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Showell MG, Mackenzie-Proctor R, Brown J, Yazdani A, Stankiewicz MT, Hart RJ

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[Intervention Review]

Antioxidants for male subfertility

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ABSTRACT

Background

Between 30% to 80% of male subfertility cases are considered to be due to the damaging effects of oxidative stress on sperm and 1 man in 20 will be affected by subfertility. Antioxidants are widely available and inexpensive when compared to other fertility treatments and many men are already using these to improve their fertility. It is thought that oral supplementation with antioxidants may improve sperm quality by reducing oxidative stress. Pentoxifylline, a drug that acts like an antioxidant, was also included in this review.

Objectives

This Cochrane review aimed to evaluate the effectiveness and safety of oral supplementation with antioxidants for subfertile male partners in couples seeking fertility assistance.

Search methods

We searched the Cochrane Menstrual Disorders and Subfertility Group Specialised Register, CENTRAL, MEDLINE, EMBASE, CINAHL, PsycINFO and AMED databases (from inception until January 2014); trial registers; sources of unpublished literature and reference lists. An updated search was run in August 2014 when potentially eligible studies were placed in 'Studies awaiting assessment'.

Selection criteria

We included randomised controlled trials (RCTs) comparing any type or dose of antioxidant supplement (single or combined) taken by the subfertile male partner of a couple seeking fertility assistance with a placebo, no treatment or another antioxidant.

Data collection and analysis

Two review authors independently selected eligible studies, extracted the data and assessed the risk of bias of the included studies. The primary review outcome was live birth; secondary outcomes included clinical pregnancy rates, adverse events, sperm DNA fragmentation, sperm motility and concentration. Data were combined, where appropriate, to calculate pooled odds ratios (ORs) or mean differences (MD) and 95% confidence intervals (CIs). Statistical heterogeneity was assessed using the I^2 statistic. We assessed the overall quality of the evidence for the main outcomes using GRADE methods.

Main results

This updated review included 48 RCTs that compared single and combined antioxidants with placebo, no treatment or another antioxidant in a population of 4179 subfertile men. The duration of the trials ranged from 3 to 26 weeks with follow up ranging from 3 weeks to 2 years. The men were aged from 20 to 52 years. Most of the men enrolled in these trials had low total sperm motility and sperm concentration. One study enrolled men after varicocelectomy, one enrolled men with a varicocoele, and one recruited men with chronic prostatitis. Three trials enrolled men who, as a couple, were undergoing in vitro fertilisation (IVF) or intracytoplasmic sperm injection (ICSI) and one trial enrolled men who were part of a couple undergoing intrauterine insemination (IUI). Funding sources were stated by 15 trials. Four of these trials stated that funding was from a commercial source and the remaining 11 obtained funding through non-commercial avenues or university grants. Thirty-three trials did not report any funding sources.

A limitation of this review was that in a sense we had included two different groups of trials, those that reported on the use of antioxidants and the effect on live birth and clinical pregnancy, and a second group that reported on sperm parameters as their primary outcome and had no intention of reporting the primary outcomes of this review. We included 25 trials reporting on sperm parameters and only three of these reported on live birth or clinical pregnancy. Other limitations included poor reporting of study methods, imprecision, the small number of trials providing usable data, the small sample size of many of the included studies and the lack of adverse events reporting. The evidence was graded as 'very low' to 'low'. The data were current to 31 January 2014.

Live birth: antioxidants may have increased live birth rates (OR 4.21, 95% CI 2.08 to 8.51, P< 0.0001, 4 RCTs, 277 men, I^2 = 0%, low quality evidence). This suggests that if the chance of a live birth following placebo or no treatment is assumed to be 5%, the chance following the use of antioxidants is estimated to be between 10% and 31%. However, this result was based on only 44 live births from a total of 277 couples in four small studies.

Clinical pregnancy rate: antioxidants may have increased clinical pregnancy rates (OR 3.43, 95% CI 1.92 to 6.11, P < 0.0001, 7 RCTs, 522 men, I^2 = 0%, low quality evidence). This suggests that if the chance of clinical pregnancy following placebo or no treatment is assumed to be 6%, the chance following the use of antioxidants is estimated at between 11% and 28%. However, there were only seven small studies in this analysis and the quality of the evidence was rated as low.

Miscarriage: only three trials reported on this outcome and the event rate was very low. There was insufficient evidence to show whether there was a difference in miscarriage rates between the antioxidant and placebo or no treatment groups (OR 1.74, 95% CI 0.40 to 7.60, P = 0.46, 3 RCTs, 247 men, I^2 = 0%, very low quality evidence). The findings suggest that in a population of subfertile men with an expected miscarriage rate of 2%, use of an antioxidant would result in the risk of a miscarriage lying between 1% and 13%.

Gastrointestinal upsets: there was insufficient evidence to show whether there was a difference in gastrointestinal upsets when antioxidants were compared to placebo or no treatment as the event rate was very low (OR 1.60, 95% CI 0.47 to 5.50, P = 0.46, 6 RCTs, 429 men, I^2 = 0%).

We were unable to draw any conclusions from the antioxidant versus antioxidant comparison as not enough trials compared the same interventions.

Authors' conclusions

There is low quality evidence from only four small randomised controlled trials suggesting that antioxidant supplementation in subfertile males may improve live birth rates for couples attending fertility clinics. Low quality evidence suggests that clinical pregnancy rates may increase. There is no evidence of increased risk of miscarriage but this is uncertain as the evidence is of very low quality. Data were lacking on other adverse effects. Further large well-designed randomised placebo-controlled trials are needed to clarify these results.

PLAIN LANGUAGE SUMMARY

Antioxidant vitamins and minerals for male subfertility

Review question: do supplementary oral antioxidants improve fertility outcomes for subfertile men when compared with placebo, no treatment or another antioxidant?

Background: many subfertile men who are part of a couple undergoing fertility treatment are also taking dietary supplements in the hope of improving their fertility. It is important that these men have access to high quality evidence that informs them on the benefits and risks of taking an antioxidant. This review aimed to assess whether oral antioxidants would increase the chances of a couple with a

subfertile male partner achieving a clinical pregnancy and ultimately a live birth. This review did not examine the use of antioxidants in men with normal sperm.

Study characteristics: the Cochrane review authors included in this updated review 48 randomised controlled trials that compared single and combined antioxidants with placebo, no treatment or another antioxidant in a population of 4179 subfertile men. The duration of the trials ranged from 3 to 26 weeks with follow up ranging from 3 weeks to 2 years. The men were aged from 20 to 52 years. Most of the men enrolled in these trials had low total sperm motility and sperm concentration. One study enrolled men after varicocelectomy (surgical removal of an engorged vein in the scrotum), one enrolled men with a varicocoele (an engorged vein in the scrotum) and one recruited men with chronic prostatitis (infection of the prostate gland). Three trials enrolled men who, as a couple, were undergoing in vitro fertilisation (IVF) or intracytoplasmic sperm injection (ICSI) and one trial enrolled men who were part of a couple undergoing intrauterine insemination (IUI). The data were current to 31 January 2014.

Funding sources were stated by 15 trials. Four of these trials stated that funding was from a commercial source and the remaining 11 obtained funding through non-commercial sources or university grants. Thirty-three trials did not report any funding sources.

Key results: antioxidants may have been effective in treating subfertile men but the reporting of studies was too inconsistent to be confident in these findings. The live birth results suggest that we would expect a live birth of a baby for 5 out of 100 subfertile men who did not take any antioxidants, compared to between 10 and 31 out of 100 men who were taking antioxidants. The results for the clinical pregnancy rate showed an expected clinical pregnancy for 6 out of 100 subfertile men who did not take any antioxidants, compared to between 11 and 28 out of 100 men who were taking antioxidants. Adverse events were poorly reported and we could not make conclusions on any harmful effects. More high quality, larger placebo-controlled trials reporting on these outcomes and adverse events are needed to draw definite conclusions.

Quality of the evidence: the quality of the evidence for live birth and clinical pregnancy was deemed 'low' while adverse events was assessed as 'very low'. These 'low' and 'very low' assessments were due to the lack of a clear description of trial methods and inconsistent, inadequate reporting of live births and clinical pregnancies. Not enough trials compared the same interventions to make any conclusions about whether one intervention worked better than the other.

SUMMARY OF FINDINGS FOR THE MAIN COMPARISON [Explanation]

Antioxidants versus placebo or no treatment for male subfertility

Patient or population: patients with male subfertility
Settings:
Intervention: Antioxidants versus placebo or no treatment

Outcomes	Illustrative comparative risks* (95% CI)		Relative effect (95% CI)	No of Participants (studies)	Quality of the evidence (GRADE)	Comments
	Assumed risk	Corresponding risk				
	Control	Antioxidants versus placebo or no treatment				
Live Birth per couple randomised Follow-up: 3 - 24 months	**50 per 1000**	**181 per 1000** (99 to 309)	**OR 4.21** (2.08 to 8.51)	277 (4 studies)	⊕⊕◯◯ **low**[1,2]	
Clinical Pregnancy rate per couple randomised Follow-up: 3-24 months	**59 per 1000**	**177 per 1000** (108 to 277)	**OR 3.43** (1.92 to 6.11)	522 (7 studies)	⊕⊕◯◯ **low**[1,3]	
Adverse event: Miscarriage rate per couple randomised Follow-up: 3-18 months	**19 per 1000**	**33 per 1000** (8 to 129)	**OR 1.74** (0.40 to 7.60)	247 (3 studies)	⊕◯◯◯ **very low**[1,4]	

*The basis for the **assumed risk** (e.g. the median control group risk across studies) is provided in footnotes. The **corresponding risk** (and its 95% confidence interval) is based on the assumed risk in the comparison group and the **relative effect** of the intervention (and its 95% CI).
CI: Confidence interval; **OR:** Odds ratio;

GRADE Working Group grades of evidence
High quality: Further research is very unlikely to change our confidence in the estimate of effect.
Moderate quality: Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low quality: Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very low quality: We are very uncertain about the estimate.

[1] Inadequate explanations of methodology and large unexplained dropouts in one study
[2] Confidence limits of one study crosses the line of no effect
[3] Wide confidence intervals. Six of the nine analyses (one trial has 3 arms) cross the line of no effect.
[4] Low event rate

BACKGROUND

Description of the condition

It is believed that about 80 million people worldwide are affected by the inability to have children (Tournaye 2006), and delayed conception affects 15% of couples trying to conceive (Attia 2007). Male factor subfertility accounts for up to 50% of these cases, and it is thought that one man in 20 will be affected by subfertility (Tremellen 2008).

Some 30% to 80% of male factor subfertility cases are believed to be due to the damaging effects of oxidative stress (Tremellen 2008). Oxidative stress occurs when reactive oxygen species (ROS) overcome the semen's natural antioxidant defences and cause cellular damage to the sperm (Tremellen 2008). This was shown in a study by Aktan 2013, where oxidative parameters in the semen of idiopathic infertile men were found to be significantly higher than in fertile men, and a high correlation was seen between oxidative parameters, sperm ROS formation and DNA fragmentation levels. Some studies have suggested that sperm production and quality has decreased over time, although not all researchers agree (Stankiewicz 2003). The increased levels of ROS are thought to be potentially due to environmental factors such as high temperatures, electromagnetic radiation, pesticides and pollution; and lifestyle factors of advanced age, alcohol consumption, smoking, stress, obesity and poor diet. Other factors include infections, autoimmunity and chronic disease (Aitken 2007; Alvarez 2003; Tremellen 2008).

Seminal plasma is rich in antioxidants that support, protect and nourish the sperm. The sperm have low levels of antioxidants and DNA repair enzymes and are, therefore, very dependent on the surrounding seminal plasma for these factors (Aktan 2013). Spermatozoal membranes while being rich in polyunsaturated fatty acids are susceptible to oxygen damage from lipid peroxidation (Sheweita 2005), and abnormal spermatozoa together with contaminating leukocytes generate ROS (Sikka 1995; Walczak-Jedrzejowska 2013). An vivo study (Shiva 2011) has made the correlation between high levels of ROS and low levels of antioxidants in the seminal plasma of healthy young males. The study found a linear correlation between dietary intake of both carotenoids and lutein and sperm motility, and intake of lycopene with sperm morphology, however they did not identify any linear association between intake of antioxidant vitamins and semen quality.

Antioxidants that are naturally found in semen include vitamins E and C, folate, zinc, selenium, carnitine and carotenoids. These antioxidants act as free radical scavengers that help to overcome ROS (Talevi 2013). Healthy young men with a higher carotenoid intake have higher sperm motility, and higher lycopene intake is associated with better sperm morphology (Zareba 2013). The levels of vitamin D, as metabolised in male reproductive organs, are associated with better quality sperm parameter markers (Jensen 2014). Subfertile men have been identified as having lower levels of antioxidants in their semen compared to fertile men (Tremellen 2008). Studies have shown that ROS levels are significantly higher in infertile sperm samples when compared with healthy controls, and that the infertile men who provided these samples may benefit from an antioxidant supplement (Aktan 2013; Bykova 2007).

A study by Aktan showed that idiopathic infertile men had significantly higher levels of ROS and DNA fragmentation with no statistically significant difference in baseline seminal analysis characteristics (Aktan 2013). Men who are oligozoospermic and azoospermic have higher levels of lipid peroxidation as measured via the malondialdehyde assay (MDA) (Shiva 2011).

Varicocele, or a vascular lesion in the spermatic cord, is a risk factor for male infertility. It is thought that the varicocoele causes increased scrotal temperature, reflux of blood flow and a damaged microcirculation, all of which act to increase both germ cell death and levels of ROS. This ultimately decreases semen quality and sperm function (Oliva 2009; Zini 2011).

ROS are thought to cause fertility problems in two ways, firstly by damaging the sperm membrane thus affecting the sperm motility and the ability of the spermatozoa to break down the oocyte membrane; and secondly by altering the sperm DNA (Shiva 2011). Spermatozoal DNA integrity is one of the major determinants of normal fertilisation and embryo growth in natural and assisted conception (Agarwal 2003; Aitken 2004; Tarozzi 2007). Indeed, many men with normal seminal parameters may have a high degree of sperm DNA damage and this correlates with a poor chance of natural conception (Aktan 2013; Boe-Hansen 2006).

Sperm DNA damage or integrity can be assessed in a number of ways. These include sperm chromatin structural assay by flow cytometry (SCSA); enzymatic labelling of broken DNA strands, the terminal deoxynucleotide transferase-mediated nick end-labelling assay (TUNEL); and microscopic observations of DNA fragments, the Comet assay. The greatest experience with and standardisation of the methodology exists for the SCSA as it has been used for over 25 years, and was initially developed for animal husbandry purposes (Aitken 2007; Evenson 2007). Indeed, there are advocates who state that this should be part of a standard assessment of the male partner when a couple presents with subfertility (Boe-Hansen 2006); although it is recognised that the technique has its limitations and hence strict laboratory control and standardisation is required (Boe-Hansen 2005).

Sperm DNA fragmentation does not appear to influence fertilisation in in vitro fertilisation (IVF), although a negative correlation of sperm DNA damage with embryo or blastocyst development has been described (Evenson 2006; Li 2006; Tarozzi 2007). In addition, Talevi 2013 showed that antioxidant supplementation improved seminal parameters and decreased DNA fragmentation in vitro. Women undergoing intrauterine insemination with a sperm DNA fragmentation index < 30%, as measured by the SCSA, were seven times more likely to achieve a pregnancy than those couples where the male partner had a higher degree of sperm DNA

damage (Evenson 2006). The evidence for the effect of a high degree of sperm DNA damage upon pregnancy outcome is less clear. A meta-analysis of sperm DNA fragmentation, assessed by the SCSA, determined that if the sperm DNA fragmentation was < 30% the couple were twice as likely to conceive in an IVF cycle than if it was greater than 30%, though the evidence for a benefit in women undergoing intracytoplasmic sperm injection (ICSI) was unclear (Evenson 2006). However, a meta-analysis of SCSA papers published in the same year demonstrated conflicting results (Li 2006). This meta-analysis found that there was no effect of sperm DNA damage, assessed by the SCSA, upon the outcome of IVF or ICSI (Li 2006). The meta-analysis also reviewed the effect of sperm DNA damage as assessed by the TUNEL assay. This demonstrated a reduced pregnancy rate in women undergoing IVF when the male partner had a high degree of sperm DNA damage, but no difference if they were undergoing ICSI (Li 2006). It also appears that miscarriage is more likely in women undergoing assisted reproduction when the sperm DNA damage is high (Borini 2006; Robinson 2012).

Description of the intervention

Antioxidants are both biological (enzymes) and chemical substances that reduce oxidative damage. These chemical antioxidants are both natural and synthetic and can be derived from nutritional sources and from supplementation (Sikka 1995).

The predominant supplementary antioxidants that are studied in male subfertility clinical trials are vitamin E, vitamin C, carotenoids, ubiquinol and the micronutrients folate and zinc (Eskenazi 2005). A paper by Tremellen (Tremellen 2008) discussed the use of a combination of vitamins and minerals to improve pregnancy rates for subfertile men.

Polyunsaturated fatty acids (PUFAs) are sources of antioxidants and in the community they are commonly taken as nutritional supplements. PUFAs have varying effects in male fertility. They provide antioxidants and also increase the plasma fluidity of the sperm membrane, which acts to assist with conception; however, this fluidity makes the sperm susceptible to ROS and lipid peroxidation that can damage the sperm (Wathes 2007). Wathes states that "It appears that PUFAs are a two edged sword - some are essential, but too many are potentially harmful" (Wathes 2007, page198). An open study by Comhaire attempted to overcome the double-edged sword of essential fatty acid supplements by also treating the subfertile men in their study with antioxidant supplements of acetyl cysteine or beta-carotene and alpha-tocopherol (Comhaire 2000).

PUFAs are classified into omega-3, omega-6 and omega-9 fatty acids. Omega-9 fatty acids are synthesised by animals but omega-3 and omega-6 fatty acids need to be supplemented in the diet. The main sources of omega-6 fatty acids are vegetable oils. Sources of omega-3 fatty acids are vegetable and fish oils (Wathes 2007).

Pentoxifylline is a methylxanthine phosphodiesterase inhibitor that reduces the concentration of superoxide anions and inhibits tumour necrosis factor-alpha (TNF-alpha), responsible for DNA fragmentation and cell death (Maxwell 2002). Pentoxifylline is included in this review as it acts like an antioxidant by reducing the effect of oxidative metabolism and helps maintain antioxidant enzyme activities (Oliva 2009).

Antioxidants are widely available and inexpensive when compared to other fertility treatments, however cost benefit analysis was beyond the scope of this review.

How the intervention might work

Antioxidants are known to scavenge and dispose of ROS, suppress their formation, and also act to oppose the actions of ROS. The dietary intake of antioxidants has been shown to be strongly associated with semen quality and men with higher intake of antioxidants, both dietary and supplementary, may have less DNA damage in their sperm (Schmid 2012). Some non-controlled studies of antioxidant supplementation have shown an associated increase in fertilisation rates, possibly by reducing oxidative stress, lipid peroxidation potential and ROS levels (Eskenazi 2005; Schmid 2012; Zareba 2013).

Why it is important to do this review

Currently there is limited evidence that antioxidant supplementation improves outcomes for subfertile couples. Although some clinical trials of supplemental antioxidants have suggested benefits in treating male subfertility there are other trials that fail to demonstrate the same benefit (Agarwal 2004). A recent meta-analysis of the effects of the antioxidant Coenzyme Q10 on pregnancy rates concluded that there was no improvement with supplemented antioxidants (Lafuente 2013). However, the consensus on the treatment of unexplained male subfertility with antioxidants is that it is potentially beneficial but states a need for further evaluation (Tournaye 2006; Walczak-Jedrzejowska 2013). The purpose of this Cochrane review was to assess the effects of antioxidants on men with documented sperm DNA damage and men with impaired semen parameters from appropriate clinical trials that use the clinically relevant parameters of live birth, clinical pregnancy and adverse events. The review also assessed the effectiveness of different antioxidants and dosages on these outcomes.

OBJECTIVES

This Cochrane review aimed to evaluate the effectiveness and safety of oral supplementation with antioxidants for subfertile male partners in couples seeking fertility assistance.

Search methods

METHODS

Criteria for considering studies for this review

Types of studies

Inclusion criteria

- Only randomised controlled trials (RCTs) were eligible for inclusion. The participants were randomised to antioxidant versus placebo, no treatment or an alternative antioxidant
- Only pre-crossover data were used from randomised crossover trials, as achieving outcomes such as pregnancy and live birth precluded couples entering the next trial phase (Dias 2006)

Exclusion criteria

- Any quasi-randomised trials

Types of participants

Inclusion criteria

- Trials that included men who were part of a couple with male factor subfertility or unexplained subfertility and who were attending a fertility clinic

In situations where individuals were randomised again following failed cycles the data would not be pooled in a meta-analysis unless individual data could be excluded.

Exclusion criteria

- Trials that included men taking any other fertility enhancing drugs
- Trials that included men who had had chemotherapy treatment

Types of interventions

Trials were investigated if they included the following:

- any type of oral chemical or biological supplementary antioxidant (individual or combined) versus placebo or no treatment;
- any type or dose of oral chemical or biological supplementary antioxidant (individual or combined) versus another type or dose of antioxidant (head to head);
- pentoxifylline versus placebo or no treatment;
- pentoxifylline versus another type or dose of antioxidant (head to head).

Interventions were considered 'combined antioxidants' if they included three or more antioxidants in the intervention arm.
Trials that included antioxidants plus a plant extract (for example garlic) were included if the antioxidant agent was the main focus of the investigation. Trials that included only plant extracts were excluded.

Types of outcome measures

Primary outcomes

- Live birth rate per couple randomised (preferred definition: delivery of a live fetus at > 20 weeks)

Secondary outcomes

- Clinical pregnancy rate per couple (defined as the presence of a gestational sac, confirmed by ultrasound)
- Adverse events as reported by the trial
- Level of sperm DNA fragmentation
- Sperm motility
- Sperm concentration

Sperm parameter outcomes were analysed at the time points of three, six and nine months post-randomisation. All trials were analysed in this way regardless of whether the participants were treated for three, six or nine months. In other words, those trials treating for three months were analysed as reported by the trial at the three month point alongside those trials treating for six months that also reported the three month outcome, and this remains true for the trials reporting at six and nine months.

Search methods for identification of studies

See the Cochrane Menstrual Disorders and Subfertility Group module for the methods used in reviews (www.mrw.interscience.wiley.com/cochrane/clabout/articles/MENSTR/frame.html).
All reports that described RCTs of oral antioxidant supplementation for subfertile men were found using the following strategies.

Electronic searches

(1) The Menstrual Disorders and Subfertility Group (MDSG) Specialised Register of controlled trials was searched by the Group's Trial Search Co-ordinator (as lead author) (from inception to 25 August 2014) (Appendix 1).
This register also contains unpublished trial abstracts. These were found by handsearching 20 relevant journals and conference proceedings.
(2) The following databases were searched (from inception to 25 August 2014) using the Ovid and EBSCO platforms:

- Ovid Cochrane Central Register of Controlled Trials (CENTRAL) (Appendix 2);
- Ovid MEDLINE (Appendix 3);
- Ovid EMBASE (Appendix 4);
- EBSCO CINAHL (Cumulative Index to Nursing and Allied Health Literature) (Appendix 5);
- Ovid PsycINFO (Appendix 6);
- Ovid AMED (Allied and Complementary Medicine) (Appendix 7).

Both indexed and free text terms were used.
The MEDLINE search was combined with the Cochrane highly sensitive search strategy for identifying randomised trials, which appears in the *Cochrane Handbook for Systematic Reviews of Interventions*, Version 5.0.1 chapter 6, 6.4.11 (Higgins 2011).
The EMBASE (Ovid) and CINAHL (Ovid and EBSCO platforms) searches were combined with trial filters developed by the Scottish Intercollegiate Guidelines Network (SIGN) (www.sign.ac.uk/ mehodology/filters.html#random).
Searches were not date limited or limited to any one language.

