E for 6 months had significant improvement in sperm motility and pregnancy rate, with 21% spontaneous pregnancy rate in the treatment group compared to 0% in the placebo-treated couples. Nine of the 11 successful pregnancies led to normal term deliveries. The study, however, was relatively small with an unacceptably high dropout rate.\textsuperscript{18}

When used in conjunction with other antioxidants, improvements in one or more semen parameters were reported.\textsuperscript{13,14,19} Pregnancy rates were higher (IVF–ICSI) in one study, as mentioned previously.\textsuperscript{15}

Comhaire et al.\textsuperscript{20} in an open prospective study of 27 infertile men, showed that combined treatment with vitamin E and vitamin A with essential fatty acids resulted in improvement in sperm concentration in oligozoospermic men and significantly reduced seminal reactive oxygen species. There was, however, no change in sperm motility or morphology.\textsuperscript{20} Other studies did not show any significant effect on semen parameters using vitamin E as a single treatment\textsuperscript{21,22} or in combination with other antioxidants.\textsuperscript{16}

\section*{SELENIUM}

Selenium is an essential trace element and has an important role in normal testicular development and spermato genesis. It may also protect sperm DNA against oxidative stress damage. It is naturally present in many foods, including seafood, nuts, cereals, meat and dairy products.

Selenium has been shown to improve semen quality in subfertile men in a few studies. Safarinejad et al.,\textsuperscript{21} in a double-blind placebo-controlled trial of 468 men with OATS, showed a modest but statistically significant improvement in all semen parameters in men receiving 200 micrograms of selenium alone or in combination with N-acetyl-cysteine (NAC) for 26 weeks.\textsuperscript{21} A smaller study reported improvement only in sperm motility with no change in sperm concentration. However, only 56% of men showed a positive improvement with treatment.\textsuperscript{24} Similarly, an increase in sperm motility was also reported if selenium was used in combination with vitamin E.\textsuperscript{19,25} However, there was no increase in overall pregnancy rates.\textsuperscript{25}

\section*{FOLIC ACID}

Folic acid is a water-soluble isomer of vitamin B that plays a crucial role in the synthesis of DNA and RNA and the metabolism of amino acids. It also has potential antioxidant properties as a scavenger of free radicals. It is found in a wide variety of foods, including leafy vegetables, fruits, nuts, dairy products, poultry and meat.

Landau et al.\textsuperscript{26} demonstrated that treatment with folic acid 30mg per day for 1 month in men with oligospermia did not improve either total sperm counts or sperm motility, in spite of an increase in recordable levels of folic acid in seminal fluid.\textsuperscript{26}

A randomised double-blind, placebo-controlled trial studied the effect of folic acid supplements as a sole treatment (5mg once daily) or in combination with zinc sulphate in 103 subfertile men. After 26 weeks of treatment, the only significant change was improvement in sperm concentration in the combined treatment arm. There was no significant effect on motility or morphology. No difference was noted between either treatments (folic acid or zinc) or placebo in terms of semen variables.\textsuperscript{27} Combination treatment resulted in significantly improved pregnancy rates following assisted conception techniques.\textsuperscript{15}

\section*{N-ACETYL CYSTEINE}

N-Acetyl cysteine is an amino acid that exhibits antioxidant properties and reduces the oxidative stress by scavenging free radicals. It is converted in the body to cysteine, which is a precursor to the biological antioxidant glutathione. NAC is not available in food but cysteine is present in most high-protein foods such as meat, eggs, dairy products and vegetables (broccoli, red peppers and onions).

Çiftci\textsuperscript{28} studied 120 infertile men and showed a significant improvement in sperm motility after 3 months of treatment with NAC (600mg daily). No change in morphology or concentration was noted.\textsuperscript{28} Other studies have demonstrated improvement in sperm
Oxidative stress plays an important role in male infertility. Excessive production of reactive oxygen species, in particular hydrogen peroxide, can induce lipid peroxidation and DNA fragmentation and lead to sperm apoptosis. Only a few studies report data on pregnancy rate and live births. Data on live births are available only for vitamin E and zinc. A recent Cochrane analysis concluded that antioxidants may improve pregnancy and live birth rates in the context of assisted reproductive techniques. However, the authors recommend that the couple should be informed that the current evidence remains inconclusive. There is a need for well-designed and adequately powered randomised controlled trials investigating the impact of antioxidants on male infertility.