Searching other resources

Appropriate journals were handsearched for trial conference abstracts. These journals included *Human Reproduction*, which contains abstract supplements for the European Society of Human Reproduction and Embryology (ESHRE), and *Fertility and Sterility* that contains abstract supplements for the 'American Society for Reproductive Medicine' (ASRM). Lists of journals handsearched by the Menstrual Disorders and Subfertility Group (MDSG) are found in the MDSG module.
Research trial registers were searched for ongoing and recently completed trials:

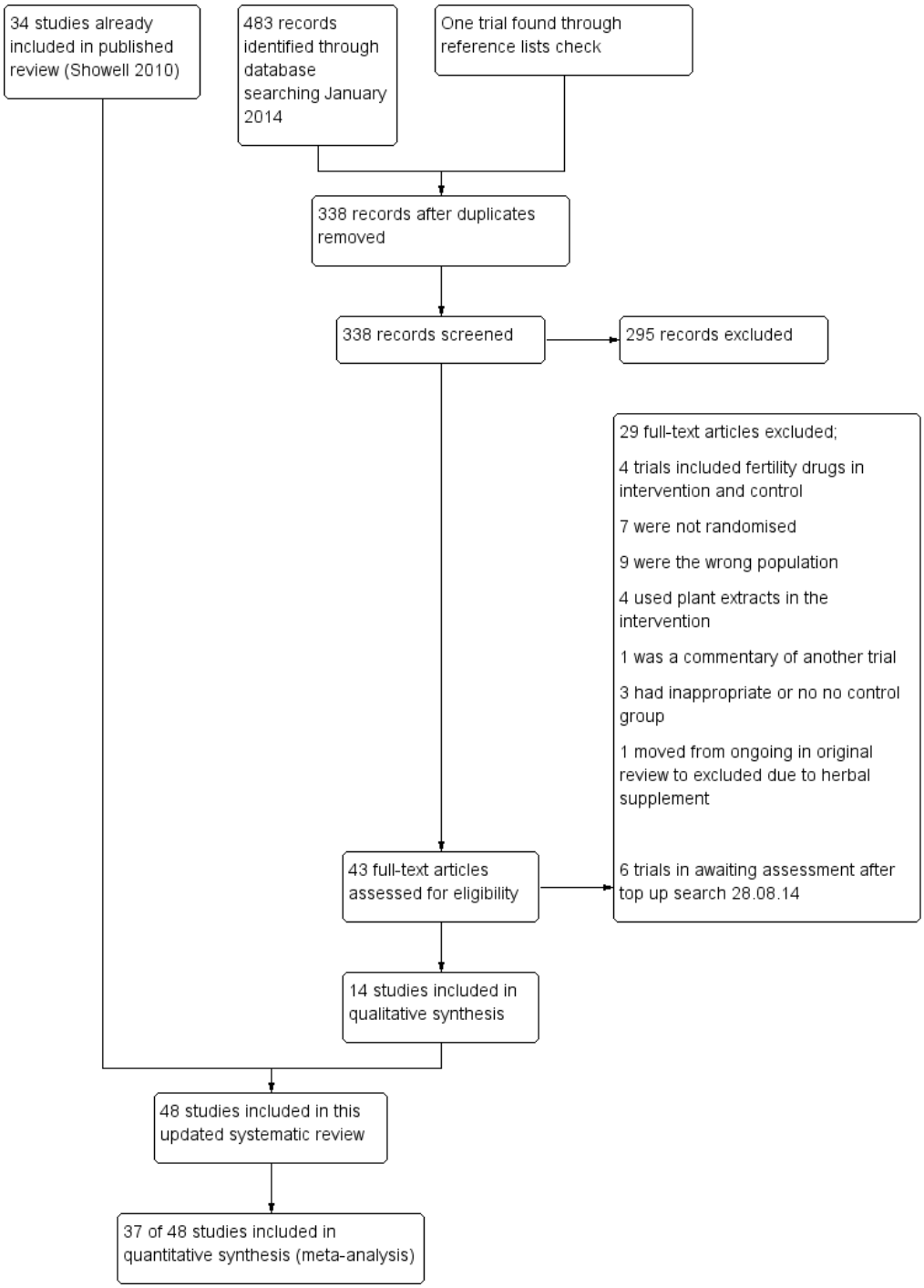
- the World Health Organization International Clinical Trials Registry Platform search portal (www.who.int/ trialsearch/ Default.aspx) (Appendix 8) (last searched 26 March 2014);
- ClinicalTrials.gov, a service of the US National Institutes of Health (http://clinicaltrials.gov/ct2/home) (Appendix 9) (last searched 26 March 2014).

The OpenGrey database was searched for European grey literature: http://www.opengrey.eu/ (Appendix 10) (last searched 26 March 2014).
ProQuest Dissertations and Theses (http://search.proquest.com.ezproxy.auckland.ac.nz/ pqdtft/advanced?accountid=8424) was also searched (Appendix 11) (last searched 26 March 2014).
Conference abstracts and other full text trials were found on the Web of Science http:// apps.webofknowledge.com.ezproxy.auckland.ac.nz/Search.do? product=UA&SID=Z2ojv8eYDcaIsDBypVq&search_mode= Refine&prID=b49cbec7-992d-4595-902e-f7228dc06050 (Appendix 12) (last searched 26 March 2014).
Reference lists from review articles and other relevant publications were handsearched.
Personal communication was undertaken with specialists in the field.

Data collection and analysis

We conducted data collection and analysis in accordance with the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011). The flow of information through the different phases of this systematic review can be seen in Figure 1.

Figure 1. Study flow diagram.



Selection of studies

Trials for inclusion in the review were selected, at different times, by two review authors (MS, RH, JB or RMP) after the search strategy was run. Titles and abstracts from the searches were scanned. We obtained the full texts of those articles that appeared to be eligible for inclusion. On assessment they were then placed in included (Characteristics of included studies), excluded (Characteristics of excluded studies) or ongoing (Characteristics of ongoing studies) studies or studies awaiting assessment (Characteristics of studies awaiting classification). Two of these individuals are content experts. Any disagreements were resolved through consensus or by another review author.

Studies were appraised in an unblinded fashion, as recommended by the Cochrane Menstrual Disorders and Subfertilty Group. Further information, where required, was sought from the authors.

Data extraction and management

The studies that appeared to meet the inclusion criteria were independently assessed by the three review authors (MS, RMP and JB) using data extraction forms. Any discrepancies were resolved with discussion.

The data extraction forms included methodological quality and allocation information. This information was included in the review and presented in the characteristics of included and excluded studies tables (see Characteristics of included studies; Characteristics of excluded studies) following the guidance of the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011).

Assessment of risk of bias in included studies

The included studies were assessed for risk of bias using the Cochrane risk of bias assessment tool to assess: sequence generation; allocation concealment; blinding of participants, providers and outcome assessors; completeness of outcome data; selective outcome reporting; and other potential sources of bias. MS, RMP and JB assessed these six criteria; any disagreements were resolved by consensus or by discussion with the third author. The conclusions were presented in the 'Risk of bias' table (Characteristics of included studies) and incorporated into the interpretation of review findings by means of sensitivity analyses. Where identified studies failed to report the primary outcome of live birth, but did report interim outcomes such as pregnancy, informal assessment was undertaken on whether those studies reporting the primary outcomes had typical values for the interim outcomes.

Those studies with a high risk of bias, whereby methods of randomisation and allocation concealment were not adequately explained, underwent a sensitivity analysis to assess the effect that these studies may have had on the results of the meta-analysis.

Measures of treatment effect

The dichotomous data for live birth, pregnancy rate, miscarriage and adverse events were expressed as odds ratios (OR) with 95% confidence intervals (95% CI) and combined in a meta-analysis with Review Manager 5 (RevMan 5) software using the Peto method and a fixed-effect model (Higgins 2011). A random-effects model was be used if there was high heterogeneity. The OR has mathematically sound properties that are consistent with benefit or harm and work well in small samples with rare events. This effect measure is appropriate when considering subfertility. For continuous data (for example sperm quality measurements) mean differences (MD) between treatment groups were calculated with associated standard deviations (SDs) and 95% CIs. The results were displayed on forest plots, where possible.

Attempts were made to contact all authors of the included trials that reported data in a form that was not suitable for meta-analysis, for example data reported as medians or ranges. Where additional data were not forthcoming, the available data were included in 'other data' under the particular outcome reported in the analyses.

Unit of analysis issues

The outcomes of live birth and pregnancy were analysed as per couple randomised. Multiple live births (for example twins or triplets) were counted as one live birth event. The sperm outcome analyses were per man randomised. We included both parallel group and crossover trials, using only the first phase of the crossover trial.

Dealing with missing data

We attempted to contact the authors of the trials with any missing data by e-mail or post. If there was no reply, and if possible, we reported the data in terms of intention to treat. If this was not possible the trials were included in the narrative of the review but not in the meta-analysis.

Assessment of heterogeneity

The authors considered whether the clinical and methodological characteristics of the included studies were sufficiently similar to provide a meaningful summary in a meta-analysis.

Heterogeneity between the treatment effects of different studies were studied by looking at the points on the forest plot, the overlap of confidence intervals (a poor overlap indicates heterogeneity) and the Chi^2 statistical test for heterogeneity. A low P value (or a large Chi^2 statistic relative to its degree of freedom) shows evidence of heterogeneity of the treatment effect, or that the differences were not likely to be by chance (Higgins 2011). To more

formally quantify the variations between the studies the I^2 statistic was used (Higgins 2011). This statistic describes the variation in effect estimates due to heterogeneity rather than by chance, as a percentage. If a value over 50% was found we assumed that there was large heterogeneity and a random-effects model was used in a sensitivity analysis in order to assess possible reasons for the high heterogeneity between the studies.

Assessment of reporting biases

A comprehensive search, covering multiple sources, for eligible studies was carried out. There were no language or publication restrictions on these searches. We were also alert to the possibility of duplication of data. We planned to perform a funnel plot in order to explore the possibility of small study effects (a tendency for estimates of the intervention effect to be more beneficial in smaller studies). Care was taken to search for within study reporting bias, such as trials failing to report obvious outcomes or reporting them in insufficient detail to allow analysis.

Data synthesis

Statistical analysis of the data was carried out using RevMan 5.3. Pregnancy outcomes were considered positive and higher numbers of pregnancy rates were considered a benefit; while miscarriage, adverse events and DNA fragmentation outcomes were negative effects and higher numbers would be considered harmful. An increase in the odds of a particular outcome, which may be beneficial (for example live birth) or detrimental (for example adverse effects), were displayed graphically in the meta-analyses to the right of the centre line, and a decrease in the odds of an outcome to the left of the centre line.These aspects have to be considered when assessing the summary graphs (Attia 2007).

The data from primary studies were combined using a fixed-effect model in the following planned comparisons.

1. Antioxidants versus placebo or no treatment:
1.1 stratified by type of antioxidant,
1.2 stratified by IVF or ICSI. This comparison was only appropriate for the outcomes of live birth and clinical pregnancy.
2. Antioxidants versus antioxidants (head to head):
2.1 stratified by type of antioxidant,
2.2 stratified by IVF or ICSI.
3. Pentoxifylline versus placebo or no treatment:
3.1 stratified by placebo or no treatment,
3.2 stratified by IVF or ICSI.

Adverse events as reported in the trials were included in the three comparisons above.

Further analyses of sperm motility and concentration were carried out by sub-grouping trials over time: at three, six and nine months. The aim here was to define analyses that were comprehensive and mutually exclusive so that all eligible study results could be be slotted into one stratum only. Comparisons were specified so that any trials falling within each stratum could sensibly be pooled for meta-analysis. Stratification allowed for consideration of effects within each stratum as well as, or instead of, an overall estimate for the comparison.

Subgroup analysis and investigation of heterogeneity

Subgroup analysis was performed on the following.

- Subgroup analyses in the outcomes of live birth and clinical pregnancy were performed on those trials that included couples who were also having IVF or ICSI.
- Subgroup analysis was performed on those studies that reported both live birth and clinical pregnancy rate in order to assess any overestimation of effect and reporting bias.
- Subgroup analysis was also used in the sperm outcomes of motility and concentration over time, at three, six and nine months.

Sensitivity analysis

We performed sensitivity analyses (using the random-effects model in RevMan software if heterogeneity was high (that is the I^2 was over 50%) for the outcomes to assess whether the findings from the analysis were robust.

- Sensitivity analysis was performed excluding those studies considered to be at a high risk of bias i.e. those studies that did not explain their methods of randomisation and allocation concealment. Here we considered whether the conclusions would be any different if eligibility was restricted to studies without high risk of bias.

We planned to perform sensitivity analysis on unpublished studies as these studies may not have been peer reviewed and thus could be of lower quality.

- Sensitivity analysis was performed on those trials enrolling men undergoing IUI.

- Sensitivity analysis was performed on those trials not using a placebo as a control in order to assess whether their exclusion would have altered the conclusions.
- A post hoc sensitivity analysis was conducted to examine the effect of excluding from analysis those studies which reported remarkably low SDs as the review authors considered that these data were potentially erroneous.

Overall quality of the body of evidence: summary of findings table

We prepared a summary of findings table using GRADEpro. This table evaluated the overall quality of the body of evidence for the primary review outcomes (live birth, clinical pregnancy and miscarriage), using GRADE criteria (study limitations (that is risk of bias), consistency of effect, imprecision, indirectness and publication bias). Judgements about evidence quality (high, moderate,

low or very low) were justified, documented, and incorporated into reporting of results for each outcome.

RESULTS

Description of studies

Results of the search

2011 version of review

Abstracts and titles were screened from the results of the search strategies. The MEDLINE search produced 406 abstracts; there were six abstracts from CENTRAL, 3 from CINAHL, 62 from EMBASE, 107 from the MSDG database and 3 from PsycINFO. Two conference abstracts were found from handsearching the conference proceedings of the European Society for Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM). One title was found from reference lists in reviews.

Five non-English trials were assessed for inclusion: two Chinese, one Bulgarian, one Japanese and one Iranian. The two Chinese trials (Li 2005; Li 2005a), the Japanese trial (Akiyama 1999) and the Iranian trial (Peivandi 2010) were included in the analysis. The Bulgarian study (Nikolova 2007) was excluded as it did not have random allocation (see Characteristics of excluded studies).

After removal of inappropriate and duplicate studies 53 trials remained: 34 trials were included, 15 were excluded and 4 trials were ongoing.

2014 update

A further 483 abstracts were assessed for inclusion in a search that was date limited from 1 August 2010 until 30 January 2014. After duplicates were removed 338 remained; 34 full text papers were retrieved from these and 14 trials were included and 20 were excluded, see the PRISMA flow chart (Figure 1). An updated search was run in August 2014 where six studies (Anarte 2013a; Gopinath 2013; Iacono 2014; Nadjarzadeh 2014; Nashivochnikova 2014; Nematollahi-Mahani 2014) were placed in 'studies awaiting assessment'.

Eleven of the full text assessed trials were in a language other than English and required translation, five of these were in Chinese, two in Persian and one each in Japanese, Russian, Italian, and Portuguese (see Acknowledgements for those who helped with translation). Five of the Chinese trials were excluded: three (Chen 2012; Tang 2011; Wang 2010a) due to an inappropriate intervention, one was not randomised (Wu 2012) and one had an inappropriate population (Lu 2010). The Portuguese trial (Verzeletti 2012) was excluded as it used a herbal intervention. Five non-English trials were included: one in Persian (Eslamian 2012), one Japanese (Kumamoto 1988), one Italian (Morgante 2010), one Russian (Sivkov 2011) and one Chinese (Wang 2010).

Fourteen new trials were included in the 2014 update. Six ongoing trials were found in the new searches.

Thus a total of 48 trials have been included in this review update (Characteristics of included studies).

Included studies

Full details of the 48 included trials can be seen in the table of included studies (Characteristics of included studies). The trials came from 23 different countries and randomised 4179 men. Nine trials were based in Italy (Balercia 2005; Balercia 2009; Biagiotti 2003; Cavallini 2004; Galatioto 2008; Lenzi 2003; Lenzi 2004; Lombardo 2002; Morgante 2010). Eight trials were from Iran (Azizollahi 2013; Eslamian 2012; Nadjarzadeh 2011; Peivandi 2010; Safarinejad 2011; Safarinejad 2012; Safarinejad 2009; Safarinejad 2009a). Three trials were from the UK (Kessopoulou 1995; Pryor 1978; Scott 1998), three from China (Li 2005; Li 2005a; Wang 2010) and three from Japan (Akiyama 1999; Dimitriadis 2010; Kumamoto 1988). Two trials each were from USA (Dawson 1990; Sigman 2006), Tunisia (Keskes-Ammar 2003; Nozha 2001), Hungary (Micic 1988; Zavaczki 2003) and Kuwait (Omu 1998; Omu 2008). A single trial was set in each of the following countries: Turkey (Ciftci 2009), Canada (Conquer 2000), France (Greco 2005), Mexico (Merino 1997), Germany (Rolf 1999), Saudi Arabia (Suleiman 1996), Australia (Tremellen 2007), Hong Kong (Wang 1983), Netherlands (Wong 2002), Belgium (Zalata 1998), Egypt (Attallah 2013), Spain (Martinez-Soto 2010), Panama Poveda 2013 and Russia (Sivkov 2011).

Design

All included trials were randomised. Five trials (Akiyama 1999; Kessopoulou 1995; Lenzi 2003; Peivandi 2010; Pryor 1978) had a randomised crossover design. In the meta-analysis only the first phase data were used as all trials reported first and second phase data separately. The remaining 43 trials had a randomised parallel group design.

One study (Li 2005) had a large imbalance between the intervention and control groups at the randomisation stage; 150 men were randomised, 90 into the treatment group and 60 into the control group. This appeared to be a blocked 3:2 allocation ratio. This method of randomisation was not explained in the report. Attempts were made to contact the author but there has been no reply.

Seven trials (Biagiotti 2003; Cavallini 2004; Conquer 2000; Dawson 1990; Kumamoto 1988; Scott 1998; Zalata 1998) were three-armed trials and six (Azizollahi 2013; Balercia 2005; Omu 2008; Poveda 2013; Safarinejad 2009; Wong 2002) were four-armed.

The duration of the treatment period ranged from 3 weeks with a 3-week follow up (Dawson 1990) to 26 weeks treatment and a 24-month follow up (Wong 2002). The longest follow-up period was in the trial by Kessopoulou (Kessopoulou 1995), with a three-week treatment period and a two-year follow-up period.

Participants

The 48 studies included 4179 men in total, 2466 in the intervention groups and 1713 men in the control groups. Trials included couples who had attended a fertility clinic, with a fertile partner and had been trying to conceive with regular intercourse for over one year.

The age of the participants ranged from 20 to 52 years. Most men in the included trials had a deficient level of spermatozoa in the seminal fluid (oligospermia) or a low motility of sperm in the seminal fluid (asthenospermia). Trials excluded men with any inflammatory disease, antibody problems or chromosomal problems; and most trials stated that they did not enrol men who smoked, took any additional medication or drank alcohol. One trial (Cavallini 2004) enrolled men with varicocoele, Azizollahi 2013 enrolled men post-varicocelectomy and Sivkov 2011 enrolled men with chronic prostatitis. Three trials (Kessopoulou 1995; Sigman 2006; Tremellen 2007) enrolled men who, as a couple, were undergoing IVF or ICSI; and one trial (Attallah 2013) enrolled men who were part of a couple undergoing IUI.

Details of the inclusion and exclusion criteria for each trial are found in Characteristics of included studies.

Interventions

- 24/48 trials compared antioxidants versus placebo
- 7/48 trials compared antioxidants to no treatment
- 7/48 trials compared antioxidants to antioxidants (or head to head)
- 10/48 multi-arm trials: nine of these compared antioxidants versus placebo and one compared antioxidants versus no treatment

A wide variety of antioxidants were used in the included trials. The comparison 'antioxidants versus placebo or no treatment' included the antioxidants: combined antioxidants plus minerals, magnesium, zinc, folic acid, N-acetylcysteine, Coenzyme Q10, vitamins E and C, selenium, docosahexaenoic acid (DHA) and carnitines (these included L-acetylcarnitine, L-carnitine, L-acetyl carnitine plus L-carnitine). The trials that reported on pentoxifylline were included here.

The second comparison, head to head, was implemented in an attempt to assess whether one antioxidant may be more effective than another, as stated in the protocol. Antioxidants used in the trials were: L-acetyl carnitine, L-carnitine, L-acetyl carnitine plus L-carnitine, ethyl cysteine, vitamin E, DHA, vitamin C, selenium, vitamin B, zinc, folic acid, combined antioxidants plus minerals and N-acetyl cysteine.

Outcomes

The primary outcome for this review was as follows.

- Live birth per couple. Four trials reported data for live birth (Kessopoulou 1995; Omu 1998; Suleiman 1996; Tremellen 2007) in the antioxidant versus placebo or no treatment comparison.

Secondary outcomes for this review were as follows.

- Clinical pregnancy rate per couple, as reported by seven trials (Attallah 2013; Azizollahi 2013; Kessopoulou 1995; Omu 1998; Suleiman 1996; Tremellen 2007; Zavaczki 2003). No trials reported clinical pregnancy rates in the head to head or pentoxifylline comparisons. Data for biochemical and undefined pregnancy can be seen in Table 1.

- Adverse events (miscarriage, gastrointestinal upsets and euphoria) were reported by eight trials (Cavallini 2004; Kessopoulou 1995; Omu 1998; Safarinejad 2009a; Sigman 2006; Suleiman 1996; Tremellen 2007; Zavaczki 2003) in the antioxidant versus placebo or no treatment comparison. No adverse events were reported in the trials in the head to head comparisons. The trial by Li (Li 2005) reported that no side effects were found in either the treatment or control groups. There was only one trial (Safarinejad 2011) that reported adverse events (vomiting, dyspepsia, headache, diarrhoea, tremor, dizziness and vertigo) in the pentoxifylline versus placebo or no treatment comparison.

- DNA fragmentation was reported by two trials (Greco 2005; Martinez-Soto 2010), comparing antioxidants versus placebo or no treatment. It was not reported in either the head to head or pentoxifylline versus placebo or no treatment comparisons.

The total sperm motility and concentration outcomes were divided into three groups: measurement after starting treatment, at three, six and nine months or more as reported by the trials. Trials were analysed together if they reported these outcomes at the same point in time, for example a trial that stopped treatment at three months but measured at six or nine months was measured in the same analysis as those that were treated for six or nine months.

- Sperm motility at three months or less was reported by 16 trials in the antioxidants versus placebo or no treatment comparison (Attallah 2013; Azizollahi 2013; Ciftci 2009; Conquer 2000; Dawson 1990; Dimitriadis 2010; Greco 2005; Martinez-Soto 2010; Morgante 2010; Nadjarzadeh 2011; Omu 2008; Peivandi 2010; Rolf 1999; Scott 1998; Sigman 2006; Zavaczki 2003), by eight trials (Akiyama 1999; Azizollahi 2013; Conquer 2000; Dawson 1990; Keskes-Ammar 2003; Li 2005; Omu 2008; Scott 1998) in the head to head comparison and by one trial (Micic 1988) in the pentoxifylline comparison.

- Sperm motility at six months was measured by nine trials (Azizollahi 2013; Balercia 2005; Balercia 2009; Lenzi 2004; Safarinejad 2009; Safarinejad 2009a; Safarinejad 2012; Sigman 2006; Suleiman 1996) in the antioxidants versus placebo or no treatment comparison. Three trials (Azizollahi 2013; Balercia 2005; Safarinejad 2009) reported this in the head to head comparison and one (Safarinejad 2011) in the pentoxifylline comparison.

- Sperm motility at nine months or more was reported by four trials (Balercia 2005; Balercia 2009; Safarinejad 2009a; Safarinejad 2012) in the antioxidants versus placebo or no treatment comparison. One trial (Balercia 2005) in the head to head comparison and one (Safarinejad 2011) in the pentoxifylline comparison.

- Sperm concentration at three months or less was reported by 13 trials in total (Attallah 2013; Azizollahi 2013; Conquer 2000; Ciftci 2009; Dimitriadis 2010; Greco 2005; Martinez-Soto 2010; Morgante 2010; Nadjarzadeh 2011; Peivandi 2010; Rolf 1999; Scott 1998; Zavaczki 2003) in the antioxidants versus placebo or no treatment comparison, six (Akiyama 1999; Azizollahi 2013; Conquer 2000; Li 2005a; Scott 1998; Wang 2010) in the head to head comparison and one (Wang 1983) in the pentoxifylline comparison.

- Sperm concentration at six months was reported as an outcome by a total of seven trials (Balercia 2005; Balercia 2009; Lenzi 2004; Li 2005a; Safarinejad 2009; Safarinejad 2009a; Safarinejad 2012) in the antioxidants versus placebo or no treatment comparison, three (Azizollahi 2013; Balercia 2005; Safarinejad 2009) in the head to head comparison and two (Safarinejad 2011; Wang 1983) in the pentoxifylline comparison.

- Sperm concentration at nine months or more was reported on by four trials (Balercia 2005; Balercia 2009; Safarinejad 2009a; Safarinejad 2012) in the antioxidants versus placebo or no treatment comparison, one (Balercia 2005) in the head to head comparison and one (Safarinejad 2011) in the pentoxifylline comparison.

Data were extracted from 37 of these included trials. The 11 remaining trials either did not report any data or the continuous data were reported in medians or ranges (Biagiotti 2003; Eslamian 2012; Kumamoto 1988; Lombardo 2002; Merino 1997; Nozha 2001; Poveda 2013; Pryor 1978; Sivkov 2011; Wong 2002; Zalata 1998), see Characteristics of included studies and the analyses (Analysis 1.8; Analysis 1.10; Analysis 1.14; Analysis 1.16; Analysis 2.7; Analysis 2.3). Table 2 also described the outcomes and conclusions of all included trials.
Attempts were made to contact all authors of the included trials for further details and clarification.

Excluded studies

The reasons for exclusions were: non-randomisation, incorrect populations, incorrect interventions, a missing control group (see details in Characteristics of excluded studies and Figure 1). Two trials (Dimitriadis 2010; Wang 2010) moved from Studies awaiting classification to be included and one ongoing trial (Kumar 2011) was excluded due to a herbal intervention (therefore a total of 29 trials were excluded in the update). An ongoing trial (Hekmatdoost NCT01846325) was found to be the same trial as the included Eslamian 2012, therefore it became a sub-study of this trial. Overall:

- 4 trials included fertility drugs in the intervention and control groups;
- 7 were not randomised;
- 9 were the wrong population;
- 1 was a commentary of another trial;
- 4 used plant extracts in the intervention;
- 3 had inappropriate control groups;
- 1 moved from ongoing in the original review to excluded due to herbal supplement.

Studies awaiting classification

An updated search was run in August 2014 when six potential included studies were placed in 'studies awaiting assessment' (Anarte 2013a; Gopinath 2013; Iacono 2014; Nadjarzadeh 2014; Nashivochnikova 2014; Nematollahi-Mahani 2014).

Ongoing studies

Eight trials were found in the searches of the trials registers ClinicalTrials.gov and the World Health Organization International Clinical Trials Registry Platform search portal (AGUNCO 2012; Gonzalez 2009; Jensen 2011; Kamath 2014; Palumbo 2012; Rigshospitalet 2011; Sadeghi 2008; Sadeghi 2009), see Characteristics of ongoing studies for details of these studies. Where possible, trial authors were contacted regarding the ongoing registered trial. Two authors from the ongoing studies of Revel 2006 and Tsafrir 2010 responded saying that their trials never proceeded beyond registration. The author from the ongoing trial Martinez- Soto 2011 replied saying that this trial was actually the published included trial Martinez-Soto 2010. One ongoing trial author (Kamath 2014) replied saying that they were still in the recruitment phase and were hoping to finish the trial in 2015.

Risk of bias in included studies

See Figure 2 for a summary of each risk of bias item across all included studies and Figure 3 for a summary of each risk of bias in individual trials.

Figure 2. Methodological quality graph: review authors' judgements about each methodological quality item presented as percentages across all included studies.

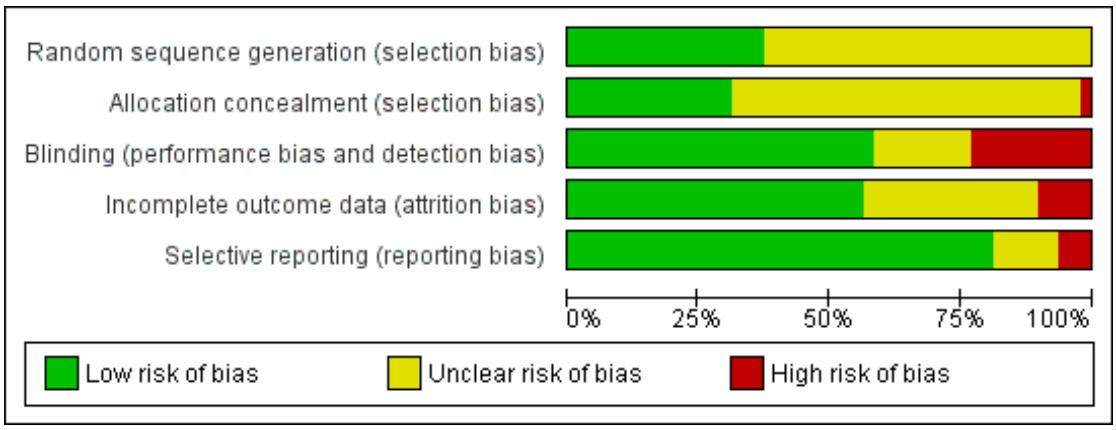
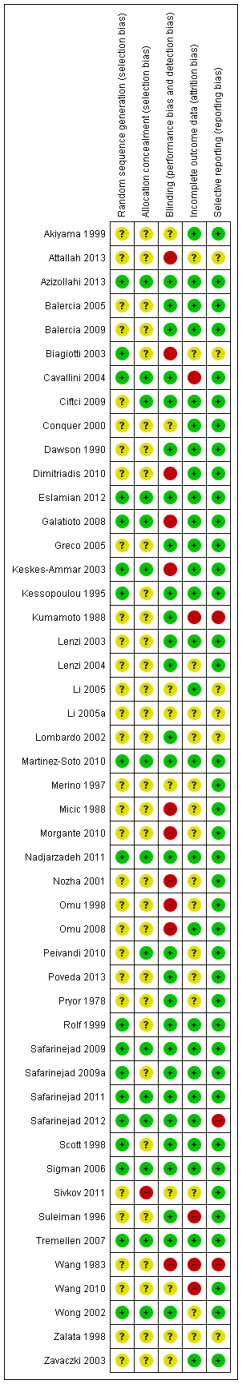


Figure 3. Methodological quality summary: review authors' judgements about each methodological quality item for each included study.



Allocation

Sequence generation

All 48 included trials were randomised: five of these were crossover (Akiyama 1999; Kessopoulou 1995; Lenzi 2003; Peivandi 2010; Pryor 1978) and the remaining were parallel design. Only 18 trials described their methods of randomisation and were rated as low risk in this domain (Azizollahi 2013; Biagiotti 2003; Cavallini 2004; Eslamian 2012; Galatioto 2008; Kessopoulou 1995; Keskes-Ammar 2003; Martinez-Soto 2010; Nadjarzadeh 2011; Rolf 1999; Safarinejad 2009; Safarinejad 2009a; Safarinejad 2011; Safarinejad 2012; Scott 1998; Sigman 2006; Tremellen 2007; Wong 2002) (see Figure 2 and Figure 3). The remaining 30 studies were rated as unclear risk. The predominant method of randomisation was by computer generated blocks. One trial (Tremellen 2007) reported a 2:1 ratio randomisation schedule and Li 2005 appeared to have a blocked 3:2 allocation.

Allocation concealment

The methods of allocation concealment were generally quite poorly described in the included studies. Thirteen trials (Cavallini 2004; Galatioto 2008; Safarinejad 2009; Sigman 2006; Tremellen 2007; Keskes-Ammar 2003; Azizollahi 2013; Eslamian 2012; Martinez-Soto 2010; Nadjarzadeh 2011; Safarinejad 2011; Safarinejad 2012; Wong 2002) described both their methods of randomisation and allocation concealment and were rated as low risk in this domain. However two (Ciftci 2009; Peivandi 2010) trials reported only on their allocation concealment and not the methods of randomisation. The remaining 33 trials were rated as unclear risk. The methods of allocation concealment included anonymous coloured boxes, sealed opaque envelopes, and numbered bottles.

Blinding

Twenty-seven trials (Azizollahi 2013; Balercia 2005; Balercia 2009; Cavallini 2004; Ciftci 2009; Dawson 1990; Eslamian 2012; Greco 2005; Kessopoulou 1995; Kumamoto 1988; Lenzi 2003; Lenzi 2004; Lombardo 2002; Martinez-Soto 2010; Nadjarzadeh 2011; Peivandi 2010; Poveda 2013; Pryor 1978; Rolf 1999; Safarinejad 2009; Safarinejad 2009a; Safarinejad 2011; Safarinejad 2012; Scott 1998; Sigman 2006; Tremellen 2007; Wong 2002) were described as randomised, double blind controlled trials and were rated as low risk. The researchers and patients were blinded (see Figure 2 and Figure 3).

The double blinded trial Suleiman 1996 reported that if a couple became pregnant then the treatment was stopped; however they did not appear to stop the placebo. This could suggest that the investigators had knowledge of whether the patients were in the placebo or antioxidant group, therefore this trial was rated as high risk. Eleven other trials (Attallah 2013; Biagiotti 2003; Dimitriadis 2010; Galatioto 2008; Keskes-Ammar 2003; Micic 1988; Morgante 2010; Nozha 2001; Omu 1998; Omu 2008; Wang 1983) were also rated high risk as they had used 'no treatment' as their comparator.

Nine trials (Akiyama 1999; Conquer 2000; Li 2005; Li 2005a; Merino 1997; Sivkov 2011; Wang 2010; Zalata 1998; Zavaczki 2003) were rated as unclear risk of bias due to no statement regarding blinding.

Two trials (Ciftci 2009; Dawson 1990) stated that a placebo was used as the control but only the patients were blinded. Other trials (Conquer 2000; Greco 2005; Merino 1997; Wang 1983; Zavaczki 2003) used a placebo as the control but did not discuss blinding.

Four trials (Attallah 2013; Galatioto 2008; Keskes-Ammar 2003; Sivkov 2011) were described as open label however the Galatioto 2008 study stated that the pharmacy was blinded during the randomisation process.

Incomplete outcome data

Twenty-five studies (Akiyama 1999; Azizollahi 2013; Balercia 2005; Balercia 2009; Ciftci 2009; Conquer 2000; Dawson 1990; Dimitriadis 2010; Eslamian 2012; Galatioto 2008; Greco 2005; Keskes-Ammar 2003; Kessopoulou 1995; Lenzi 2003; Martinez-Soto 2010; Nadjarzadeh 2011; Omu 2008; Rolf 1999; Safarinejad 2009; Safarinejad 2009a; Safarinejad 2011; Safarinejad 2012; Scott 1998; Sigman 2006; Zavaczki 2003) were rated as low risk for attrition bias.

Seventeen trials were rated as unclear (Attallah 2013; Biagiotti 2003; Lenzi 2004; Li 2005a; Lombardo 2002; Merino 1997; Micic 1988; Morgante 2010; Nozha 2001; Omu 1998; Peivandi 2010; Poveda 2013; Pryor 1978; Sivkov 2011; Tremellen 2007; Wong 2002; Zalata 1998).

Six trials (Cavallini 2004; Kumamoto 1988; Li 2005; Suleiman 1996; Wang 1983; Wang 2010) were rated as high risk of attrition bias.

Only two trials (Balercia 2009; Galatioto 2008) actually stated that they used intention to treat in their analysis, however most of the included trials accounted for the participants that withdrew from their trials and then analysed the groups in an intention-to-treat fashion (ITT). Two trials (Azizollahi 2013; Wang 2010) did not use ITT, however the numbers of dropouts were given for each intervention and control group and therefore we were able to use ITT in the data analysis by making the assumption of no event for the binary outcomes. No imputation was carried out on the continuous outcome data, these were analysed as they were reported in the trials.

Six trials had over 20% withdrawal from their trials. One trial (Keskes-Ammar 2003) had over 50% dropout rate; all these men were accounted for and the main reason given was non-compliance. Cavallini 2004 had 30% dropout rate and reasons were provided for only 53 out of the 55 dropouts; these reasons included refusal due to the chance of taking a placebo and preference for assisted reproduction techniques. There also remained some confusion in this trial on the total numbers randomised and analysed. Azizollahi 2013 had a 30% dropout rate; Li 2005a; Suleiman 1996 and Nadjarzadeh 2011 had slightly over 20% withdrawal from their trials.

One trial (Suleiman 1996) had a large imbalance in numbers. There were found to be 52 in the treatment group and 35 in the placebo once the code had been broken at the end of the trial. There was no indication of how the randomisation was performed. The dropout reasons were only accounted for broadly: many couples had left the region and some simply failed to continue, no numbers were given for individual dropout reasons (see Figure 2 and Figure 3). The numbers of participants that were initially randomised to each group were not available, so intention to treat for the dichotomous outcomes was not possible.

Selective reporting

Trial protocols were unavailable for all the 48 included trials so it was difficult to assess reporting bias.

The majority of the trials (33) included in this review reported only on sperm parameters, therefore we assumed that may constitute some degree of reporting bias, in that these trials could have reported on the clinical outcomes of live birth and clinical pregnancy but did not. Failure to report live birth or pregnancy is common and is a major source of bias, as ultimately for couples these are the most meaningful outcomes.

Twenty-two of the 33 trials provided suitable data for meta-analysis on sperm motility, concentration and DNA fragmentation, and the data that could not be used in the forest plots can be seen in Analysis 1.8; Analysis 1.10; Analysis 1.14; Analysis 1.16; Analysis 2.3; Analysis 2.7; Analysis 3.2; Analysis 3.4 and Table 2.

Only four studies reported live birth (Kessopoulou 1995; Omu 1998; Suleiman 1996; Tremellen 2007). The author of Tremellen 2007 provided live birth data for this update. No new studies in the update reported on live birth.

Only 20 of the 48 trials reported on pregnancy. Seven reported on clinical pregnancy (Attallah 2013; Azizollahi 2013; Kessopoulou 1995; Omu 1998; Suleiman 1996; Tremellen 2007; Zavaczki 2003). Therefore 41 of the 48 included trials did not report on clinical pregnancy rate: 12 of these reported on biochemical pregnancy or undefined pregnancy (Balercia 2005; Balercia 2009; Cavallini 2004; Galatioto 2008; Lenzi 2003; Lenzi 2004; Peivandi 2010; Pryor 1978; Rolf 1999; Safarinejad 2009a; Sigman 2006; Wang 1983) (Table 1). There were five trials that reported on pregnancy rates even though this was not stated a priori in the methods sections of the papers (Balercia 2005; Balercia 2009; Kessopoulou 1995; Lenzi 2004; Omu 1998) (Table 2). Two of these (Kessopoulou 1995; Omu 1998) were included in both the clinical pregnancy and the live birth analyses.

Three trials were rated at high risk of reporting bias, for the following reasons; Kumamoto 1988 performed subgroup analysis post-treatment, Safarinejad 2012 did not pre-specify outcomes and Wang 1983 did not provide control data. Four trials Attallah 2013; Biagiotti 2003; Lombardo 2002; Zalata 1998 were rated as unclear risk as they were conference abstracts, and two trials (Li 2005; Li 2005a) were rated as unclear as it was possible that these were two publications of the same trial that were reporting on different intervention arms. Obtaining help with Chinese translation did not clarify this and attempts to contact the authors were unsuccessful. The remaining 39 trials were rated as low risk in this domain.

Adverse events were poorly reported. Only seven trials reported on side effects, which were mainly gastrointestinal (Cavallini 2004; Kessopoulou 1995; Safarinejad 2009a; Safarinejad 2011; Sigman 2006; Tremellen 2007; Zavaczki 2003). Cavallini (Cavallini 2004) also reported euphoria as a side effect. Only three trials reported on the adverse event of miscarriage (Omu 1998; Suleiman 1996; Tremellen 2007).

We were unable to assess publication bias by using a funnel plot as none of the analyses included sufficient studies. Only seven included studies were able to be analysed in the outcome of clinical pregnancy, 10 studies are the minimum requirement for constructing a funnel plot (Higgins 2011).

Other potential sources of bias

Funding sources were stated by 15 trials (Conquer 2000; Eslamian 2012; Kessopoulou 1995; Lenzi 2003; Lombardo 2002; Martinez-Soto 2010; Nadjarzadeh 2011; Omu 1998; Peivandi 2010; Poveda 2013; Rolf 1999; Safarinejad 2012; Wang 1983; Wang 2010; Zavaczki 2003). Four of these trials (Conquer 2000; Martinez-Soto 2010; Safarinejad 2012; Wang 1983) stated that funding was from a commercial source and the remaining 11 obtained funding through non-commercial avenues or university grants (see Characteristics of included studies). Thirty-three trials did not report any funding sources.

Two of the trials included in the analysis of the semen parameter outcomes (Safarinejad 2009; Safarinejad 2009a) had consistently reported SDs very much smaller than those reported by most of the other included trials. The review authors considered that these were potentially erroneous, but an attempt to check with the study authors was unsuccessful. One other trial (Peivandi 2010) also had very small SDs when compared to data in the other trials but the authors confirmed, when contacted, that they are indeed SDs and not standard errors. We tried to manage these analyses in two different ways: firstly by imputing SDs from studies of a similar size and secondly by treating the data as SEs and converting back

to SDs, however heterogeneity remained high in both situations so for the final analyses we reverted to the SDs as reported in the studies. The low SDs may have been due to the strict inclusion and exclusion criteria indicating that the trial was homogenous in nature, however we were unable to carry out a sensitivity analysis on these trials as pooling was not possible due to high heterogeneity.

Twenty-seven of the 48 included trials were small in size (randomising between 20 and 60 men), and the estimates of the intervention effect tend to be more beneficial in smaller studies. Smaller studies also may not be as rigorous as the larger studies in their methodology (Higgins 2011).

The trial by Cavallini (Cavallini 2004) had unexplained differences in randomisation and analysis numbers and this may have introduced some reporting bias.

There may have been some publication bias in this review as, although we performed a comprehensive and wide ranging search including both full text and conference abstracts, we did not find any unpublished trials.

Effects of interventions

See: **Summary of findings for the main comparison** Antioxidants versus placebo or no treatment for male subfertility

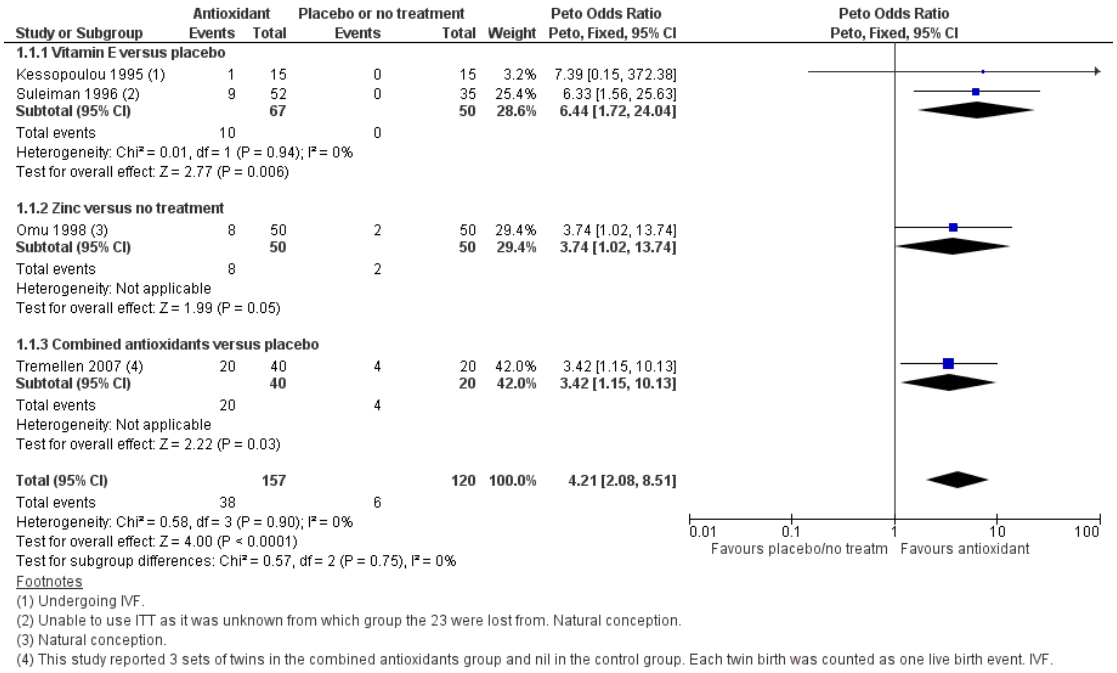
1 Antioxidants versus placebo or no treatment (natural conception and undergoing fertility treatment)

1.1 Live birth; type of antioxidant

See Analysis 1.1

Only four trials reported on live birth, three of these had methodological inadequacies as they did not describe their methods of randomisation or allocation concealment. The meta-analysis of these trials showed that antioxidants were associated with an increased live birth rate compared with placebo or no treatment (OR 4.21, 95% CI 2.08 to 8.51, P < 0.0001, 4 RCTs, 277 men, I^2 = 0%, low quality evidence) (Figure 4). This meant that within this population of subfertile men with an expected live birth rate of 5%, use of an antioxidant increased this rate to between 10% and 31% (Summary of findings for the main comparison).

Figure 4. Forest plot of comparison: 1 Antioxidant(s) versus placebo or no treatment, outcome: 1.1 Live birth; type of antioxidant.



Footnotes

(1) Undergoing IVF.

(2) Unable to use ITT as it was unknown from which group the 23 were lost from. Natural conception.

(3) Natural conception.

(4) This study reported 3 sets of twins in the combined antioxidants group and nil in the control group. Each twin birth was counted as one live birth event. IVF.

1.1.1 Two trials (Kessopoulou 1995; Suleiman 1996) reported on this outcome comparing vitamin E versus placebo. There was an association with increased live birth rate (OR 6.44, 95% CI 1.72 to 24.04, 2 RCTs, 117 men, P = 0.006, I^2 = 0%) and vitamin E, favouring vitamin E over the placebo.
1.1.2 One trial (Omu 1998) compared zinc versus no treatment on this outcome. As there was only one trial in this subgroup meta-analysis was not possible. Zinc was associated with an increased live birth rate when compared to no treatment (OR 3.74, 95% CI 1.02 to 13.74, P = 0.05, 1 trial, 100 men).
1.1.3 Tremellen 2007 compared combined antioxidants versus placebo (OR 3.42, 95% CI 1.15 to 10.13, P = 0.03, 1 trial, 60 men). Combined antioxidants were associated with an increased live birth rate when compared to placebo. The results from this study also included 3 sets of twins in the combined antioxidant group and nil in the placebo group. Each twin birth was counted as one event as stated in the methods section in the review protocol.

1.2 Live birth; IVF or ICSI

See Analysis 1.2
Only two studies (Kessopoulou 1995; Tremellen 2007) reported live birth in this subgroup . Antioxidants were associated with an increased live birth rate, in those couples undergoing IVF/ICSI, when compared with placebo (OR 3.61, 95% CI 1.27 to 10.29, P = 0.02, 2 RCTs, 90 men, I^2 = 0%).

Sensitivity analysis for trials reporting live birth and clinical pregnancy

The four trials that reported live birth had an OR for live birth of 4.21, and in these same trials the OR for clinical pregnancy was 4.00. When we pooled all seven trials reporting the clinical pregnancy rate the OR was lower at 3.43. This might suggest that the clinical pregnancy rate in these four trials that reported live birth may have overestimated the effect of the antioxidants, and therefore the live birth rate in these trials may also be a slight overestimate. This could have been due to the uneven dropout rates between the intervention and control groups in Suleiman 1996. The true effect was unknown unless all trials reporting on clinical pregnancy rate also reported on live birth rate.

Sensitivity analysis for trials rated as high risk of bias

Two trials (Omu 1998; Suleiman 1996) in this comparison were rated as 'unclear risk of bias' as their methods of randomisation and allocation concealment were not described. However when these two studies were removed from the analysis there remained an association between the use of antioxidants and live birth when compared with placebo (OR 3.61, 95% CI 1.27 to 10.29, P = 0.02, 2 RCTs, 90 men, I^2 = 0%).