**KEY POINTS**

- Oxidative stress plays an important role in male infertility.
- Excessive production of reactive oxygen species, in particular hydrogen peroxide, can induce lipid peroxidation and DNA fragmentation and lead to sperm apoptosis.
- Only a few studies report data on pregnancy rate and live births.
- Data on live births are available only for vitamin E and zinc.
- A recent Cochrane analysis concluded that antioxidants may improve pregnancy and live birth rates in the context of assisted reproductive techniques. However, the authors recommend that the couple should be informed that the current evidence remains inconclusive.
- There is a need for well-designed and adequately powered randomised controlled trials investigating the impact of antioxidants on male infertility.

A randomised controlled trial studied the effect of combination antioxidant therapy (NAC 600mg and vitamin–minerals) in 42 men with persistent oligospermia after varicocele embolisation. It reported improvement in sperm concentration; however, this was not associated with a significant increase in spontaneous pregnancies after 12 months. No change in sperm morphology or motility was noted.

Similar results were also reported by Comhaire et al in a small, open, prospective study of 27 infertile men, which concluded that the combination of NAC with essential fatty acids may lead to an increase in sperm concentration in men with oligozoospermia. However, no change in sperm motility or morphology could be demonstrated.

**ZINC**

Zinc is an essential trace mineral that has a role in the metabolism of DNA and RNA and has anti-apoptotic and antioxidant properties with a potential positive effect on spermatogenesis. It has also been shown that the seminal plasma concentration of zinc is significantly lower in infertile men compared to their fertile counterparts. A prospective study comparing a single treatment arm with no therapy showed that men with asthenozoospermia treated with zinc supplements (250mg twice daily) for 3 months had a statistically significant improvement in sperm motility and concentration with a better fertilising capacity. They also reported higher pregnancy and live birth rates in the treatment group.

In a more recent randomised study, Omu et al reported a modest improvement in all semen parameters and decreased DNA fragmentation after zinc therapy alone or in combination with vitamin E and vitamin C. However, the only statistically significant improvement was in sperm motility.

When combined with other antioxidants, Wong (zinc with folic acid) and Galatioti (zinc plus NAC, vitamins and minerals) reported a statistically significant improvement in sperm concentration but no effect on motility or morphology.

**COENZYME Q10**

Coenzyme Q10 (CoQ-10) is an essential antioxidant that inhibits lipid peroxidation of the sperm membrane. It is also highly concentrated in the mitochondria of the sperm mid-piece and has an important role in the energy production pathways. Dietary sources include fish, meat, whole grains and certain vegetables such as parsley and cabbage.

CoQ-10 supplements have been shown significantly to improve sperm motility and concentration compared to placebo. In a more recent prospective open-label series, a significant improvement in all semen parameters following treatment with 600mg/day of CoQ-10 for 12 months was reported. A potential beneficial effect on spontaneous pregnancy rate was also observed. A small case series of 17 patients showed an improvement in fertilisation rate after ICSI following treatment with 60mg/day of oral CoQ-10. However, this study did not report any significant change in semen parameters.

**CONCLUSIONS**

The observational and randomised controlled trial data available in this area remain conflicting. Antioxidant supplements, including vitamins E and C plus mineral elements, show null, or even some harmful, effects of supplementation on a range of outcomes. This highlights the often underestimated problems of confounding and reverse causality that can lead to premature causal inferences in such studies. Specific factors may include: circulating androgen concentrations, concomitant health and lifestyle factors (eg smoking, drinking, drug use, obesity, varicocele and increasing age), semen testing variables (eg reproducibility, inter- and intra-individual bias) and environmental factors (eg biohazard exposure, diet and social status).

Although we report on predominantly level 1b and 2a evidence here, the majority of studies are limited by their short duration, small patient numbers and variable endpoints. Unfortunately, only a minority of studies investigated pregnancy rates as a focused clinical endpoint. Fundamentally, the mantra that these compounds may provoke benefit while harbouring low clinical risk should be moderated; as all medicines have potential side-effects and can be harmful in higher doses.

We will continue to learn from the experiences of studying these compounds in...
other conditions (e.g., inflammation, cancer and ageing) with regards to improving study design and execution; while identifying biologically active molecules, based on the principal chemistry underpinning antioxidant mechanisms in animal and human systems. In the future, this may lead to novel targeted inhibitors and disease-modulating drugs that can be evaluated through large, pharmaceutical clinical trials.

Declaration of interests: none declared.

REFERENCES