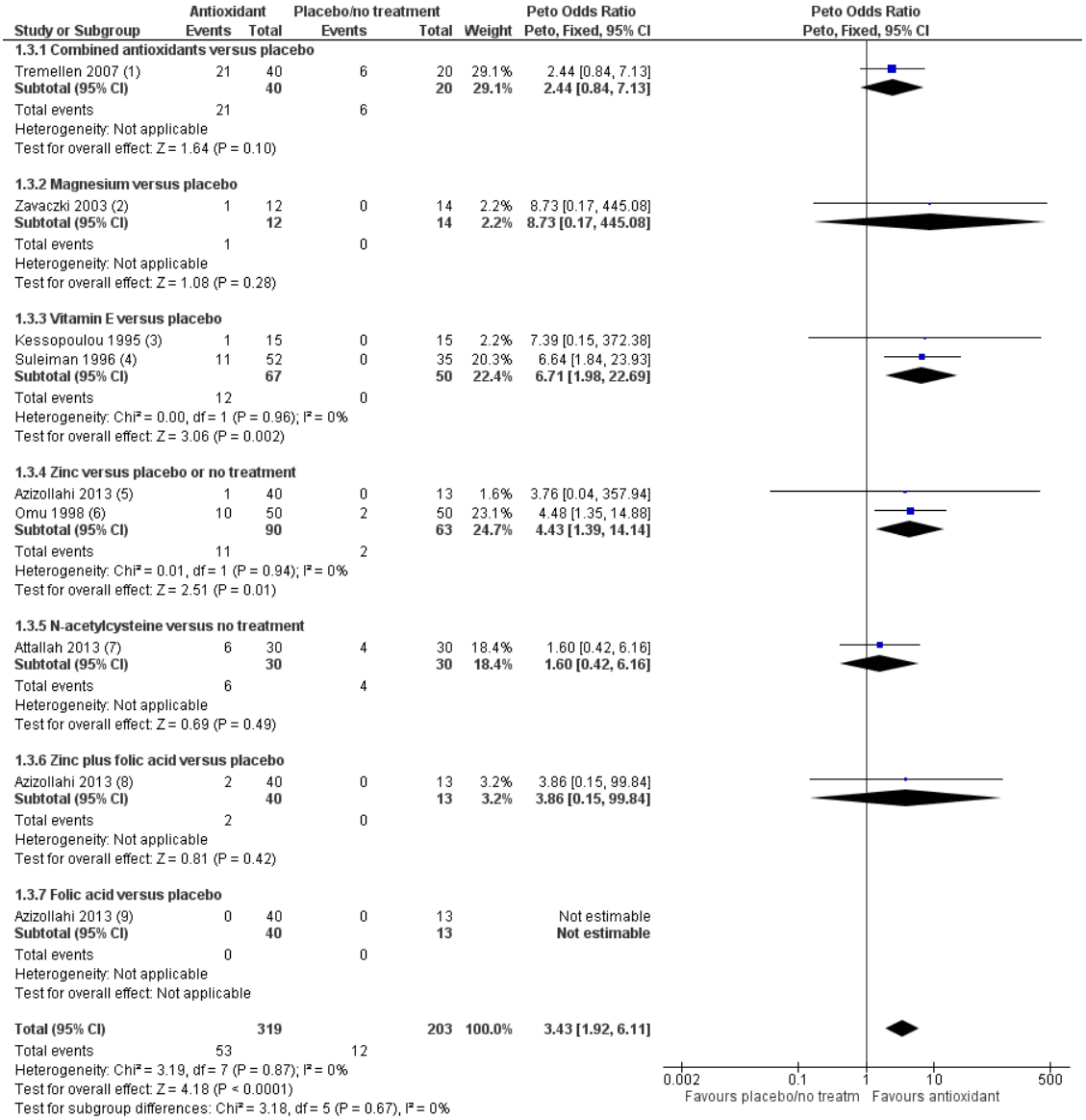
Sensitivity analysis for trials using placebo and no treatment controls

Only one trial (Omu 1998) used 'no treatment' as the control. When this trial was removed from the analysis, an association with live birth remained when compared with placebo only (OR 4.42, 95% CI 1.91 to 10.21, P = 0.0005, 3 RCTs, 177 men, I^2 = 0%). Antioxidants were also associated with an increase in live birth rate in the trial by Omu 1998, when compared to 'no treatment' (OR 3.74, 95% CI 1.02 to 13.74, P = 0.05).

1.3 Clinical pregnancy; type of antioxidant

See Analysis 1.3
Antioxidants were associated with an increased clinical pregnancy rate when compared to placebo or no treatment (OR 3.43, 95% CI 1.92 to 6.11, P < 0.0001, 7 RCTs, 522 men, I^2 = 0%, low quality evidence) (Figure 5). This meant that within this population of subfertile men with the expected clinical pregnancy rate of 6%, use of an antioxidant increased this rate to between 11% and 28% (Summary of findings for the main comparison).

Figure 5. Forest plot of comparison: 1 Antioxidant(s) versus placebo or no treatment, outcome: 1.3 Clinical pregnancy; type of antioxidant.



Footnotes

(1) IVF. This study reported 3 sets of twin pregnancies in the combined antioxidants group and nil in the control group. Each twin pregnancy was counted as one...
(2) Natural conception.
(3) IVF.
(4) Natural conception.
(5) Placebo group divided into three for this 4 arm trial. Post varicocelectomy. Natural conception. Control is placebo
(6) Natural conception. Control is no treatment
(7) IUI.
(8) Post varicocelectomy. Natural conception.
(9) Post varicocelectomy. Natural conception.

Two subgroups of 'type of antioxidant' each contained two trials:

- vitamin E (Kessopoulou 1995; Suleiman 1996) was associated with an increased clinical pregnancy rate when compared to placebo (OR 6.71, 95% CI 1.98 to 22.69, P = 0.002, 2 RCTs, 117 men, I^2 = 0%); and
- zinc (Azizollahi 2013; Omu 1998) was also associated with an increased clinical pregnancy rate when compared to placebo or no treatment (OR 4.43, 95% CI 1.39 to 14.14, P = 0.01, 2 RCTs, 153 men, I^2 = 0%).

The five remaining 'type of antioxidant' subgroups contained only one trial each.

Four subgroups showed no association with clinical pregnancy rate when compared to a control: combined antioxidants (Tremellen 2007) (OR 2.44, 95% CI 0.84 to 7.13, P = 0.10, 1 RCT, 60 men), magnesium versus placebo (Zavaczki 2003) (OR 8.73, 95% CI 0.17 to 445.08, P = 0.28, 1 RCT, 26 men), N-acetylcysteine versus no treatment (Attallah 2013) (OR 1.60, 95% CI 0.42 to 6.16, P = 0.49, 1 RCT, 60 men), zinc plus folic acid versus placebo (Azizollahi 2013) (OR 3.86, 95% CI 0.15 to 99.84, P = 0.42, 1 RCT, 53 men) and the folic acid versus placebo subgroup (Azizollahi 2013) was not estimable due to no pregnancies occurring in the treatment and the control groups.

Sensitivity analysis for trials using placebo and no treatment controls

Antioxidants were associated with an increase in clinical pregnancy rate in the trials (Azizollahi 2013; Kessopoulou 1995; Suleiman 1996; Tremellen 2007; Zavaczki 2003) that compared antioxidants with placebo (OR 3.92, 95% CI 1.84 to 8.33, P = 0.0004, 5 RCTs, 362 men, I^2 = 0%). Antioxidants were also associated with an increase in clinical pregnancy rate in those trials (Attallah 2013; Omu 1998) that compared antioxidants versus no treatment (OR 2.84, 95% CI 1.16 to 6.96, P = 0.02, 2 RCTs, 213 men, I^2 = 20%).

Sensitivity analysis for trials rated as unclear or high risk of bias

When the four trials (Attallah 2013; Omu 1998; Suleiman 1996; Zavaczki 2003) rated with an unclear risk of bias, in both the domains of randomisation and allocation concealment, were removed from the analysis there remained an association between antioxidants and an increased clinical pregnancy rate (OR 2.77, 95% CI 1.06 to 7.25, P = 0.04, 3 RCTs, 249 men, I^2 = 0%).

Sensitivity analysis for trials enrolling men with varicocoele

When the trial that enrolled men with varicocoele (Azizollahi 2013) (post-variocoele removal) was removed from the analysis, antioxidants remained associated with increased clinical pregnancy rate when compared to placebo or no treatment (OR 3.41, 95% CI 1.89 to 6.16, P < 0.0001, 6 RCTs, 363 men, I^2 = 0%).

Sensitivity analysis for trials enrolling men in couples undergoing IUI

Only one trial Attallah 2013 reported on men in couples undergoing IUI. When this trial was removed from the analysis there remained an association between the use of antioxidants and increased clinical pregnancy rate when compared to no treatment (OR 4.07, 95% CI 2.15 to 7.71, P < 0.0001, 6 RCTs, 462 men, I^2 = 0%).

1.4 Clinical pregnancy; IVF or ICSI

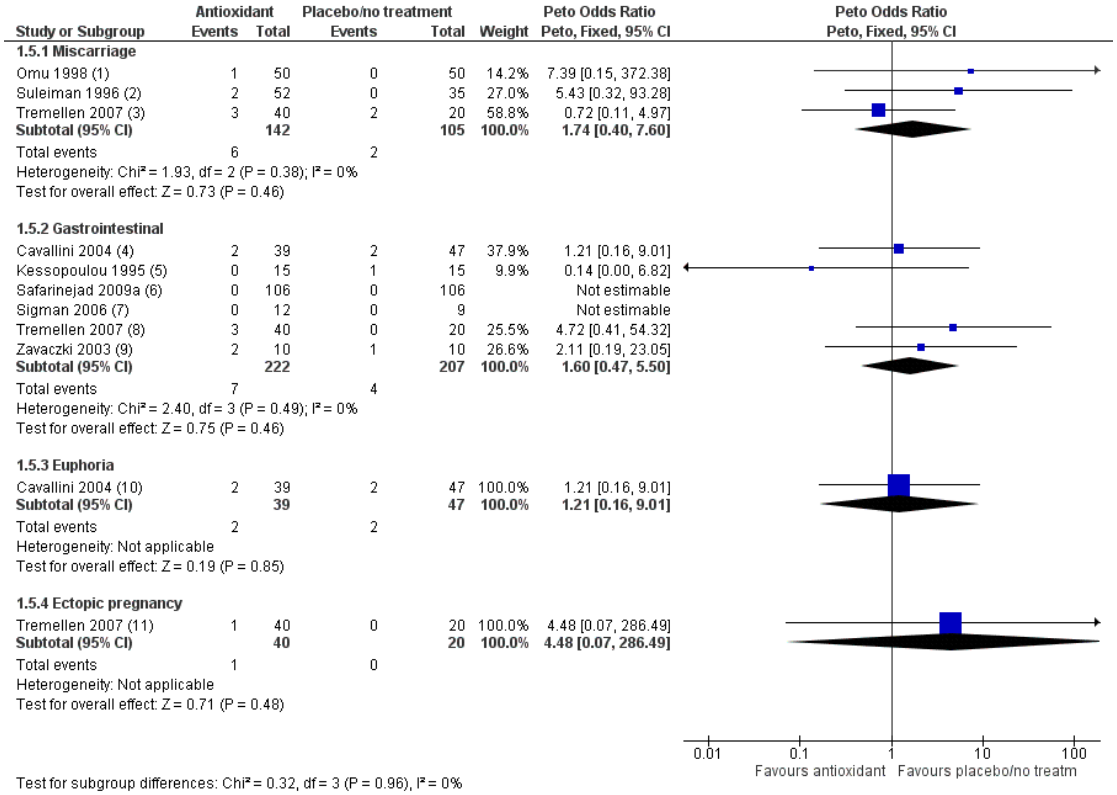
See Analysis 1.4

Kessopoulou 1995 and Tremellen 2007 enrolled men who were part of a couple undergoing IVF. Antioxidants were not associated with an increase in clinical pregnancy rate versus placebo in those couples undergoing IVF or ICSI (OR 2.64, 95% CI 0.94 to 7.41, P = 0.07, 2 RCTs, 90 men, I^2 = 0%).

1.5 Adverse events

See Analysis 1.5 and Figure 6

Figure 6. Forest plot of comparison: 1 Antioxidant(s) versus placebo or no treatment, outcome: 1.5 Adverse events.



The only adverse events reported in the trials were miscarriage, gastrointestinal disorders, euphoria and ectopic pregnancy.

1.5.1 Miscarriage. Only three trials (Omu 1998; Suleiman 1996; Tremellen 2007) reported on miscarriage and the event rate was very low (eight miscarriages from 247 couples). The analysis of these three trials showed no association between the use of antioxidants and miscarriage when compared to placebo or no treatment (OR 1.74, 95% CI 0.40 to 7.60, P = 0.46, 3 RCTs, 247 men, I^2 = 0%, very low quality evidence). This meant that within this population of subfertile men, with an expected miscarriage rate of 2%, the chances of having a miscarriage lay between 1% and 13% with the use of an antioxidant (Summary of findings for the main comparison).

1.5.2 Gastrointestinal. The analysis of six trials (Cavallini 2004; Kessopoulou 1995; Safarinejad 2009a; Sigman 2006; Tremellen 2007; Zavaczki 2003) showed no association between the use of antioxidants and gastrointestinal upsets when compared to placebo or no treatment (OR 1.60, 95% CI 0.47 to 5.50, P = 0.46, 6 RCTs, 429 men, I^2 = 0%). However, the event rate was very low so we could not be sure of these results.

1.5.3 Euphoria. Only one trial (Cavallini 2004) reported on this adverse event and there was no association between antioxidants and euphoria (OR 1.21, 95% CI 0.16 to 9.01, P = 0.85, 1 RCT, 86 men).

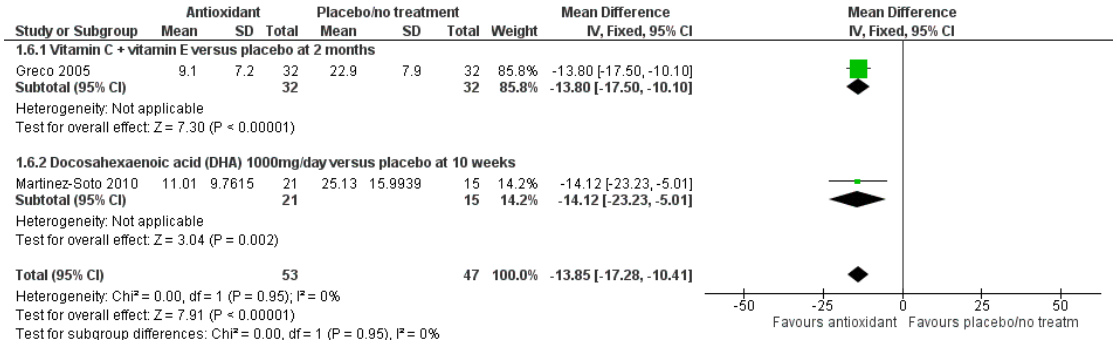
1.5.4 Ectopic pregnancy. Only one trial (Tremellen 2007) reported

on this adverse event and there was no association between antioxidants and ectopic pregnancy (OR 4.48, 95% CI 0.07 to 286.49, P = 0.48, 1 RCT, 60 men).
It was unlikely that these last two adverse events, euphoria and ectopic pregnancy, were related to intake of antioxidants.

1.6 Sperm DNA fragmentation; type of antioxidant

See Analysis 1.6
Two trials (Greco 2005; Martinez-Soto 2010) reported on DNA fragmentation and found that there was an association between antioxidants and a lower DNA fragmentation rate when compared to placebo (MD -13.85, 95% CI -17.28 to -10.41, P < 0.00001, 2 RCTs, 100 men, I^2 = 0%) (Figure 7).

Figure 7. Forest plot of comparison: 1 Antioxidant(s) versus placebo or no treatment, outcome: 1.6 Sperm DNA fragmentation; type of antioxidant.

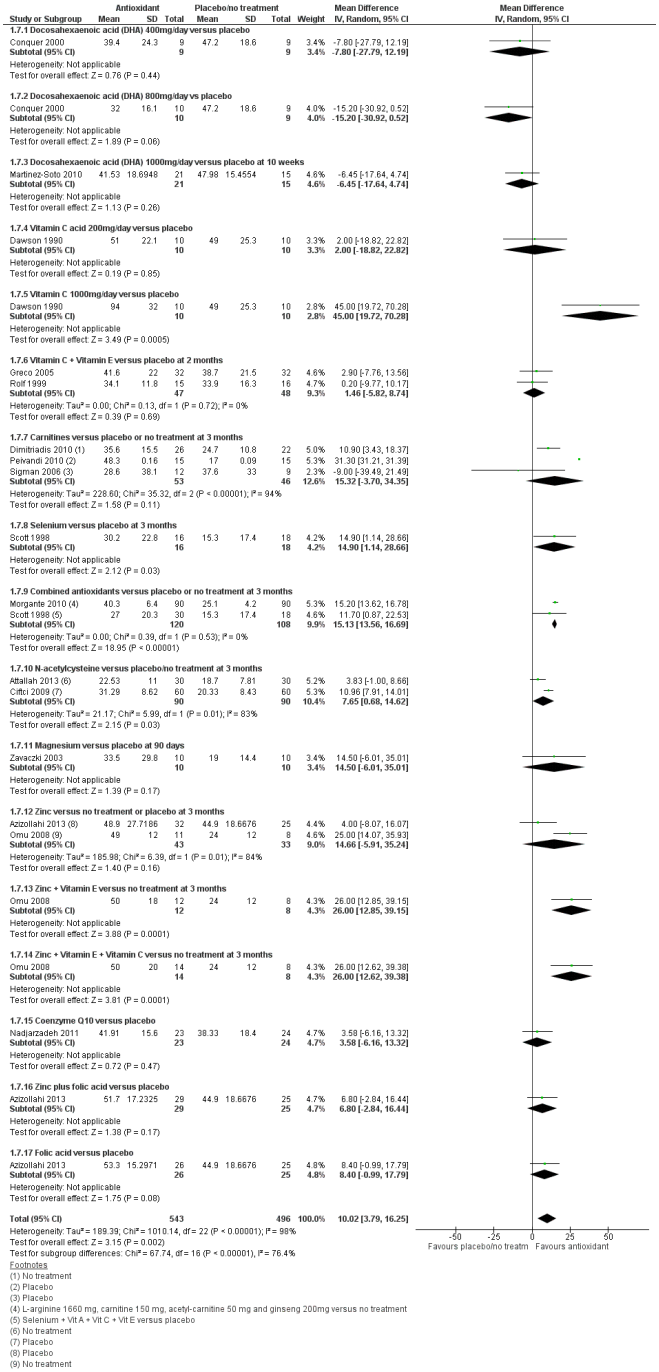


The two trials in this analysis looked a two different antioxidants: Greco 2005 reported that vitamin C + vitamin D was associated with a lower DNA fragmentation rate when compared to placebo (MD -13.80, 95% CI -17.50 to -10.10, P < 0.00001, 1 RCT, 64 men), and Martinez-Soto 2010 reported that docosahexaenoic acid was also associated with a lower DNA fragmentation rate when compared to placebo (MD -14.12, 95% CI -23.23 to -5-01, P < 0.002, 1 RCT, 36 men).

1.7 Total sperm motility at three months or less; type of antioxidant

See Analysis 1.7
Using a fixed-effect model and inverse variance statistical method this analysis had an I^2 statistic of 98%, indicating that were was extremely high heterogeneity overall and within the subgroups. Therefore, we analysed this outcome using a random-effects model (MD 10.02, 95% CI 3.79 to 16.25, P = 0.002, 16 RCTs, 1039 men, I^2 = 98%) and used subtotals as pooling was not possible (Figure 8).

Figure 8. Forest plot of comparison: 1 Antioxidant(s) versus placebo or no treatment, outcome: 1.7 Total sperm motility at 3 months or less; type of antioxidant.



1.7.1, 1.7.2, 1.7.3 Docosahexaenoic acid (DHA). Single trials only were available in the following subgroups, at three months:

- DHA 400 mg/day (Conquer 2000) (MD -7.80, 95% CI -27.79 to 12.19, P = 0.44, 18 men) showed no association with sperm motility when compared to placebo;
- DHA 800 mg/day (Conquer 2000) (MD -15.20, 95% CI -30.92 to 0.52, P = 0.06, 19 men) showed no association with sperm motility when compared to placebo;
- DHA 1000 mg/day (Martinez-Soto 2010) (MD -6.45, 95% CI -17.64 to 4.74, P = 0.26, 36 men) showed no association with sperm motility when compared to placebo.

1.7.4, 1.7.5 Vitamin C. A single trial (Dawson 1990) reported on:

- vitamin C 200 mg/day (MD 2.00, 95% CI -18.82 to 22.82, P = 0.85, 20 men) and there was no association with sperm motility when compared to placebo;
- vitamin C 1000 mg/day (MD 45.00, 95% CI 19.72 to 70.28, P = 0.0005, 20 men) and did show an association with improved sperm motility at three months when compared to placebo.

1.7.6 Vitamin C plus vitamin E. Two trials (Greco 2005; Rolf 1999) reported on vitamin C 1000 mg/day + vitamin E. There was no association with sperm motility when compared to placebo (MD 1.46, 95% CI -5.82 to 8.74, P = 0.69, 2 RCTs, 95 men, I^2 = 0%).

1.7.7 Carnitines. Three trials reported on carnitines (MD 15.32, 95% CI -3.70 to 34.35, P = 0.11, 3 RCTs, 99 men, I^2 = 94%); as heterogeneity was high we were unable to pool these trials and the individual results were:

- Dimitriadis 2010 showed an association with improved sperm motility at three months when compared to no treatment (MD 10.90, 95% CI 3.43 to 18.37, 48 men);
- Peivandi 2010 showed an association with improved sperm motility at three months when compared to placebo (MD 31.30, 95% CI 31.21 to 31.39, 30 men);
- Sigman 2006 showed no association with improved sperm motility at three months when compared to placebo (MD -9.00, 95% CI -39.49 to 21.49, 21 men).

1.7.8 Selenium. There was an association with the use of selenium and increased sperm motility when compared to placebo (MD 14.90, 95% CI 1.14 to 28.66, P = 0.03, 34 men) (Scott 1998).

1.7.9 Combined antioxidants. Two trials (Morgante 2010; Scott 1998) reported on the use of combined antioxidants. There was an association with improvement in sperm motility when compared to placebo or no treatment (MD 15.13, 95% CI 13.56 to 16.69, P < 0.00001, 2 RCTs, 228 men, I^2 = 0%). However, as one trial (Morgante 2010) had not described the method of randomisation and carried 97.9% of the weight in this analysis, the true association between the combined antioxidants and improved sperm motility remained unclear. Sensitivity analysis for this risk of bias was not possible as there were only two trials in this subgroup.

1.7.10 N-acetylcysteine. Two trials (Attallah 2013; Ciftci 2009) reported on N-acetylcysteine; heterogeneity was extremely high (MD 7.65, 95% CI 0.68 to 14.62, P = 0.03, 2 RCTs, 180 men, I^2 = 83%) therefore we could not pool these studies:

- Attallah 2013 showed no association with improved sperm motility at three months when compared to no treatment (MD 3.83, 95% CI -1.00 to 8.66, 60 men);
- Ciftci 2009 showed an association with improved sperm motility at three months when compared to placebo (MD 10.96, 95% CI 7.91 to 14.01, 120 men).

1.7.11 Magnesium. There was no association (MD 14.50, 95% CI -6.01 to 35.01, P = 0.17, 20 men) between the use of magnesium and sperm motility when compared to placebo in the single trial (Zavaczki 2003).

1.7.12 Zinc. Two trials reported on zinc (MD 14.66, 95% CI -5.91 to 35.24, P = 0.16, 2 RCTs, 76 men, I^2 = 84%). Here again heterogeneity was high so we couldn't pool the trial results:

- Azizollahi 2013 showed no association with improved sperm motility at three months when compared to placebo (MD 4.00, 95% CI -8.07 to 16.07, 57 men);
- Omu 2008 showed an association with improved sperm motility at three months when compared to no treatment (MD 25.00, 95% CI 14.07 to 35.93, 19 men).

1.7.13, 1.7.14 Zinc plus vitamin E, zinc plus vitamin E plus vitamin C. A single trial (Omu 2008) reported on both zinc plus vitamin E (MD 26.00, 95% CI 12.85 to 39.15, P = 0.0001, 20 men) and zinc plus vitamin E and vitamin C (MD 26.00, 95% CI 12.62 to 39.38, P = 0.0001, 22 men). A association with improved sperm motility was seen for both of these interventions when compared to no treatment.

1.7.15 Coenzyme Q10. Nadjarzadeh 2011 found no association between coenzyme Q10 and sperm motility when compared to placebo (MD 3.58, 95% CI -6.16 to 13.32, P = 0.47, 47 men).

1.7.16 Zinc plus folic acid. There was no association between the use of zinc plus folic acid (Azizollahi 2013) and sperm motility when compared to placebo (MD 6.80, 95% CI -2.84 to 16.44, P = 0.17, 54 men).

1.7.17 Folic acid. There was no association between the use of folic acid (Azizollahi 2013) and sperm motility when compared to placebo (MD 8.40, 95% CI -0.99 to 17.79, P = 0.08, 51 men).

1.8 Other data

Analysis 1.8

1.8.1, 1.8.2, 1.8.3, 1.8.4, 1.8.5 L-carnitine + Acetyl-carnitine, combined antioxidants, vitamin E, L-carnitine and selenium plus

zinc. Five studies (Cavallini 2004; Galatioto 2008; Kessopoulou 1995; Lenzi 2003; Sivkov 2011) provided data as medians, no standard deviations or percentages, and therefore they could not be used in the forest plot. Three of these trials (Galatioto 2008; Kessopoulou 1995; Sivkov 2011) found no difference between the intervention and control or no treatment for this outcome. Two (Cavallini 2004; Lenzi 2003) indicated that there might be some improvement in sperm motility in the intervention group when measured at three months, however these data were not rigorous and no conclusions could be made.

1.9 Total sperm motility at six months or less; type of antioxidant

See Analysis 1.9

Using a fixed-effect model and inverse variance statistical method this analysis had an I^2 statistic of 97%, indicating that were was an extremely high heterogeneity overall and within the subgroups. Therefore, we analysed this outcome using a random-effects model (MD 5.93 95% CI 3.52 to 8.35, P < 0.00001, 9 RCTs, 1203 men, I^2 = 97%) and used subtotals as pooling was not possible.

1.9.1 Carnitines. Three trials (Balercia 2005; Lenzi 2004; Sigman 2006) reported on carnitines (MD 7.28, 95% CI -9.47 to 24.02, P = 0.39, 3 RCTs, 107 men, I^2 = 90%). As the heterogeneity was high we were unable to pool these trials, individually their results were:

- Balercia 2005 showed an association with improved sperm motility at six months when compared to placebo (MD 21.13, 95% CI 14.58 to 27.68, 30 men);
- Lenzi 2004 showed no association with improved sperm motility at six months when compared to placebo (MD 1.56, 95% CI -4.48 to 7.60, 56 men);
- Sigman 2006 showed no association with improved sperm motility at six months when compared to placebo (MD -7.70, 95% CI -33.24 to 17.84, 21 men).

1.9.2, 1.9.3, 1.9.4 Selenium, N-acetyl-cysteine and selenium plus N-acetyl-cysteine. A single trial (Safarinejad 2009) reported:

- selenium did show an association with improved sperm motility when compared to placebo (MD 3.20, 95% CI 2.28 to 4.12, P ≤ 0.00001, 140 men);
- N-acetyl-cysteine did show an association with improved sperm motility when compared to placebo (MD 1.90, 95% CI 0.98 to 2.82, P ≤ 0.0001, 140 men);
- selenium plus N-acetyl-cysteine did show an association with improved sperm motility when compared to placebo (MD 6.30, 95% CI 5.38 to 7.22, P ≤ 0.00001, 140 men).

1.9.5 Coenzyme Q10. Three trials (Balercia 2009; Safarinejad 2009a; Safarinejad 2012) reported on coenzyme Q10 (MD 6.58, 95% CI 1.80 to 11.37, P = 0.007, 3 RCTs, 479 men, I^2 = 99%). As the heterogeneity was extremely high we were unable to pool these trials, individually their results were:

- Balercia 2009 did show an association with improved sperm motility when compared to placebo (MD 4.48, 95% CI 0.71 to 8.25, 60 men);
- Safarinejad 2009a did show an association with improved sperm motility when compared to placebo (MD 4.50, 95% CI 3.89 to 5.11, 194 men);
- Safarinejad 2012 did show an association with improved sperm motility when compared to placebo (MD 10.40, 95% CI 9.77 to 11.03, 225 men).

1.9.6 Vitamin E. There was an association between the use of vitamin E (Suleiman 1996) and sperm motility when compared to placebo (MD 13.00, 95% CI 7.02 to 18.98, P < 0.0001, 87 men).

1.9.7, 1.9.8, 1.9.9 Zinc, zinc plus folic acid and folic acid. A single trial (Azizollahi 2013) reported on:

- zinc did not show an association with improved sperm motility when compared to placebo (MD 0.00, 95% CI -10.19 to 10.19, P = 1.00, 40 men);
- zinc plus folic acid did not show an association with improved sperm motility when compared to placebo (MD 2.60, 95% CI -8.82 to 14.02, P = 0.66, 37 men);
- folic acid did not show an association with improved sperm motility when compared to placebo (MD 1.70, 95% CI -8.49 to 11.89, P = 0.74, 34 men).

1.10 Other data

See Analysis 1.10

1.10.1, 1.10.2, 1.10.3, 1.10.4 L-carnitine + acetyl-carnitine, folic acid, zinc, zinc plus folic acid. Two studies (Cavallini 2004; Wong 2002) provided data data as medians, no standard deviations or percentages, and therefore could not be used in the forest plot. Both trials indicated that there might be some improvement in sperm motility in the intervention group when measured at six months, however these data are not rigorous and no conclusions could be made.

1.11 Total sperm motility at nine months or more; type of antioxidant

See Analysis 1.11

Using a fixed-effect model and inverse variance statistical method this analysis had an I^2 statistic of 95%, indicating that were was high heterogeneity overall and within the subgroups. Therefore, we analysed this outcome using a random-effects model (MD 3.36, 95% CI 0.33 to 6.40, P = 0.03, 4 RCTs, 539 men, I^2 = 95%) and used subtotals as pooling was not possible.

1.11.1, 1.11.2, 1.11.3 L-carnitine, L-acetyl carnitine, L-carnitine + L-acetyl carnitine. A single trial (Balercia 2005) reported on:

- L-carnitine did show an association with improved sperm motility when compared to placebo (MD 11.54, 95% CI 1.66 to 21.42, P = 0.02, 20 men);

- L-acetyl carnitine did not show an association with improved sperm motility when compared to placebo (MD 7.84, 95% CI -1.41 to 17.09, P = 0.10, 20 men);
- L-carnitine + L-acetyl carnitine did not show an association with improved sperm motility when compared to placebo (MD 6.27, 95% CI -3.36 to 15.90, P = 0.20, 20 men).

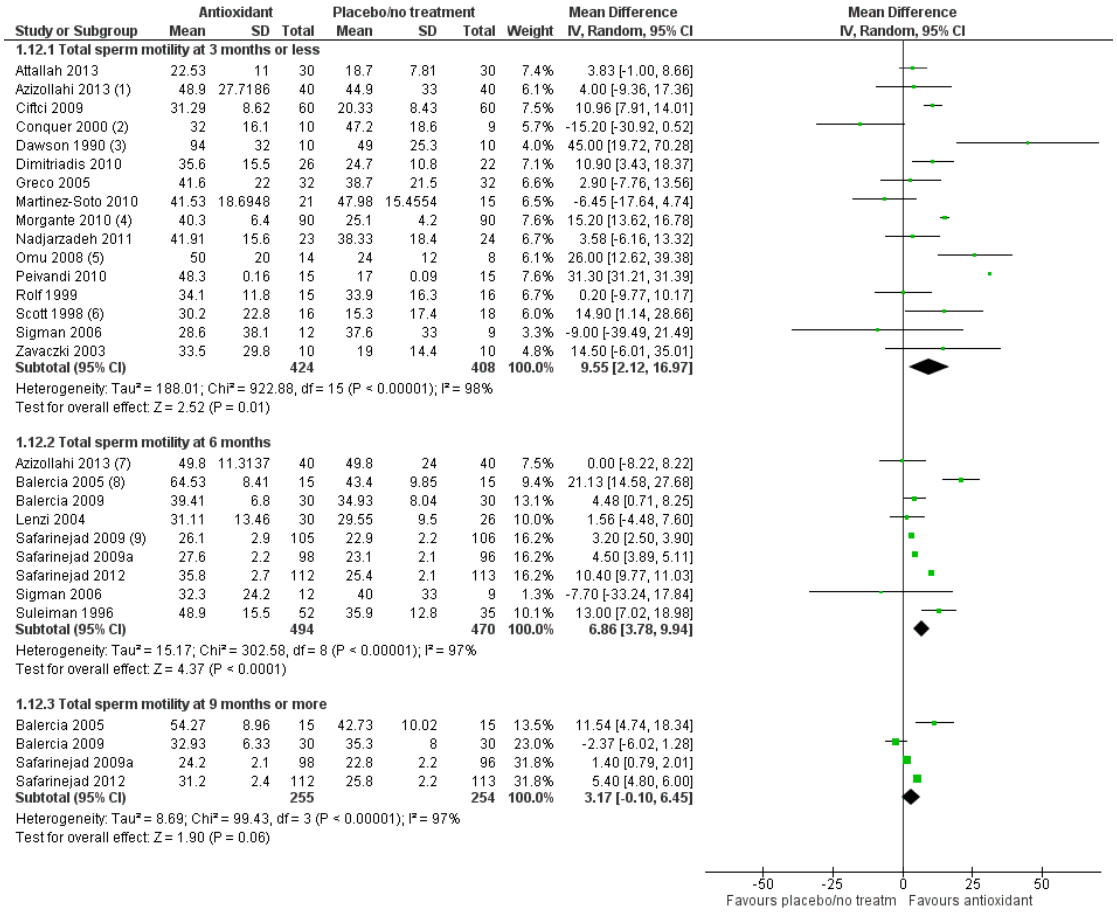
1.11.4 Coenzyme Q10. Three trials (Balercia 2009; Safarinejad 2009a; Safarinejad 2012) reported on coenzyme Q10 (MD 1.88 95% CI -1.58 to 5.34, P = 0.29, 3 RCTs, 479 men, I^2 = 98%). As the heterogeneity was extremely high we were unable to pool these trials, individually their results were:

- Balercia 2009 did not show an association with improved sperm motility when compared to placebo (MD -2.37, 95% CI -6.02 to 1.28, 60 men);
- Safarinejad 2009a did show an association with improved sperm motility when compared to placebo (MD 1.40, 95% CI 0.79 to 2.01, 194 men);
- Safarinejad 2012 did show an association with improved sperm motility when compared to placebo (MD 5.40, 95% CI 4.80 to 6.00, 225 men).

1.12 Total sperm motility over time

See Analysis 1.12 and Figure 9

Figure 9. Forest plot of comparison: 1 Antioxidant(s) versus placebo or no treatment, outcome: 1.12 Total sperm motility over time.



Footnotes

(1) Zinc versus placebo arm from 4 arm trial used here
(2) trial with 3 arms: Docosahexaenoic acid (DHA) 800mg/day arm used in this analysis
(3) trial with 3 arms: Ascorbic acid 1000mg/day arm used in this analysis
(4) L-arginine 1660 mg, carnitine 150 mg, acetyl-carnitine 50 mg and ginseng 200mg versus no treatment
(5) trial with 3 arms: Zinc+ vitamin E + vitamin C arm used in this analysis
(6) Selenium versus placebo used in this analysis from a three arm trial
(7) Zinc versus placebo used here from a 4 arm trial
(8) trial with 4 arms: L-carnitine versus placebo arm used in this analysis for 6 and 9 months
(9) trial with 4 arms: Selenium versus placebo arm used in this analysis

This analysis was only useful in directly comparing the same trials reporting at the three time points and not in comparing results of meta-analyses that included different subsets of trials.

1.12.1 Total sperm motility at three months or less. Using a fixed-effect model and inverse variance statistical method this analysis had an I^2 statistic of 98%, indicating that were was extremely high heterogeneity, therefore we analysed this outcome using a random-effects model (MD 9.55, 95% CI 2.12 to 16.97, P = 0.01, 16 RCTs, 832 men, I^2 = 98%) and used subtotals (Attallah 2013; Azizollahi 2013; Ciftci 2009; Conquer 2000; Dawson 1990; Dimitriadis 2010; Greco 2005; Martinez-Soto 2010; Morgante 2010; Nadjarzadeh 2011; Omu 2008; Peivandi 2010; Rolf 1999; Scott 1998; Sigman 2006; Zavaczki 2003).

1.12.2 Total sperm motility at six months. In this analysis the heterogeneity was also very high (I^2 = 97%) therefore a random-effects model was used (MD 6.86, 95% CI 3.78 to 9.94, P < 0.0001, 9 RCTs, 964 men, I^2 = 97%). We were unable to pool these trials (Azizollahi 2013; Balercia 2005; Balercia 2009; Lenzi 2004; Safarinejad 2009; Safarinejad 2009a; Safarinejad 2012; Sigman 2006; Suleiman 1996).

1.12.3 Total sperm motility at nine months or more. Using a fixed-effect model and inverse variance statistical method this analysis had an I^2 statistic of 97%, indicating extremely high heterogeneity overall and within the subgroups. We analysed this outcome using a random-effects model (MD 3.17, 95% CI -0.10 to 6.45, P = 0.06, 4 RCTs, 509 men, I^2 = 97%). We were unable to pool these trials (Balercia 2005; Balercia 2009; Safarinejad 2009a; Safarinejad 2012).

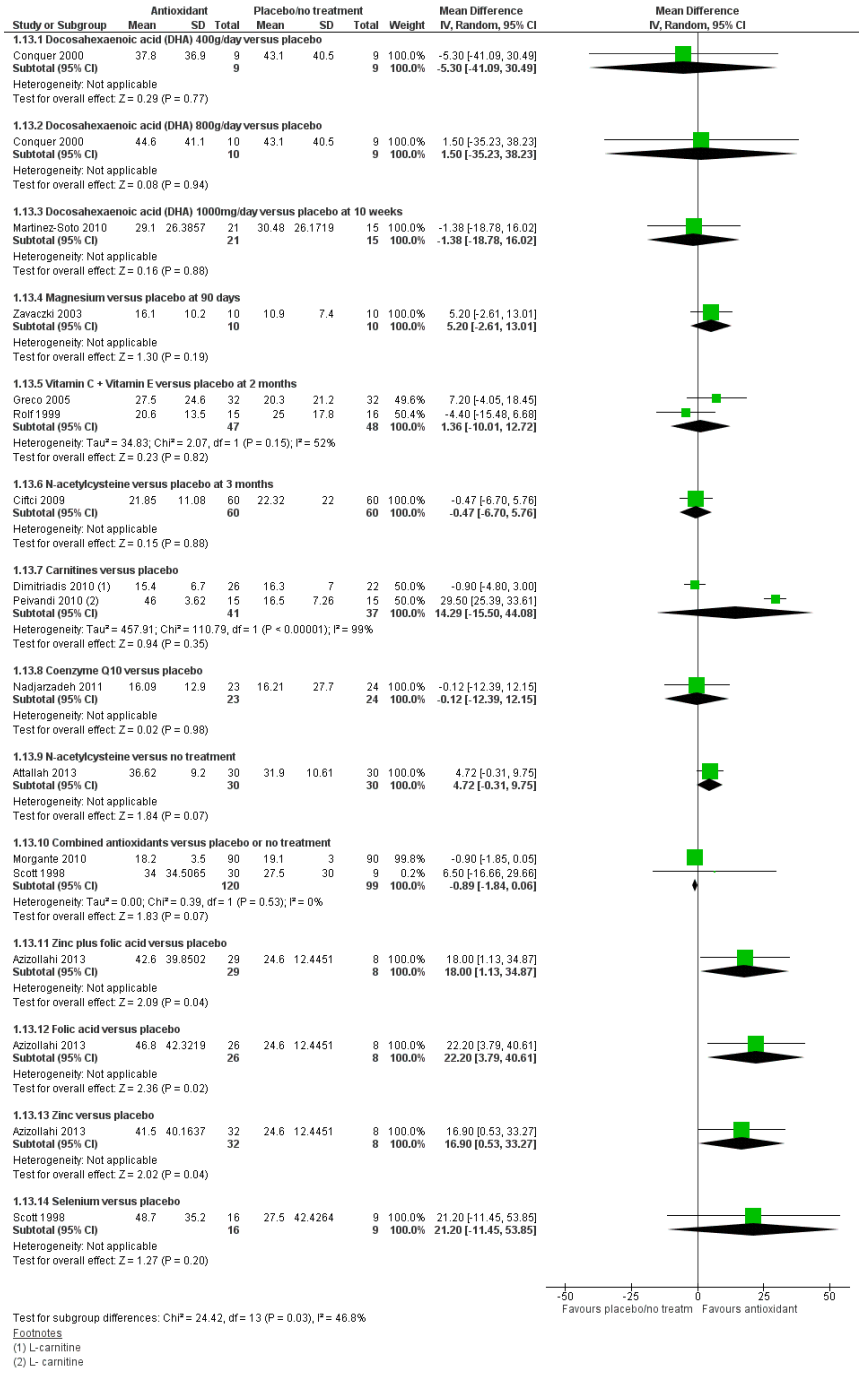
Using only one arm of the multi-arm trials in these analyses meant that the numbers differed slightly from the total sperm motility analyses (Analysis 1.7; Analysis 1.9; Analysis 1.11). which have incorporated all arms of each multi-arm trial in the analysis with the control arms being divided accordingly.

1.13 Sperm concentration at three months or less; type of antioxidant

See Analysis 1.13

Using a fixed-effect model and inverse variance statistical method this analysis had an I^2 statistic of 93%, indicating that there was high heterogeneity overall and within the subgroups, therefore we analysed this outcome using a random-effects model (MD 6.79, 95% CI 0.50 to 13.08, P = 0.03, 13 RCTs, 848 men, I^2 = 93%). We used only subtotals in this analysis (Figure 10).

Figure 10. Forest plot of comparison: 1 Antioxidant(s) versus placebo or no treatment, outcome: 1.13 Sperm concentration at 3 months or less; type of antioxidant.



1.13.1, 1.13.2, 1.13.3 Docosahexaenoic acid (DHA). Single trials only were available in these subgroups:

- DHA 400 mg/day (Conquer 2000) showed no association with sperm concentration when compared to placebo at three months (MD -5.30, 95% CI -41.09 to 30.49, P = 0.77, 18 men);
- DHA 800 mg/day (Conquer 2000) showed no association with sperm concentration when compared to placebo at three months (MD 1.50, 95% CI -35.23 to 38.23, P = 0.94, 19 men);
- DHA 1000 mg/day (Martinez-Soto 2010) showed no association with sperm concentration when compared to placebo at three months (MD -1.38, 95% CI -18.78 to 16.02, P = 0.88, 36 men).

1.13.4 Magnesium. There was no association between the use of magnesium and increased sperm concentration when compared to placebo (MD 5.20, 95% CI -2.61 to 13.01, P = 0.19, 20 men) (Zavaczki 2003).

1.13.5 Vitamin C + vitamin E. Two trials (Greco 2005; Rolf 1999) reported on vitamin C + vitamin E (MD 1.36, 95% CI -10.01 to 12.72, P = 0.82, 2 RCTs, 95 men, I^2 = 52%) and showed no association with sperm concentration when compared to placebo at three months.

1.13.6 N-acetylcysteine. There was no association between the use of N-acetylcysteine and increased sperm concentration when compared to placebo (MD -0.47, 95% CI -6.70 to 5.76, P = 0.88, 120 men) (Ciftci 2009).

1.13.7 Carnitines. Two trials (Dimitriadis 2010; Peivandi 2010) reported on carnitines (MD 14.29, 95% CI -15.50 to 44.08, P = 0.35, 2 RCTs, 78 men, I^2 = 99%). As the heterogeneity was high we were unable to pool these trials. Individually their results were:

- Dimitriadis 2010 showed no association with improved sperm concentration at three months when compared to no treatment (MD -0.90, 95% CI -4.80 to 3.00, 48 men);
- Peivandi 2010 showed an association with improved sperm concentration at three months when compared to placebo (MD 29.50, 95% CI 25.39 to 33.61, 30 men).

1.13.8 Coenzyme Q10. There was no association between the use of coenzyme Q10 and increased sperm concentration when compared to placebo (MD -0.12, 95% CI -12.39 to 12.15, P = 0.98, 47 men) (Nadjarzadeh 2011).

1.13.9 N-acetylcysteine. There was no association between the use of N-acetylcysteine and increased sperm concentration when compared to no treatment (MD 4.72, 95% CI -0.31 to 9.75, P = 0.07, 60 men) (Attallah 2013).

1.13.10 Combined antioxidants. Two trials (Morgante 2010; Scott 1998) reported on combined antioxidants (MD -0.89, 95% CI -1.84 to 0.06, P = 0.07, 2 RCTs, 219 men, I^2 = 0%) and showed no association with sperm concentration when compared to placebo and no treatment at three months.

1.13.11, 1.13.12, 1.13.13 Zinc plus folic acid, folic acid, zinc. A single trial (Azizollahi 2013) reported:

- zinc plus folic acid did show an association with improved sperm concentration when compared to placebo (MD 18.00, 95% CI 1.13 to 34.87, P = 0.04, 37 men);
- folic acid did show an association with improved sperm concentration when compared to placebo (MD 22.20, 95% CI 3.79 to 40.61, P = 0.02, 34 men);
- zinc did show an association with improved sperm concentration when compared to placebo (MD 16.90, 95% CI 0.53 to 32.27, P = 0.04, 40 men).

1.13.14 Selenium. There was no association between the use of selenium and increased sperm concentration when compared to placebo (MD 21.20, 95% CI -11.45 to 53.85, P = 0.20, 25 men) (Scott 1998).

1.14 Other data

See Analysis 1.14

1.14.1, 1.14.2, 1.14.3 L-carnitine + acetyl-carnitine, vitamin E and L-carnitine. Two trials (Cavallini 2004; Kessopoulou 1995) provided data as medians and interquartile ranges and therefore could not be used in the forest plot. Both trials indicated that there might be some improvement in sperm concentration in the intervention group when measured at three months, however these data were not rigorous and no conclusions could be made. One trial (Lenzi 2003) provided data as the mean with no standard deviation, the P value was 0.03 indicating that there may have been an association between L-carnitine and improved sperm concentration at three months.

1.15 Sperm concentration at six months; type of antioxidant

See Analysis 1.15

Using a fixed-effect model and inverse variance statistical method this analysis had an I^2 statistic of 88%, indicating that there was high heterogeneity overall and within the subgroups, therefore we analysed this outcome using a random-effects model (MD 6.46, 95% CI 3.53 to 9.40, P < 0.00001, 7 RCTs, 1125 men, I^2 = 88%). We used only subtotals in this analysis.

1.15.1 Carnitines. Two trials (Balercia 2005; Lenzi 2004) reported on carnitines (MD 2.59, 95% CI -3.11 to 8.30, P = 0.37, 2 RCTs, 116 men, I^2 = 0%) and showed no association with sperm concentration when compared to placebo or no treatment at six months.

1.15.2, 1.15.3, 1.15.4, Selenium, N-acetyl-cysteine, selenium plus N-acetyl-cysteine. A single trial (Safarinejad 2009) reported:

- selenium did show an association with improved sperm concentration when compared to placebo (MD 4.10, 95% CI 1.82 to 6.38, P = 0.0004, 140 men);

- N-acetyl-cysteine did show an association with improved sperm concentration when compared to placebo (MD 3.30, 95% CI 1.13 to 5.47, P = 0.003, 140 men);
- selenium plus N-acetyl-cysteine did show an association with improved sperm concentration when compared to placebo (MD 8.60, 95% CI 6.28 to 10.92, P < 0.00001, 139 men).

1.15.5 Coenzyme Q10. Three trials reported on coenzyme Q10 (MD 6.88, 95% CI 1.20 to 12.56, P = 0.02, 3 RCTs, 479 men, I^2 = 96%). As the heterogeneity was high we were unable to pool these trials. Individually their results were:

- Balercia 2009 showed no association with improved sperm concentration at six months when compared to placebo (MD -1.44, 95% CI -11.33 to 8.45, 60 men);
- Safarinejad 2009a showed an association with improved sperm concentration at six months when compared to placebo (MD 5.60, 95% CI 4.38 to 6.82, 194 men);
- Safarinejad 2012 showed an association with improved sperm concentration at six months when compared to placebo (MD 11.90, 95% CI 10.72 to 13.08, 225 men).

1.15.6, 1.15.7, 1.15.8 Zinc plus folic acid, folic acid, zinc. A single trial (Azizollahi 2013) reported that:

- zinc plus folic acid did not show an association with improved sperm concentration when compared to placebo (MD 17.70, 95% CI -1.88 to 37.28, P = 0.08, 37 men);
- folic acid did show an association with improved sperm concentration when compared to placebo (MD 19.20, 95% CI 4.74 to 33.66, P = 0.009, 34 men);
- zinc did not show an association with improved sperm concentration when compared to placebo (MD 9.70, 95% CI -7.01 to 26.41, P = 0.26, 40 men).

1.16 Other data

Analysis 1.16,
1.16.1, 1.16.2, 1.16.3, 1.16.4 L-carnitine + acetyl-carnitine, folic acid, zinc, zinc plus folic acid. Two studies (Cavallini 2004; Wong 2002) provided data as medians with no standard deviations or percentages, and therefore could not be used in the forest plot. Both of these trials indicated that there might be some improvement in sperm concentration in the intervention group when measured at six months.

1.17 Sperm concentration at nine months; type of antioxidant

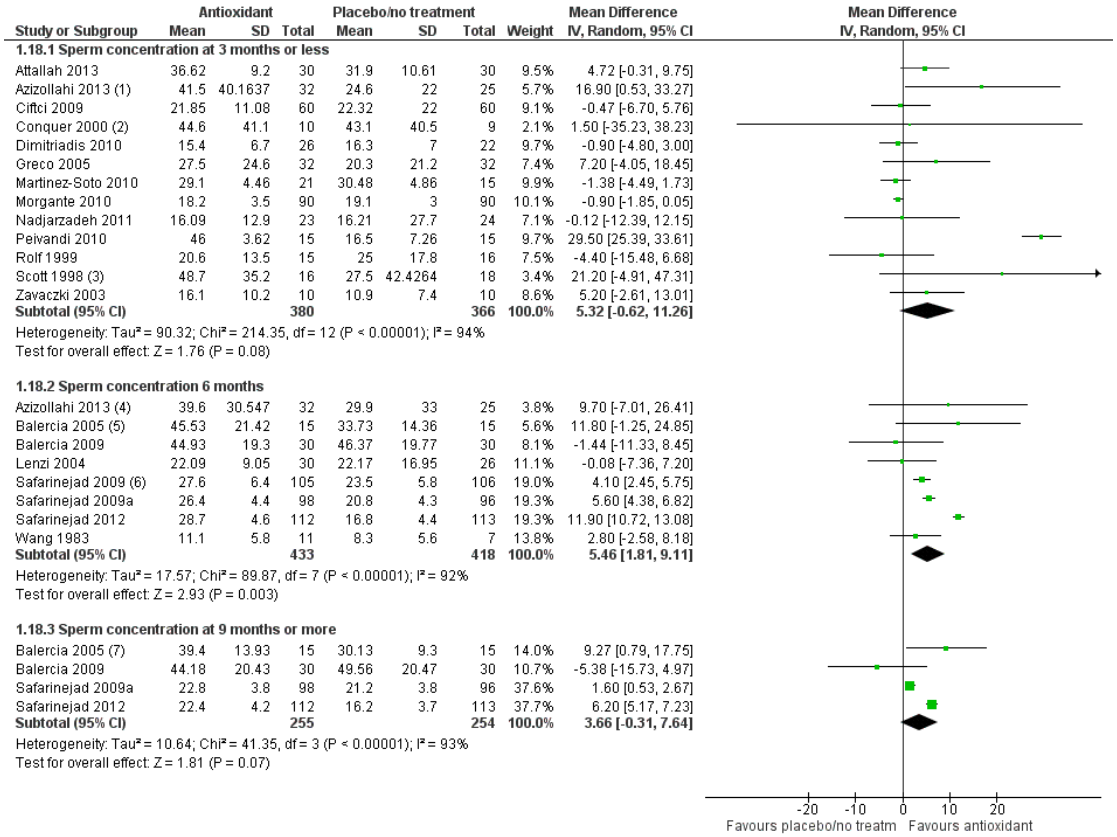
See Analysis 1.17
Using a fixed-effect model and inverse variance statistical method this analysis had an I^2 statistic of 88%, indicating high heterogeneity overall and within the subgroups. Therefore, we analysed this outcome using a random-effects model (MD 3.18, 95% CI -0.36 to 6.73, P = 0.08, 4 RCTs, 539 men, I^2 = 88%). We used only subtotals in this analysis.
1.17.1 Carnitines: L-acetyl carnitine, L-carnitine plus L-acetyl carnitine and L-carnitine. There was no association between the use of carnitines and increased sperm concentration when compared to placebo (MD 4.12, 95% CI -1.74 to 9.99, P = 0.17, 60 men) (Balercia 2005).
1.17.2 Coenzyme Q10. Three trials reported on coenzyme Q10 (MD 2.74, 95% CI -1.56 to 7.05, P = 0.21, 3 RCTs, 479 men, I^2 = 95%). As the heterogeneity was high we were unable to pool these trials, individually their results were:

- Balercia 2009 showed no association with improved sperm concentration at nine months when compared to placebo (MD -5.38, 95% CI -15.73 to 4.97, 60 men);
- Safarinejad 2009a showed an association with improved sperm concentration at nine months when compared to placebo (MD 1.60, 95% CI 0.53 to 2.67, 194 men);
- Safarinejad 2012 showed an association with improved sperm concentration at nine months when compared to placebo (MD 6.20, 95% CI 5.17 to 7.23, 225 men).

1.18 Sperm concentration over time

See Analysis 1.18, Figure 11

Figure 11. Forest plot of comparison: 1 Antioxidant(s) versus placebo or no treatment, outcome: 1.18 Sperm concentration over time.



Footnotes
(1) Zinc versus placebo used here from a 4 arm trial
(2) trial with 3 arms: Docosahexaenoic acid (DHA) 800mg/day arm used in this analysis
(3) Selenium versus placebo used in this analysis from a three arm trial
(4) Zinc versus placebo used here from a 4 arm trial
(5) trial with 3 arms: L-carnitine versus placebo arm used in this analysis
(6) trial with 3 arms: Selenium versus placebo arm used in this analysis
(7) trial with 4 arms: L-carnitine versus placebo arm used in this analysis

This analysis was only useful in directly comparing the same trials reporting at the three time points and not in comparing results of meta analyses that included different subsets of trials.

1.18.1 Total sperm concentration at three months or less. Using a fixed-effect model and inverse variance statistical method this analysis had an I^2 statistic of 94%, indicating extremely high heterogeneity. Therefore, we analysed this outcome using a random-effects model (MD 5.32, 95% CI -0.62 to 11.26, P = 0.08, 13 RCTs, 746 men, I^2 = 94%). We were unable to pool these trials (Attallah 2013; Azizollahi 2013; Ciftci 2009; Conquer 2000; Dimitriadis 2010; Greco 2005; Martinez-Soto 2010; Morgante 2010; Nadjarzadeh 2011; Peivandi 2010; Rolf 1999; Scott 1998; Zavaczki 2003).

1.18.2 Total sperm concentration at six months. In this analysis the heterogeneity was also very high (I^2 = 92%), therefore a random-effects model was used (MD 5.46, 95% CI 1.81 to 9.11, P = 0.003, 8 RCTs, 851 men, I^2 = 92%). We were unable to pool these trials (Azizollahi 2013; Balercia 2005; Balercia 2009; Lenzi 2004; Safarinejad 2009; Safarinejad 2009a; Safarinejad 2012; Wang 1983).

1.18.3 Total sperm concentration at nine months or more. Using a fixed-effect model and inverse variance statistical method this analysis had an I^2 statistic of 93%, indicating extremely high heterogeneity. Therefore, we analysed this outcome using a random-effects model (MD 3.66, 95% CI -0.31 to 7.64, P = 0.07, 4 RCTs, 509 men, I^2 = 93%). We were unable to pool these trials (Balercia 2005; Balercia 2009; Safarinejad 2009a; Safarinejad 2012).

Using only one arm of the multi-arm trials in these analyses meant that the numbers differed slightly from the total sperm concentration analyses (Analysis 1.13; Analysis 1.15; Analysis 1.17). These analyses have incorporated all arms of each multi-arm trial in the analysis with the control arms being divided accordingly.

2 Head to head antioxidants (natural conception and undergoing fertility treatment)

The trials included in this comparison did not report on live birth, clinical pregnancy, adverse events or sperm DNA fragmentation.

2.1 Total sperm motility at three months or less; type of antioxidant

See Analysis 2.1

Totals were not used in this analysis as, of the eight trials included, there were data for one trial only per subgroup, and therefore pooling was not possible.

2.1.1 Ethyl cysteine 600 mg/day versus vitamin E. There was no association between the use of ethyl cysteine and increased sperm motility when compared to vitamin E (MD -1.90, 95% CI -41.97 to 38.17, P = 0.93, 10 men) (Akiyama 1999).

2.1.2 Docosahexaenoic acid (DHA) 400 g/day versus docosahexaenoic acid 800 mg/day. There was no association between the use of docosahexaenoic acid 400 g/day and increased sperm motility when compared to docosahexaenoic acid 800 mg/day (MD 7.40, 95% CI -11.35 to 26.15, P = 0.44, 19 men) (Conquer 2000).

2.1.3 Vitamin C 200 mg/day versus ascorbic acid 1000 mg/day. There was an association between the use of ascorbic acid 200 mg/day and decreased sperm motility when compared to ascorbic acid 1000 mg/day (MD -43.00, 95% CI -67.10 to -18.90, P = 0.0005, 20 men) (Dawson 1990).

2.1.4 Vitamin E + selenium versus vitamin B. There was no association between the use of vitamin E and increased sperm motility when compared to selenium versus vitamin B (MD 0.00, 95% CI -10.71 to 10.71, P = 1.00, 54 men) (Keskes-Ammar 2003).

2.1.5 Zinc versus zinc + vitamin E. There was no association between the use of zinc and increased sperm motility when compared to zinc + vitamin E (MD -1.00, 95% CI -15.00 to 13.00, P = 0.89, 18 men) (Omu 2008).

2.1.6 Zinc versus zinc + vitamin E + vitamin C. There was no association between the use of zinc and increased sperm motility when compared to zinc + vitamin E + vitamin C (MD -1.00, 95% CI -19.66 to 17.66, P = 0.89, 12 men) (Omu 2008).

2.1.7 Zinc + vitamin E versus zinc + vitamin E + vitamin C. There was no association between the use of zinc + vitamin E and increased sperm motility when compared to zinc + vitamin E + vitamin C (MD -0.00, 95% CI -18.97 to 18.97, P = 1.00, 18 men) (Omu 2008).

2.1.8 Selenium versus combined antioxidants. There was no association between the use of selenium and increased sperm motility when compared to combined antioxidants (MD 3.20, 95% CI -10.13 to 16.53, P = 0.64, 46 men) (Scott 1998).

2.1.9 L acetyl carnitine + L carnitine versus vitamin E + vitamin C. There was an association between the use of L acetyl carnitine + L carnitine and increased sperm motility when compared to vitamin E + vitamin C (MD 23.05, 95% CI 20.09 to 26.01, P < 0.00001, 138 men) (Li 2005).

2.1.10 Zinc + folic acid versus folic acid. There was no association between the use of zinc + folic acid and increased sperm motility when compared to folic acid alone (MD -0.60, 95% CI -7.74 to 6.54, P = 0.87, 80 men) (Azizollahi 2013).

2.1.11 Zinc versus zinc + folic acid. There was no association between the use of zinc and increased sperm motility when compared to zinc + folic acid (MD -2.80, 95% CI -12.91 to 7.31, P = 0.59, 80 men) (Azizollahi 2013).

2.1.12 Zinc versus folic acid. There was no association between the use of zinc and increased sperm motility when compared to folic acid (MD -4.40, 95% CI -14.21 to 5.41, P = 0.38, 80 men) (Azizollahi 2013).

2.2 Total sperm motility at six months or less; type of antioxidant

See Analysis 2.2

Pooling was not possible in this analysis as of the three trials included in this analysis there were data for one trial per subgroup.

2.2.1 L-acetyl carnitine + L-carnitine versus L-carnitine. There was no association between the use of L-acetyl carnitine + L-carnitine and increased sperm motility when compared to L-carnitine (MD -3.46, 95% CI -9.72 to 2.80, P = 0.28, 30 men) (Balercia 2005).

2.2.2 L-acetyl carnitine + L-carnitine versus L-acetyl carnitine. There was no association between the use of L-acetyl carnitine + L-carnitine and increased sperm motility when compared to L-acetyl carnitine (MD 0.64, 95% CI -6.37 to 7.65, P = 0.86, 30 men) (Balercia 2005).

2.2.3 Selenium versus N-acetyl-cysteine. There was an association between the use of selenium and increased sperm motility when compared to N-acetyl-cysteine (MD 1.30, 95% CI 0.56 to 2.04, P = 0.0006, 234 men) (Safarinejad 2009).

2.2.4 Selenium versus selenium + N-acetyl-cysteine. There was an association between the use of selenium and decreased sperm motility when compared to selenium + N-acetyl-cysteine (MD -3.10, 95% CI -3.85 to -2.35, P < 0.00001, 232 men) (Safarinejad 2009).

2.2.5 N-acetyl-cysteine versus selenium plus N-acetyl-cysteine. There was an association between the use of N-acetyl-cysteine and decreased sperm motility when compared to selenium + N-acetyl-cysteine (MD -4.40, 95% CI -5.14 to -3.66, P < 0.00001, 234 men) (Safarinejad 2009).

2.2.6 Zinc + folic acid versus folic acid. There was no association between the use of zinc + folic acid and increased sperm motility when compared to folic acid (MD 0.90, 95% CI -5.45 to 7.25, P

= 0.78, 80 men) (Azizollahi 2013).
2.2.7 Zinc versus zinc + folic acid. There was no association between the use of zinc and increased sperm motility when compared to zinc + folic acid (MD -2.60, 95% CI -9.13 to 3.93, P = 0.44, 80 men) (Azizollahi 2013).
2.2.8 Zinc versus folic acid. There was no association between the use of zinc and increased sperm motility when compared to folic acid (MD -1.70, 95% CI -6.42 to 3.02, P = 0.48, 80 men) (Azizollahi 2013).

2.3 Other data

See Analysis 2.3
2.3.1, 2.3.2, 2.3.3 Zinc versus folic acid, zinc versus zinc + folic acid, folic acid versus zinc + folic acid. One trial Wong 2002 reported data as medians and ranges for these three subgroups. There was no indication of any difference in effect for total sperm motility at six months between the intervention and control groups, however these data were not rigorous and no conclusions could be made.

2.4 Total sperm motility at nine months or more; type of antioxidant

See Analysis 2.4
Pooling was not possible in this analysis as it included only one trial.
2.4.1, 2.4.2 L-aceytl carnitine + L-carnitine versus L-carnitine, L-acetyl carnitine + L-carnitine versus L-acetyl carnitine. A single trial (Balercia 2005) reported on:

- L-aceytl carnitine + L-carnitine versus L-carnitine (MD -5.27, 95% CI -11.28 to 0.74, P = 0.09, 30 men), L-aceytl carnitine + L-carnitine did not show an association with improved sperm motility when compared to L-carnitine;
- L-acetyl carnitine + L-carnitine versus L-acetyl carnitine (MD -1.57, 95% CI -6.46 to 3.32, P = 0.53, 34 men), L-acetyl carnitine + L-carnitine did show an association with improved sperm motility when compared to L-acetyl carnitine.

2.5 Sperm concentration at three months or less; type of antioxidant

See Analysis 2.5
Pooling was not possible in this analysis as the six trials included in this analysis reported on single subgroups.
2.5.1 Ethyl cysteine 600 mg/day versus vitamin E. There was no association between the use of ethyl cysteine and increased sperm concentration when compared to vitamin E (MD 2.20, 95% CI -16.65 to 21.05, P = 0.82, 10 men) (Akiyama 1999).
2.5.2 Docosahexaenoic acid (DHA) 400 g/day versus docosahexaenoic acid 800 g/day. There was no association between the use of DHA 400 g and increased sperm concentration when compared to DHA 800 g (MD -6.80, 95% CI -41.87 to 28.27, P = 0.70, 19 men) (Conquer 2000).
2.5.3 L-carnitine versus vitamin E + vitamin C. There was an association between the use of L-carnitine and increased sperm concentration when compared to vitamin E + vitamin C (MD 15.50, 95% CI 12.49 to 18.51, P < 0.00001, 63 men) (Li 2005a).
2.5.4 L-carnitine plus vitamin E versus vitamin E. There was no association between the use of L-carnitine plus vitamin E and increased sperm concentration when compared to vitamin E (MD 1.90, 95% CI -10.52 to 14.32, P = 0.76, 113 men) (Wang 2010).
2.5.5 Selenium versus combined antioxidants. There was no association between the use of selenium and increased sperm concentration when compared to combined antioxidants (MD 14.70, 95% CI -6.51 to 35.91, P = 0.17, 46 men) (Scott 1998).
2.5.6, 2.5.7, 2.5.8 Zinc + folic acid versus folic acid, zinc versus zinc + folic acid, zinc versus folic acid. A single trial Azizollahi 2013 reported on:

- zinc + folic acid versus folic acid (MD -4.20, 95% CI -22.21 to 13.81, P = 0.65, 80 men), zinc + folic acid did not show an association with improved sperm concentration when compared to folic acid alone;
- zinc versus zinc + folic acid (MD -1.10, 95% CI -18.63 to 16.43, P = 0.90, 80 men), zinc did not show an association with improved sperm concentration when compared to zinc + folic acid;
- zinc versus folic acid (MD -5.30, 95% CI -23.38 to 12.78, P = 0.57, 80 men), zinc did not show an association with improved sperm concentration when compared to folic acid.

2.6 Sperm concentration at six months or less; type of antioxidant

See Analysis 2.6
Pooling was not possible in this analysis as of the three trials included in this analysis there were data for only one trial per subgroup.
2.6.1, 2.6.2 L-aceytl carnitine + L-carnitine versus L-carnitine, L-acetyl carnitine + L-carnitine versus L-acetyl carnitine. Balercia 2005 reported on:

- L-aceytl carnitine + L-carnitine versus L-carnitine (MD -8.13, 95% CI -21.79 to 5.53, P = 0.24, 30 men), L-aceytl carnitine + L-carnitine did not show an association with improved sperm concentration when compared to L-carnitine;
- L-acetyl carnitine + L-carnitine versus L-acetyl carnitine (MD -2.17, 95% CI -15.26 to 10.92, P = 0.75, 30 men), L-aceytl carnitine + L-carnitine did not show an association with improved sperm concentration when compared to L-acetyl carnitine.

2.6.3, 2.6.4, 2.6.5 Selenium versus N-acetyl-cysteine, selenium versus selenium + N-acetyl-cysteine, N-acetyl-cysteine versus selenium + N-acetyl-cysteine. A single trial (Safarinejad 2009) reported on:

- selenium versus N-acetyl-cysteine (MD 0.80, 95% CI -0.71 to 2.31, P = 0.30, 234 men), selenium did not show an association with improved sperm concentration when compared to N-acetyl-cysteine;
- selenium versus selenium + N-acetyl-cysteine (MD -4.50, 95% CI -6.20 to -2.80, P < 0.00001, 232 men), selenium showed an association with decreased sperm concentration when compared to N-acetyl-cysteine;
- N-acetyl-cysteine versus selenium + N-acetyl-cysteine (MD -5.30, 95% CI -6.86 to -3.74, P < 0.00001, 234 men), N-acetyl-cysteine showed an association with decreased sperm concentration when compared to selenium + N-acetyl-cysteine.

2.6.6, 2.6.7, 2.6.8 Zinc + folic acid versus folic acid, zinc versus zinc + folic acid, zinc versus folic acid. Azizollahi 2013 reported on:

- zinc + folic acid versus folic acid (MD -1.50, 95% CI -15.06 to 12.06, P = 0.83, 80 men), zinc + folic acid did not show an association with improved sperm concentration when compared to folic acid;
- zinc versus zinc + folic acid (MD -8.00, 95% CI -23.69 to 7.69, P = 0.32, 80 men), zinc did not show an association with improved sperm concentration when compared to zinc + folic acid;
- zinc versus folic acid (MD -9.50, 95% CI -20.31 to 1.31, P = 0.08, 80 men), zinc did not show an association with improved sperm concentration when compared to folic acid.

2.7 Other data

See Analysis 2.7
2.7.1, 2.7.2, 2.7.3 Zinc versus folic acid, zinc versus zinc + folic acid, folic acid versus zinc + folic acid. One trial Wong 2002 reported data as medians and ranges for these three subgroups. There may have been an association with improved sperm concentration at six months for the intervention groups when compared to the control groups, however these data were not rigorous and no conclusions could be made.

2.8 Sperm concentration at nine months or more; type of antioxidant

See Analysis 2.8
Pooling was not possible in this analysis as only one trial reported on two subgroups.
2.8.1, 2.8.2, L-aceytl carnitine +L-carnitine versus L-carnitine, L-acetyl carnitine + L-carnitine versus L-acetyl carnitine. One trial Balercia 2005 reported on:

- L-aceytl carnitine + L-carnitine versus L-carnitine (MD -6.13, 95% CI -15.99 to 3.73, P = 0.22, 30 men), L-aceytl carnitine + L-carnitine did not show an association with improved sperm concentration when compared to L-carnitine;
- L-acetyl carnitine + L-carnitine versus L-acetyl carnitine (MD 2.06, 95% CI -6.09 to 10.21, P = 0.62, 30 men), L-aceytl carnitine + L-carnitine did not show an association with improved sperm concentration when compared to L-acetyl carnitine.

3 Pentoxifylline versus placebo or no treatment

The trials included in this comparison did not report on the live birth, clinical pregnancy, adverse events or sperm DNA fragmentation.

3.1 Total sperm motility at three months or less; pentoxifylline versus placebo or no treatment

See Analysis 3.1
Pooling was not possible in this analysis as only one trial reported sperm motility at three months.
3.1 Pentoxifylline versus no treatment. There was an association between the use of pentoxifylline and increased sperm motility when compared to no treatment (MD 12.77, 95% CI 9.23 to 16.31, P < 0.00001, 90 men) (Micic 1988).

3.2 Other data

See Analysis 3.2
3.2.1 Pentoxifylline versus placebo. One trial (Merino 1997) reported data as medians and ranges for this subgroup. There may have been an association with improved sperm motility at three months for pentoxifylline when compared to placebo (P < 0.01), however these data were not rigorous and no conclusions could be made.

3.3 Total sperm motility at six months; pentoxifylline versus placebo or no treatment

See Analysis 3.3
Pooling was not possible in this analysis as only one trial reported sperm motility at six months.
There was an association between the use of pentoxifylline and increased sperm motility when compared to placebo (MD 10.10, 95% CI 9.09 to 11.11, P < 0.00001, 229 men) (Safarinejad 2011).

3.4 Other data

See Analysis 3.4
3.4.1 Pentoxifylline versus placebo. One trial (Merino 1997) reported data as medians and ranges for this subgroup. There may have been an association with improved sperm motility at six months for pentoxifylline compared to placebo (P < 0.00001), however these data were not rigorous and no conclusions could be made.

3.5 Sperm total motility at nine months; pentoxifylline versus placebo or no treatment

See Analysis 3.5

There was an association between the use of pentoxifylline and increased sperm motility when compared to placebo (MD 3.10, 95% CI 1.93 to 4.27, P < 0.00001, 221 men) (Safarinejad 2011).

3.6 Total sperm motility over time

See Analysis 3.6

Only one trial was available for analysis in each subgroup.

3.6.1 Total sperm motility at three months (Micic 1988).

3.6.2 Total sperm motility at six months (Safarinejad 2011).

3.6.3 Total sperm motility at nine months (Safarinejad 2011).

Results for these individual analyses are reported in sections 3.1, 3.2 and 3.3.

3.7 Sperm concentration at three months

See Analysis 3.7

There was no association with the use of pentoxifylline and increased sperm concentration when compared to placebo (MD 4.30, 95% CI -0.69 to 9.29, P = 0.09, 18 men) (Wang 1983).

3.8 Sperm concentration at six months

See Analysis 3.8

In this analysis the heterogeneity was very high (I^2 = 85%) therefore a random-effects model was used (MD 6.90, 95% CI -0.09 to 13.89, P = 0.05, 2 RCTs, 247 men, I^2 = 85%). As heterogeneity remained unchanged we were unable to pool these trials (Safarinejad 2011; Wang 1983).

3.9. Sperm concentration at nine months

See Analysis 3.9

There was an association between the use of pentoxifylline and increased sperm concentration when compared to placebo (MD 1.70, 95% CI 0.62 to 2.78, P = 0.002, 221 men) (Safarinejad 2011).

3.10 Sperm concentration over time

See Analysis 3.10

Subtotals only were in this analysis.

3.10.1 Total sperm concentration at three months (Wang 1983).

3.10.2 Total sperm concentration at six months (Safarinejad 2011; Wang 1983). This subgroup could not be pooled due to high heterogeneity.

3.10.3 Total sperm concentration at nine months (Safarinejad 2011).

Results for these individual analyses are reported in sections 3.7, 3.8 and 3.9.

3.11 Adverse events

See Analysis 3.11

Only one trial Safarinejad 2011 reported adverse effects.

3.11.1, 3.11.2, 3.11.3, 3.11.4, 3.11.5, 3.11.6, 3.11.7 Pentoxifylline versus placebo.

- Vomiting: there was an association between the use of pentoxifylline and vomiting when compared to placebo (OR 4.98, 95% CI 1.32 to 18.81, P = 0.02, 254 men).
- Dyspepsia: there was an association between the use of pentoxifylline and dyspepsia when compared to placebo (OR 4.68, 95% CI 1.15 to 19.07, P = 0.03, 254 men).
- Headache: there was no association between the use of pentoxifylline and headache when compared to placebo (OR 2.41, 95% CI 0.54 to 10.78, P = 0.25, 254 men).
- Diarrhoea: there was an association between the use of pentoxifylline and diarrhoea when compared to placebo (OR 7.63, 95% CI 1.30 to 44.67, P = 0.02, 254 men).
- Tremor: there was no association between the use of pentoxifylline and tremor when compared to placebo (OR 7.45, 95% CI 0.46 to 119.73, P = 0.16, 254 men).
- Dizziness: there was no association between the use of pentoxifylline and tremor when compared to placebo (OR 7.45, 95% CI 0.46 to 119.73, P = 0.16, 254 men).
- Vertigo: there was no association between the use of pentoxifylline and tremor when compared to placebo (OR 1.96, 95% CI 0.20 to 18.99, P = 0.56, 254 men).

DISCUSSION

Summary of main results

Effectiveness of antioxidants versus placebo or no treatment

Live birth

The findings of this review suggest that for subfertile men the use of antioxidants may be effective in increasing a couple's chances of having a live birth when compared to placebo and when compared to no treatment. It was found that within this population of subfertile men with an expected live birth rate of 5% the use of antioxidant would increase this rate to between 10% and 31%. However there were only four trials with a total of 277 men reporting on live birth and the quality of this evidence was considered to be low (Summary of findings for the main comparison). The methods were not well explained in three out of four of these trials and one (Suleiman 1996) had a significant number of participants who dropped out of the study. The broad reasons for dropouts

were explained, but not the individual reasons, and we were unaware of how many of the dropouts were from the treatment or control group.

Two trials reported on the use of vitamin E versus placebo and there appeared to be an association with vitamin E and an increased live birth rate but the two trials reporting this both had a high risk of bias.

There was also a possible overestimation of the effect of antioxidants when compared to placebo or no treatment in the live birth trials as shown in a sensitivity analysis. This overestimation may have been due to one of the trials enrolling men who were part of a couple undergoing IVF and the trial with uneven dropouts. The true effect will not be known until all the trials that report clinical pregnancy also report on live birth. All trials should report both these outcomes.

The benefit from antioxidants persisted when analyses were restricted to studies at low risk of bias, placebo controlled, and studies enrolling men undergoing IVF/ICSI.

Clinical pregnancy

The use of an antioxidant was also associated with an increased clinical pregnancy rate versus placebo or no treatment (7 trials with 522 men). The results suggest that for subfertile men with an expected clinical pregnancy rate of 6%, the use of antioxidants increased this rate to between 11% and 28%. The quality of this evidence was however judged as low (Summary of findings for the main comparison) because four of the seven trials had high risk of bias with unexplained methodologies.

The benefit from antioxidants persisted when analyses were restricted to studies at lower risk of bias, studies of men not undergoing ART, and studies of men with post-varicocelectomy. This benefit was not seen in the men undergoing IVF or ICSI.

In the analysis of type of specific antioxidants, sample numbers were generally very small but the evidence suggested that the following antioxidants were associated with an increased clinical pregnancy rate: vitamin E and zinc.

Adverse events

There was no association seen between the use of antioxidants and increased miscarriage risk when compared to placebo or no treatment (3 trials, 247 men). There were only eight events in this analysis so no conclusions could be drawn. Within this population of subfertile men with an expected miscarriage rate of 2%, use of an antioxidant would mean that the chances of having a miscarriage were between 1% and 13%. However the quality of this evidence was very low quality (Summary of findings for the main comparison) due to the high risk of bias within these studies.

There was also no evidence that the risk of other adverse events differed between the groups, but samples were too small to draw any conclusions.

Sperm DNA fragmentation

Only two trials (100 men) reported on sperm DNA fragmentation. There was an association of antioxidant use and a lowered sperm DNA fragmentation when compared to placebo although the two trials were reporting on different antioxidants, one was vitamin C + vitamin E and the other; DHA.

Sperm parameters

The findings for total sperm motility and concentration at three, six and nine months were inconsistent and inconclusive as heterogeneity was extremely high in each analysis. The only subgroups within the analyses with low heterogeneity reported the following;

- Vitamin C 100 mg/day plus vitamin E (2 trials, 95 men) found no association with increased sperm motility at three months when compared to placebo.
- Combined antioxidants (2 trials, 228 men) found an association with increased sperm motility at three months compared to placebo or no treatment.
- Vitamin C plus vitamin E (2 trials, 95 men) found no association with increased sperm concentration at three months compared to placebo.
- Carnitines (2 trials, 116 men) found no association with increased sperm concentration at six months compared to placebo.

Effectiveness of antioxidants versus antioxidants (head to head)

The head to head trials did not report on live birth, clinical pregnancy rate or adverse effects and we were unable to perform a meta-analysis on any of the sperm parameter outcomes.

Effectiveness of pentoxifylline versus placebo or no treatment

None of the relevant trials reported on live birth, clinical pregnancy or adverse effects and we were unable to perform a meta-analysis on any of the sperm parameter outcomes due to inconsistencies.

Overall completeness and applicability of evidence

Of the 48 trials included in this review only seven reported on clinical pregnancy rate and then only four went on to report live birth. Live birth and clinical pregnancy rate are the outcomes of most interest to subfertile couples and until these are robustly reported by all subfertility trials we will not be able to draw clear conclusions for the use of antioxidants for subfertile men. Adverse events of miscarriage, ectopic pregnancy and side effects appear to be poorly reported. The high heterogeneity may be an artefact caused by some of the trials reporting very small and potentially

erroneous standard deviations. This undermines the credibility of the data.

Three trials (Morgante 2010; Scott 1998; Tremellen 2007) used combined antioxidants (three or more antioxidants) versus placebo or no treatment but only Tremellen 2007 reported on live birth and clinical pregnancy rate. Morgante 2010 and Scott 1998 reported only on sperm motility and concentration at three months.

We tried to assess which type of antioxidant might have a beneficial effect on the outcomes of interest in this review, however only three trials at the most could be pooled in any antioxidant subgrouping.

The head to head comparison does not provide constructive information as we could not pool direct comparisons. Subgrouping of antioxidants, or different doses of antioxidants, was unable to be performed in the treatment versus treatment groups as there were only single trials analysing these differences. Therefore this review was unable to show any difference in effect between different antioxidants or different doses of the same antioxidant.

There were only three trials that reported the use of pentoxifylline versus placebo or no treatment. We could not pool two trials in any of the subgroup analysis due to high heterogeneity.

There were eight trials (Cavallini 2004; Galatioto 2008; Kessopoulou 1995; Kumamoto 1988; Lenzi 2003; Merino 1997; Sivkov 2011; Wong 2002) that contained data that were unusable in the analysis, with either some or all of their data. The reasons for this were presentation of medians, percentages or ranges, and in some cases no standard deviations or standard errors were given (Analysis 1.8; Analysis 1.10; Analysis 1.14; Analysis 1.16). Attempts were made to contact these authors regarding the data.

Quality of the evidence

The evidence was graded as very low to low quality. Limitations included poor reporting of study methods, imprecision, the number of small studies, reporting bias and lack of data about adverse events.

None of the included studies reported live birth as a primary outcome, very few reported clinical pregnancy as a primary outcome, and studies were small with few events. Three of the four studies reporting live birth had unclear methods of sequence generation and allocation concealment. One trial (Suleiman 1996) also had some imbalance in numbers at analysis due to dropouts; and Omu 1998 used 'no treatment' as the control, which introduced a degree of performance bias.

Figure 2 shows the review authors' judgements about the methodological quality of the trials included in this review. All included trials were described as randomised, however only 35% gave information on how the randomisation was achieved. Allocation concealment was described in only 31% of the trials. Blinding was better described with over 56% of the trials being double blinded or occasionally single blinded; 8% of trials stated that there was no blinding and 21% of included trials used no treatment as a control.

Dropout rates were high in some studies and dropout rates tended to be higher in the control groups, which created a potential for differential follow-up with better reporting of clinical pregnancies in the intervention groups.

Potential biases in the review process

Some bias in the review process may have arisen due to the inclusion of trials that have had dropouts of participants of > 20%, and subsequent imbalances in the number of participants between the treatment and control groups.

Agreements and disagreements with other studies or reviews

Two other reviews described the effects of L-carnitine and L-acetylcarnitine on subfertile men. The systematic review and meta-analysis by Zhou (Zhou 2007) compared L-carnitine and L-acetylcarnitine therapy to placebo treatment and found improvements in pregnancy rate and total sperm motility. Our review was unable to pool the results of the carnitine trials due to inconsistencies between the trials. The descriptive review by Patel (Patel 2008) discusses the improvement in pregnancy rates with oral intake of antioxidants, however Patel states that RCTs have not shown an effect on sperm motility and that there is a need for more RCTs in men with oxidative stress.

Agarwal (Agarwal 2004) discusses in an overview of the literature a range of antioxidants, and combinations of these, and their effect on male subfertility. Agarwal notes that vitamin E and a combination of vitamin E with other antioxidants such as N-acetylcysteine, vitamin A and fatty acids appears to improve pregnancy rates in astheno-zoospermic men. Carnitines also appear to have an effect on pregnancy rates. This review also found an association of vitamin E and clinical pregnancy. We also found an association between the use of combined antioxidants and sperm motility at three months when compared to placebo.

Another review (Ross 2010) showed improvement in pregnancy rate and sperm quality after antioxidant therapy. This is in agreement with our review, although we are uncertain of the sperm parameter outcomes due to the extreme heterogeneity.

A systematic review (Lafuente 2013) looking at the effect of Coenzyme Q10 and male subfertility found an association between this antioxidant and improved pregnancy rate, sperm concentration and motility. We did agree on the effect of Coenzyme Q10 on sperm motility and concentration at six months, however we could not draw clear conclusions due to the heterogeneity in these analyses.

These systematic reviews all reported on pregnancy rates, whereas this updated Cochrane review reported on clinical pregnancy rates (as confirmed by the identification of a gestational sac on ultra-

sound at ≥ 7 weeks gestation) so fewer studies were available for analysis.

A Cochrane review of antioxidants for female subfertility has been published (Showell 2013) showing that there is no evidence of an effect of antioxidants on female subfertility.

Evidence to date suggests that the side effect profile of antioxidants is low. However, as there were few studies reporting side effects, more data are required to evaluate fully any adverse events and the side effect profile of these supplements.

AUTHORS' CONCLUSIONS

Implications for practice

Clinicians could consider recommending antioxidants for subfertile men whose partners are trying to conceive as part of an assisted reproduction program. However, subfertile couples should be advised that current evidence is inconclusive and that further well-designed placebo-controlled trials reporting on pregnancy and live births are required to clarify the role of antioxidants. This review did not examine the use of antioxidants in men with normal sperm.

Implications for research

In this review there were only four small trials (three comparing antioxidant versus placebo and one versus no treatment) reporting on live birth, the most important outcome from the perspective of the couple experiencing difficulty with conception, and the number of events was very small. Only two trials reported on DNA fragmentation. A low degree of DNA fragmentation is thought to increase the likelihood of achieving a pregnancy. Further large well-designed placebo-controlled randomised trials with live birth and clinical pregnancy as primary outcomes are needed.

Four trials (Galatioto 2008; Morgante 2010; Scott 1998; Tremellen 2007) used combined antioxidants (three or more antioxidants) versus control but reported on different outcomes. The results were generally in favour of the antioxidant over the control. However, there is a need for more randomised controlled trials in order to make any conclusions on whether a combination of antioxidants would have a statistically significant benefit over a single antioxidant versus placebo.

If evidence emerges from placebo-controlled randomised trials which shows that antioxidant supplements improve clinical outcomes (pregnancy and live birth) then randomised head to head trials will be needed to assess whether one antioxidant is more effective than another.

There is also a gap in the evidence as to whether different doses of an antioxidant have different effects. This review was only able to include single trials measuring different doses and therefore meta-analysis of this comparison was unable to be performed.

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Further information for the trials was received from:

Dr Nematollahi-mahani (Azizollahi 2013),

Associate Professor Kelton Tremellen (Tremellen 2007).

Dr Mohan Kamath (Kamath 2014)

Dr S Peivandi (Peivandi 2010)

Dr E El Gindy (Elgindy 2008)

Dr M Sigman (Sigman 2006)

Professor Niewchlag (Rolf 1999)

Dr G Cavallini (Cavallini 2004)

Dr C Wang (Wang 1983)

Dr Martinez-Soto (Martinez-Soto 2010)

Dr G Morgante (Morgante 2010)

Dr A Nadjarzadeh (Nadjarzadeh 2011)

Dr MR Safarinejad (Safarinejad 2009; Safarinejad 2009a).

REFERENCES

References to studies included in this review

Akiyama 1999 *{published data only}*

Akiyama M. In vivo scavenging effect of ethylcysteine on reactive oxygen species in human semen. Japanese Journal of Urology 1999; Vol. 90, issue 3:421–8.

Attallah 2013 *{published data only}*

Attallah D, El Nashar IH, Mahmoud R, Shaaban OM, Salman SA. N-acytelcysteine prior to intrauterine insemination in couples with isolated athenozospermia: a randomized controlled trial. Fertility and Sterility 2013; Vol. 100, issue 3 Suppl:S462.

Azizollahi 2013 *{published data only}*

* Azizollahi G, Azizollahi S, Babaei H, Kianinejad M, Baneshi MR, Nematollahi-mahani SN. Effects of supplement therapy on sperm parameters, protamine content and acrosomal integrity of varicocelectomized subjects. *Journal of Assisted Reproduction and Genetics* 2013; **30**(4):593–9.

Azizollahi S, Azizollahi G, Nematollahi SN, Babaei H, Rastegari A, Maghsudi S. The effect of folic acid administration on protamine deficiency, acrosomal activity and semen parameters in varicocelectomized patients. *Human Reproduction* 2011;**26 Suppl 1 Abstract no: P-57**: i340.

Balercia 2005 *{published data only}*

Balercia G, Regoli F, Armeni T, Koverech A, Mantero F, Boscaro M. Placebo-controlled double-blind randomized trial on the use of L-carnitine, L-acetylcarnitine, or combined L-carnitine and L-acetylcarnitine in men with idiopathic asthenozoospermia. Fertility and Sterility 2005; Vol. 84, issue 3:662–71.

Balercia 2009 *{published data only}*

Balercia G, Buldreghini E, et al. Coenzyme Q10 treatment in infertile men with idiopathic asthenozoospermia: a placebo-controlled, double-blind randomized trial. *Fertility and Sterility* 2009;**91**(5):1785–92.

Biagiotti 2003 *{published data only}*

Biagiotti G, Cavallini G, Modenini F, Vitali G, Magli C, Ferraretti A. Prostaglanadins pulsed down-regulation enhances carnitine therapy performance in severe idiopathic oligoasthenospermia. Human Reproduction 2003; Vol. 18 Suppl 1:202.

Cavallini 2004 *{published data only}*

Cavallini G, Ferraretti AP, Gianaroli L, Biagiotti G, Vitali G. Cinnoxicam and L-carnitine/acetyl-L-carnitine treatment for idiopathic and varicocoele associated oligoasthenospermia [see comment]. Journal of Andrology 2004; Vol. 25, issue 5:761–70.

* Cavallini G, Ferraretti AP, Gianaroli L, Magli MC, Biagiotti G, Vitali G. Cinnoxicam + carnitines for idiopathic dyspermia. The 20th Annual Meeting of the European Society of Human Reproduction and Embryology. *Human Reproduction* 2004:i23–4.

Ciftci 2009 *{published data only}*

Ciftci H, Verit A, Savas M, Yeni E, Erel O. Effects of N-acetylcysteine on semen parameters and oxidative/antioxidant status. *Urology* 2009;**74**(1):73–6.

Conquer 2000 *{published data only}*

Conquer JA, Martin JB, Tummon I, Watson L, Tekpetey F. Effect of DHA supplementation on DHA status and sperm motility in asthenozoospermic males. *Lipids* 2000;**35**(2): 149–54.

Dawson 1990 *{published data only}*

Dawson EB, Harris WA, Powell LC. Relationship between ascorbic acid and male fertility. World Review of Nutrition and Dietetics 1990; Vol. 62:1–26.

Dimitriadis 2010 *{published data only}*

Dimitriadis F, Tsambalas S, Tsounapi P, Kawamura H, Vlachopoulou E, Haliasos N, et al. Effects of phosphodiesterase-5 inhibitors on Leydig cell secretory function in oligoasthenospermic infertile men: A randomized trial. *BJU International* 2010;**106**(8):1181–5.

Eslamian 2012 *{published data only}*

* Eslamian G, Amirjannati N, Sadeghi MR, Rashidkhani B, Pahlavan S, Hooshangi A, et al. The effects of combined supplementation of docosahexaenoic acid and vitamin E on fatty acid changes in sperm membrane in asthenozoospermic men. *Iranian Journal of Nutrition Sciences & Food Technology* 2013;**8**(1):23–37.

Hekmatdoost A. The effects of administration of combined docosahexaenoic acid and vitamin E supplements on spermatogram and seminal plasma oxidative stress in infertile men with asthenozoospermia. www.clinicaltrials.gov. [ClinicalTrials.gov: NCT01846325]

Galatioto 2008 *{published data only}*

Galatioto GP, Gravina GL, Angelozzi G, Sacchetti A, Innominato PF, Pace G, et al. May antioxidant therapy improve sperm parameters of men with persistent oligospermia after retrograde embolization for varicocele?. World Journal of Urology 2008; Vol. 26, issue 1:97–102.

Greco 2005 *{published data only}*

Greco E, Iacobelli M, Rienzi L, Ubaldi F, Ferrero S, Tesarik J. Reduction of the incidence of sperm DNA fragmentation by oral antioxidant treatment. Journal of Andrology 2005; Vol. 26, issue 3:349–53.

Keskes-Ammar 2003 *{published data only}*

Keskes-Ammar L, Feki-Chakroun N, Rebai T, Sahnoun Z, Ghozzi H, Hammami S, et al. Sperm oxidative stress and the effect of an oral vitamin E and selenium supplement on semen quality in infertile men. Archives of Andrology 2003; Vol. 49, issue 2:83–94.

Kessopoulou 1995 *{published data only}*

Kessopoulou E, Powers HJ, Sharma KK, Pearson MJ, Russell JM, Cooke ID, et al. A double-blind randomized placebo cross-over controlled trial using the antioxidant vitamin E to treat reactive oxygen species associated male

infertility. Fertility and Sterility 1995; Vol. 64, issue 4: 825–31.

Kumamoto 1988 *{published data only}*
Kumamoto Y, Maruta H, Ishigami J, Kamidono S, Orikasa S, Kimura M, et al. Clinical efficacy of mecobalamin in the treatment of oligozoospermia--results of double-blind comparative clinical study. *Hinyokika Kiyo. Acta Urologica Japonica* 1988;**34**:1109–32.

Lenzi 2003 *{published data only}*
Lenzi A, Lombardo F, Sgro P, Salacone P, Caponecchia L, Dondero F, et al. Use of carnitine therapy in selected cases of male factor infertility: a double-blind crossover trial [see comment]. Fertility and Sterility 2003; Vol. 79, issue 2: 292–300.

Lenzi 2004 *{published data only}*
Lenzi A, Sgro P, Salacone P, Paoli D, Gilio B, Lombardo F, et al. A placebo-controlled double-blind randomized trial of the use of combined l-carnitine and l-acetyl-carnitine treatment in men with asthenozoospermia [see comment]. Fertility and Sterility 2004; Vol. 81, issue 6:1578–84.

Li 2005 *{published data only}*
Li Z, Chen GW, Shang XJ, Bai WJ, Han YF, Chen B, et al. A controlled randomized trial of the use of combined L-carnitine and acetyl-L-carnitine treatment in men with oligoasthenozoospermia. Zhong Hua Nan Ke Xue 2005; Vol. 11, issue 10:761–4.

Li 2005a *{published data only}*
Li Z, Gu R, Liu Y, Xiang Z, Cao X, Han Y, et al. Curative effect of L-carnitine supplementation in the treatment of male infertility. Academic Journal of Shanghai Second Medical University 2005; Vol. 25, issue 3:292–4.

Lombardo 2002 *{published data only}*
Lombardo F, Gandini L, Agarwal A, Sgro P, Dondero F, Lenzi A. A prospective double blind placebo controlled cross over trial of carnitine therapy in selected cases of male infertility. Fertility and Sterility 2002; Vol. 78 Suppl 1: 68–9.

Martinez-Soto 2010 *{published data only}*
Martinez-Soto JC, Domingo JC, Cardobilla LP, et al. Effect of dietary DHA supplementation on sperm DNA integrity. *Fertility and Sterility* September 2010;**94**(4):S235–6.

Merino 1997 *{published data only}*
Merino G, Martinez Chequer JC, Barahona E, Bermudez JA, Moran C, Carranza-Lira S. Effects of pentoxifylline on sperm motility in normogonadotropic asthenozoospermic men. Archives of Andrology 1997; Vol. 39, issue 1:65–9.

Micic 1988 *{published data only}*
Micic S, Hadzi-Djokic J, Dotlic R, Tulic C. Pentoxifyllin treatment of oligoasthenospermic men. Acta Europaea Fertilitatis 1988; Vol. 19, issue 3:135–7.

Morgante 2010 *{published data only}*
Morgante G, Scolaro V, Tosti C, Di Sabatino A, Piomboni P, De Leo V. Treatment with carnitine, acetyl carnitine, L-arginine and ginseng improves sperm motility and sexual health in men with asthenopermia. *Minerva Urologica e Nefrologica* 2010;**62**(3):213–8.

Nadjarzadeh 2011 *{published data only}*
Nadjarzadeh A, Shidfar F, Amirjannati N, Vafa MR, Motevalian SA, Gohari MR, et al. Coenzyme Q10 improves seminal oxidative defense but does not affect on seman in idiopathic oligoasthenoteratozoospermia: A randomized double- blind placebo controlled trial. *Journal of Endocrinological Investigation* 2011;**34**:e224–8.

Nozha 2001 *{published data only}*
Nozha CF, Leila AK, Zouhir S, Hanen G, Khled Z, Tarek R. Oxidative stress and male infertility: comparative study of combined vitamin E/selenium treatment versus vitamin B. Human Reproduction 2001; Vol. 17, issue Abstract book 1:111–2.

Omu 1998 *{published data only}*
Omu AE, Dashti H, Al-Othman S. Treatment of asthenozoospermia with zinc sulphate: andrological, immunological and obstetric outcome. European Journal of Obstetrics, Gynecology, and Reproductive Biology 1998; Vol. 79, issue 2:179–84.

Omu 2008 *{published data only}*
Omu AE, Al-Azemi MK, Kehinde EO, Anim JT, Oriowo MA, Mathew TC. Indications of the mechanisms involved in improved sperm parameters by zinc therapy. Medical Principles and Practice 2008; Vol. 17, issue 2:108–16.

Peivandi 2010 *{published data only}*
Peivandi S, Abasali K, Narges M. Effects of L-carnitine on infertile men's spermogram; a randomised clinical trial. *Journal of Reproduction and Infertility* 2010;**10**(4):331.

Poveda 2013 *{published data only}*
Poveda C, Rodriguez R, Chu EE, Aparicio LE, Gonzales IG, Moreno CJ. A placebo-controlled double-blind randomized trial of the effect of oral supplementation with spermotrend, maca extract (lepidium meyenii) or L-carnitine in semen parameters of infertile men. Fertility and Sterility 2013; Vol. 100, issue 3 Suppl:S440.

Pryor 1978 *{published data only}*
Pryor JP, Blandy JP, Evans P, Chaput de Saintonge DM, Usherwood M. Controlled clinical trial of arginine for infertile men with oligozoospermia. *British Journal of Urology* 1978;**50**:47–50.

Rolf 1999 *{published data only}*
* Rolf C. Antioxidant treatment with high dose vitamin C and vitamin E of patients with asthenozoospermia a randomized placebo controlled double blind study. ESHRE 14th annual meeting. *Human Reproduction* 1998;**72**.
Rolf C, Cooper TG, Yeung CH, Nieschlag E. Antioxidant treatment of patients with asthenozoospermia or moderate oligoasthenozoospermia with high-dose vitamin C and vitamin E: a randomized, placebo-controlled, double-blind study [see comment]. Human Reproduction 1999; Vol. 14, issue 4:1028–33.

Safarinejad 2009 *{published data only}*
Safarinejad MR, Safarinejad S. Efficacy of selenium and/or N-acetyl-cysteine for improving semen parameters in infertile men: a double-blind, placebo controlled,

randomized study. *The Journal of Urology* 2009;**181**(2): 741–51.

Safarinejad 2009a ***{published data only}***
Safarinejad ME. Efficacy of coenzyme Q10 on semen parameters, sperm function and reproductive hormones in infertile men. *The Journal of Urology* 2009;**182**:237–48.

Safarinejad 2011 ***{published data only}***
Safarinejad MR. Effect of pentoxifylline on semen parameters, reproductive hormones and seminal plasma antioxidant capacity in men with idiopathic infertility: a randomized double-blind placebo-controlled study. *International Urology and Nephrology* 2011;**43**:315–28.

Safarinejad 2012 ***{published data only}***
Safarinejad MR, Shafiei N, Safarinejad S. Effects of the reduced form of coenzyme Q10 (ubiquinol) on semen parameters in men with idiopathic infertility: A double-blind, placebo controlled, randomized study. *The Journal of Urology* 2012;**188**(2):526–31.

Scott 1998 ***{published data only}***
Scott R, MacPherson A, Yates RW, Hussain B, Dixon J. The effect of oral selenium supplementation on human sperm motility. British Journal of Urology 1998; Vol. 82, issue 1: 76–80.

Sigman 2006 ***{published data only}***
Pryor JL, Stacy L, Glass S, Campagnone J, Sigman M. Randomized double blind placebo controlled trial of carnitine for the treatment of idiopathic asthenospermia. *Fertility and Sterility* 2003;**80 Suppl 3**:48.
* Sigman M, Glass S, Campagnone J, Pryor JL. Carnitine for the treatment of idiopathic asthenospermia: a randomized, double-blind, placebo-controlled trial. *Fertility and Sterility* 2006;**85**(5):1409–14.

Sivkov 2011 ***{published data only}***
Sivkov AV, Oshchepkov VN, Evdokimov VV, Keshishev NG, Skabko OV. Selzink plus study in patients with chronic non-infectious prostatitis and abnormal fertility. [Russian]. Urologii 2011; Vol. 5:27–33.

Suleiman 1996 ***{published data only}***
Suleiman SA, Ali ME, Zaki ZM, el-Malik EM, Nasr MA. Lipid peroxidation and human sperm motility: protective role of vitamin E. Journal of Andrology 1996; Vol. 17, issue 5:530–7.

Tremellen 2007 ***{published data only}***
Tremellen K, Froiland D, Miari G, Thompson J. A randomised control trial examining the effect of an antioxidant on sperm function pregnancy outcome during IVF treatment. The Fertility Society of Australia 25th Annual scientific meeting. *The Australian & New Zealand Journal of Obstetrics & Gynaecology* 2006;**46 Suppl 2**:A.2.
* Tremellen K, Miari G, Froiland D, Thompson J. A randomised control trial examining the effect of an antioxidant (Menevit) on pregnancy outcome during IVF-ICSI treatment. *The Australian & New Zealand Journal of Obstetrics & Gynaecology* 2007;**47**:216–21.

Wang 1983 ***{published data only}***
Wang C, Chan CW, Wong KK, Yeung KK. Comparison of the effectiveness of placebo, clomiphene citrate, mesterolone, pentoxifylline, and testosterone rebound therapy for the treatment of idiopathic oligospermia. *Fertility and Sterility* 1983;**40**(3):358–65.

Wang 2010 ***{published data only}***
Wang YX, Yang SW, Qu CB, Huo HX, Li W, Li JD, et al. L-carnitine: safe and effective for asthenozoospermia. *Zhonghua nan ke xue - National Journal of Andrology* 2010; **16**(5):420–2.

Wong 2002 ***{published data only}***
Ebisch IM, Pierik FH, De Jong FH, Thomas CM, Steegers-Theunissen RP. Does folic acid and zinc sulphate intervention affect endocrine parameters and sperm characteristics in men?. *International Journal of Andrology* 2006;**29**(2):339–45.
* Wong WY, Merkus HM, Thomas CM, Menkveld R, Zielhuis GA, Steegers-Theunissen RP. Effects of folic acid and zinc sulfate on male factor subfertility: a double-blind, randomized, placebo-controlled trial. Fertility and Sterility 2002; Vol. 77, issue 3:491–8.

Zalata 1998 ***{published data only}***
* Zalata A, Christophe A, Horrbin D, Dhooge W, Comhaire F. Protection of sperm function and DNA by essential fatty acids and antioxidants dietary supplementation. *Fertility and Sterility* 1998;**70**:287.
Zalata A, Christophe A, Horrobin D, Dhooge W, Comhaire F. Effect of essential fatty acids and antioxidants dietary supplementation on the oxidative DNA damage of the human spermatozoa. ESHRE 14th annual meeting. Goteborg, 1998:270–1.

Zavaczki 2003 ***{published data only}***
Zavaczki Z, Szollosi J, Kiss S, Koloszar S, Fejes I, Kovacs L, Pal A. Magnesium-orotate supplementation for idiopathic infertile male patients: a randomized, placebo-controlled clinical pilot study. *Magnesium Research* 2003;**16**(2):131–6.

References to studies excluded from this review

Anarte 2012 ***{published data only}***
Anarte C, Calvo I, Domingo A, et al. Effect of DHA supplementation on fatty acid composition of sperm and its relation to semen quality. *Human Reproduction - Abstract book of the 28th ESHRE Annual Meeting, Turkey 1-4 July 2012* 2012;**27**(2):ii121.

Anarte 2013 ***{published data only}***
Anarte C, Domingo A, Agirregoikoa JA, De Pablo J, Barrenetxea G. Regular consumption of docosahexaenoic acid (DHA) improves semen quality. Fertility and Sterility 2013; Vol. 100 Suppl 3:S443.

Cai 2012 ***{published data only}***
Cai T, Wagenlehner F, Mazzoli S, Meacci F, Mondaini N, Nesi G, et al. Semen quality in patients with Chlamydia trachomatis genital infection treated concurrently with prulifloxacin and a phytotherapeutic agent. *Journal of Andrology* 2012;**33**(4):615–23.

Chen 2012 *{published data only}*
Chen XF, Li Z, Ping P, Dai JC, Zhang FB, Shang XJ, et al. Efficacy of natural vitamin E on oligospermia and asthenospermia: a prospective multi-centered randomized controlled study of 106 cases. *Zhong Hua Nan Ke Xue* 2012; **18**(5):428–31.

Comhaire 2005 *{published data only}*
Comhaire FH, El Garem Y, Mahmoud A, Eertmans F, Schoonjans F. Combined conventional/antioxidant "Astaxanthin" treatment for male infertility: a double blind, randomized trial. Asian Journal of Andrology 2005; Vol. 7, issue 3:257–62.

Ebisch 2003 *{published data only}*
Ebisch IM, van Heerde WL, Thomas CM, van der Put N, Wong WY, Steegers-Theunissen RP. C677T methylenetetrahydrofolate reductase polymorphism interferes with the effects of folic acid and zinc sulfate on sperm concentration. Fertility and Sterility 2003; Vol. 80, issue 5:1190–4.

Elgindy 2008 *{published data only}*
Elgindy EA, El-Huseiny AM, Mostafa MI, Gaballah AM, Amed TA. N-Acetylcysteine: Could it be an effective adjuvant therapy in ICSI cycles. *Fertility and Sterility* 2008;**90 Suppl 1**(American Society for Reproductive Medicine 64th Annual Meeting. 8-12 November 2008, San Francisco, CA):356 Abstract no: P738.

Ghanem 2010 *{published data only}*
Ghanem H, Shaeer O, El-Segini A. Combination clomiphene citrate and antioxidant therapy for idiopathic male infertility: a randomized controlled trial. Fertility and Sterility 2010; Vol. 93, issue 7:2232–5.

Hafeez 2011 *{published data only}*
Hafeez M, Ahmed A, Usmanghani K, Mohiuddin E, Asif HM, Akram M, et al. Clinical evaluation of herbal medicine for oligospermia. *Pakistan Journal of Nutrition* 2011;**10**(3): 238–40.

Jawad 2013 *{published data only}*
Jawad HM. Zinc sulfate treatment of secondary male infertility associated with positive serum and seminal plasma anti-sperm antibody test. Middle East Fertility Society Journal 2013; Vol. 18, issue 1:24–30.

Kim 2010 *{published data only}*
Kim C-H, Yoon J-W, Ahn J-W, Kang H-J, Lee J-W, Kang B-M. The effect of supplementation with omega-3-polyunsaturated fatty acids in intracytoplasmic sperm injection cycles for infertile patients with a history of unexplained total fertilization failure. *Fertility and Sterility* 2010;**94**(4 Suppl 1):S242.

Kumar 2011 *{published data only}*
Kumar R, Saxena V, Shamsi M, Venkatesh S, Dada R. Herbo-mineral supplementation in men with idiopathic oligoasthenoteratospermia: A double blind randomized placebo-controlled trial. *Indian Journal of Urolology* 2011; **27**(3):357-62.

Lenzi 1993 *{published data only}*
Lenzi A, Culasso F, Gandini L, Lombardo F, Dondero F. Placebo-controlled, double-blind, cross-over trial of glutathione therapy in male infertility. *Human Reproduction* 1993;**8**(10):1657–62.

Lu 2010 *{published data only}*
Lu SM, Li X, Zhang HB, Hu JM, Yan JH, Liu JL, et al. [Use of L-carnitine before percutaneous epididymal sperm aspiration-intracytoplasmic sperm injection for obstructive azoospermia]. *Zhonghua nan ke xue - National Journal of Andrology* 2010;**16**(10):919–21.

Micic 2001 *{published data only}*
Micic S, Lalic N, Bojanic N, Nale DJ. Oligospermic men treated by carnitine. 17th World Congress on Fertility and Sterility. Melbourne, 2001:38.

Niederberger 2011 *{published data only}*
Niederberger C. Combination clomiphene citrate and antioxidant therapy for idiopathic male infertility: A randomized controlled trial. The Journal of Urology 2011; Vol. 185, issue 1:252.

Nikolova 2007 *{published data only}*
Nikolova V, Stanislavov R, Vatev I, Nalbanski B, Punevska M. Sperm parameters in male idiopathic infertility after treatment with prelox. Akusherstvo i Ginekologiia 2007; Vol. 46, issue 5:7–12.

Pawlowicz 2001 *{published data only}*
Pawlowicz P, Stachowiak G, Bielak A, Wilczynski J. Administration of natural anthocyanins derived from chokeberry (aronia melanocarpa) extract in the treatment of oligospermia in males with enhanced autoantibodies to oxidized low density lipoproteins (oLAB). The impact on fructose levels. *Ginekologia Polska* 2001;**72**(12):983–8.

Polak 2013 *{published data only}*
Polak de Fried E, Bossi NM, Notrica JA, Vazquez Levin MH. Vitamin-d treatment does not improve pregnancy rates in patients undergoing art: a prospective, randomized, double-blind placebo-controlled trial. Fertility and Sterility 2013; Vol. 100 Suppl 3:S493–4.

Safarinejad 2011a *{published data only}*
Safarinejad MR, Shafiei N, Safarinejad S. A prospective double-blind randomized placebo-controlled study of the effect of saffron (Crocus sativus Linn.) on semen parameters and seminal plasma antioxidant capacity in infertile men with idiopathic oligoasthenoteratozoospermia. *Phytotherapy research: PTR* 2011;**25**(4):508–16.

Soylemez 2012 *{published data only}*
Soylemez H, Kilic S, Atar M, Penbegul N, Sancaktutar AA, Bozkurt Y. Effects of micronised purified flavonoid fraction on pain, semen analysis and scrotal color Doppler parameters in patients with painful varicocele; results of a randomized placebo-controlled study. *International Urology and Nephrology* 2012;**44**(2):401–8.

Stanislavov 2009 *{published data only}*
Stanislavov R, Nikolova V, Rohdewald P. Improvement of seminal parameters with Prelox®: a randomized, double-

blind, placebo controlled, cross over trial. *Phytotherapy Research* 2009;**23**:297–302. [DOI: 10.1002

Tang 2011 *{published data only}*
Tang KF, Xing Y, Wu CY, Liu RZ, Wang XY, Xing JP. [Tamoxifen combined with coenzyme Q10 for idiopathic oligoasthenospermia]. Zhong Hua Nan Ke Xue 2011; Vol. 17, issue 7:615–8.

Verzeletti 2012 *{published data only}*
Verzeletti FB, Poletto RS, Bertolin TE, Fornari F. Evaluation of sperm quality in adults after use spirulina platensis and resveratrol. *Jornal Brasileiro de Reproducao Assistida* 2012;**16** (5):271–7.

Vicari 2001 *{published data only}*
Vicari E, Calogero AE. Effects of treatment with carnitines in infertile patients with prostatovesiculoepididymitis and elevated sperm oxidative stress. ESHRE 17th Annual Meeting. Lausanne: Human Reproduction, 2001; Vol. 16 Suppl 1:106–7.

Vicari 2001a *{published data only}*
Vicari E, Rubino C, De Palma A, Longo G, Lauretta M, Consoli S, Arancio A. Antioxidant therapeutic efficiency after the use of carnitine in infertile patients with bacterial or non bacterial prostato-vesiculo-epididymitis. *Archivio Italiano di Urologia, Andrologia* 2001;**73**(1):15–25.

Vicari 2002 *{published data only}*
Vicari E, La Vignera S, Calogero AE. Antioxidant treatment with carnitines is effective in infertile patients with prostatovesiculoepididymitis and elevated seminal leukocyte concentrations after treatment with nonsteroidal anti-inflammatory compounds. *Fertility and Sterility* 2002;**78** (6):1203–8.

Wang 2010a *{published data only}*
Wang Y, Yang S, Cai W, Qu C, Li J, Chang X, et al. [Clinical efficacy of L-carnitine combined with tamoxifen in treatment of oligoasthenozoospermia] LA: Chi. Zhonghua nan ke xue - National Journal of Andrology 2010; Vol. 16, issue 5:420–2.

Wu 2012 *{published data only}*
Wu ZM, Lu X, Wang YW, Sun J, Tao JW, Yin FH, et al. Short-term medication of L-carnitine before intracytoplasmic sperm injection for infertile men with oligoasthenozoospermia. *Zhong Hua Nan Ke Xue* 2012;**18** (3):253–6.

References to studies awaiting assessment

Anarte 2013a *{published data only}*
Anarte C, Domingo A, Calvo I, Presilla N, Aguirre O, Bou R, et al. Proper dietary supplementation may improve fatty acid composition and semen quality: A prospective, randomized, double-blind trial. Human Reproduction 2013; Vol. 28:i136.

Gopinath 2013 *{published data only}*
Gopinath PM, Kalra B, Saxena A, Malik S, Kochhar K, Kalra S, et al. Fixed dose combination therapy of antioxidants in treatment of idiopathic oligoasthenozoospermia: Results of a randomized, double-blind, placebo-controlled clinical trial. International Journal of Infertility and Fetal Medicine 2013; Vol. 4, issue 1:6–13.

Iacono 2014 *{published data only}*
Iacono F, Ruffo A, Prezioso D, Illiano E, Romeo G, Romis L, et al. Combination therapy with antiestrogen and a natural composite containing tribulus terrestris, alga ecklonia bicyclis, biovis and myo-inositol in the treatment of male idiopathic infertility. Journal of Sexual Medicine 2014; Vol. 11:92.

Nadjarzadeh 2014 *{published data only}*
Nadjarzadeh A, Shidfar F, Amirjannati N, Vafa MR, Motevalian SA, Gohari MR. Effect of Coenzyme Q10 supplementation on antioxidant enzymes activity and oxidative stress of seminal plasma: a double-blind randomised clinical trial. *Andrologia* 2014;**46**(2):177–83.

Nashivochnikova 2014 *{published data only}*
Nashivochnikova NA, Krupin VN, Selivanova SA. [Efficiency of spematon in male infertility]. *Urologiia (Moscow, Russia: 1999)* 2014, (2):52, 54. [PUBMED: 24956674]

Nematollahi-Mahani 2014 *{published data only}*
Nematollahi-Mahani SN, Azizollahi GH, Baneshi MR, Safari Z, Azizollahi S. Effect of folic acid and zinc sulphate on endocrine parameters and seminal antioxidant level after varicocelectomy. Andrologia 2014; Vol. 46, issue 3:240–5.

References to ongoing studies

AGUNCO 2012 *{unpublished data only}*
Agunco. Effect of treatment with myo-inositol on human semen parameters in patients undergoing in vitro fertilization cycles. ongoing trial.

Gonzalez 2009 *{unpublished data only}*
Gonzalez Sanchez R. Assessment of the efficacy of dietary supplement spermotrend in the treatment of male infertility. ClinicalTrials.gov.

Jensen 2011 *{unpublished data only}*
Jensen MB. Vitamin D supplementation and male infertility: The CBG-study a randomized clinical trial. Ongoing trial.

Kamath 2014 *{unpublished data only}*
Mohan SK. To compare the effectiveness of antioxidants versus no treatment for male partner for improving pregnancy rates in couples undergoing In vitro fertilization (IVF) for abnormal semen analysis. www.clinicaltrials.gov.

Palumbo 2012 *{unpublished data only}*
Palumbo. Myo-inositol on human semen parameters. ongoing trial. [NCT01828710]

Rigshospitalet 2011 *{unpublished data only}*
Rigshospitalet. Vitamin D supplementation and male infertility: a randomized double blinded clinical trial. ongoing trial.

Sadeghi 2008 *{published data only}*
Sadeghi M. Effects of coenzyme Q10 (CoQ10) supplementation on semen quality and seminal oxidative

stress of idiopathic oligoasthenoteratozoospermic (iOAT) infertile men. World Health Organization International Clinical Trials Registry Platform Search Portal 2008. [ISRCTN: ISRCTN29954277]

Sadeghi 2009 *{unpublished data only}*

Sadeghi MR. Effect of ubiquinone supplementation on semen quality, antioxidant enzyme, oxidative stress and DNA fragmentation in infertile men. www.clinicaltrials.gov.

Additional references

Agarwal 2003

Agarwal A, Said T. Role of sperm chromatin abnormalities and DNA damage in male infertility. *Human Reproduction Update* 2003;**9**(4):331–45.

Agarwal 2004

Agarwal A, Nallella K, Allamaneni S, Said T. Role of antioxidants in treatment of male infertility: an overview of the literature. *Reproductive Biomedicine Online* 2004;**8**(6): 616–27.

Aitken 2004

Aitken RJ, Koopman P, Lewis SE. Seeds of concern. *Nature* 2004;**432**(7013):48–52.

Aitken 2007

Aitken RJ, De Iuliis GN. Origins and consequences of DNA damage in male germ cells. *Reproductive Biomedicine Online* 2007;**14**(6):727–33.

Aktan 2013

Aktan G, Dogru-Abbasoglu S, Kucukgergin C, Kadioglu A, Ozdemirler-Erata G, Kocak-Toker N. Mystery of idiopathic male infertility: is oxidative stress an actual risk?. *Fertility and Sterility* 2013;**99**(5):1211–5.

Alvarez 2003

Alvarez 2003. Nuture vs nature: How can we optimise sperm quality?. *Journal of Andrology* 2003;**24**(5):640–8.

Attia 2007

Attia AM, Al-Inany HG, Farquar C, Proctor M. Gonatrophins for idiopathic male factor subfertility. *Cochrane Database of Systematic Reviews* 2007, Issue 4. [DOI: 10.1002/14651858.CD005071.pub3

Boe-Hansen 2005

Boe-Hansen GB, Ersboll AK, Christensen P. Variability and laboratory factors affecting the sperm chromatin structure assay in human semen. *Journal of Andrology* 2005;**26**(3): 360–8.

Boe-Hansen 2006

Boe-Hansen GB, Fedder J, Ersboll AK, Christensen P. The sperm chromatin structure assay as a diagnostic tool in the human fertility clinic. *Human Reproduction* 2006;**21**(6): 1576–82.

Borini 2006

Borini A, Tarozzi N, Bizzaro D, Bonu MA, Fava L, Flamigni C, Coticchio G. Sperm DNA fragmentation: paternal effect on early post-implantation embryo development in ART. *Human Reproduction* 2006;**21**(11):2876–81.

Bykova 2007

Bykova M, Athayde K, Sharma R, Jha R, Sabanegh E, Agarwal A. Defining the reference value of seminal reactive oxygen species in a population of infertile men and normal healthy volunteers. *Fertility and Sterility* 2007;**88 Suppl 1** (P-597):305.

Comhaire 2000

Comhaire FH, Christophe AB, Zalata AA, Dhooge WS, Mahmoud AM, Depuydt CE. The effects of combined conventional treatment, oral antioxidants and essential fatty acids on sperm biology in subfertile men. *Prostaglandins, Leukotrienes, and Essential Fatty Acids* 2000;**63**(3):159–65.

Dias 2006

Dias S, McNamee R, Vail A. Evidence of improving quality of reporting of randomised controlled trials in subfertility. *Human Reproduction* 2006;**21**(10):2617–27.

El-Taieb 2009

El-Taieb MAA, Herwig R, Nada EA, Greilberger J, Marberger M. Oxidative stress and epididymal sperm transport, motility and morphological defects. *European Journal of Obstetrics, Gynecology, and Reproductive Biology* 2009;**144 Suppl 1**:199–203.

Eskenazi 2005

Eskanazi B, Kidd SA, Marks AR, Sloter E, Block G, Wyrobeck AJ. Antioxidant intake is associated with semen quality in healthy men. *Human Reproduction* 2005;**20**(4): 1006–12.

Evenson 2006

Evenson D, Wixon R. Meta-analysis of sperm DNA fragmentation using the sperm chromatin structure assay. *Reproductive Biomedicine Online* 2006;**12**(4):466–72.

Evenson 2007

Evenson DP, Kasperson K, Wixon RL. Analysis of sperm DNA fragmentation using flow cytometry and other techniques. *Society for Reproduction and Fertility* 2007;**65 Suppl**:93–113.

Higgins 2011

Higgins JPT, Green S, editors. Cochrane Handbook for Systematic Reviews of Interventions 5.1.0 (updated March 2011). *The Cochrane Collaboration: available from www.cochrane-handbook.org*. Chichester UK: John Wiley and Sons Ltd, 2011.

Jensen 2014

Jensen MB. Vitamin D and male reproduction. *Nature* 2014;**10**:175–86.

Lafuente 2013

Lafuente R, Gonzalez-Comadran M, Sola I, Lopez G, Brassesco M, Carreras R, et al. Coenzyme Q10 and male infertility: a meta-analysis. *Journal of Assisted Reproduction and Genetics.* 2013;**30**(9):1147–56.

Li 2006

Li Z, Wang L, Cai J, Huang H. Correlation of sperm DNA damage with IVF and ICSI outcomes: a systematic review and meta-analysis. *Journal of Assisted Reproduction and Genetics* 2006;**23**(9-10):367–76.

Martinez- Soto 2011
Martinez-Soto JC. Effect of dietary DHA supplementation on sperm quality. Clinicaltrials.gov NCT01368484.

Maxwell 2002
Maxwell DT, Jacobson JD, King A, Chan PJ. Effect of pentoxifylline on tumor suppressor and proto-oncogene apoptosis in sperm. *Journal of Assisted Reproduction and Genetics* 2002;**19**(6):279–83.

Oliva 2009
Oliva A, Dotta A, Multigner L. Pentoxifylline and antioxidants improve sperm quality in male patients with varicocele. *Fertility and Sterility* 2009;**91**(4):1536–9.

Patel 2008
Patel SR, Sigman M. Antioxidant therapy in male infertility. *Urologic Clinics of North America.* 2008;**35**(2):319–30. MEDLINE: 18423251

Revel 2006
Revel A. A preliminary study on effect of omega-3 on human sperm. ClinicalTrials.gov. [CTG: NCT00479960]

Robinson 2012
Robinson L, Gallos ID, Conner SJ, Rajkhowa M, Miller D, Lewis S, et al. The effect of sperm DNA fragmentation on miscarriage rates: a systematic review and meta-analysis. [Review]. *Human Reproduction* 2012;**27**(10):2908–17.

Ross 2010
Ross C, Morriss A, Khairy M, Khalaf Y, Braude P, Coomarasamy A, El-Toukhy T. A systematic review of the effect of oral antioxidants on male infertility. *Reproductive Biomedicine Online* 2010;**20**(6):711–23. [PUBMED: 20378409]

Schmid 2012
Schmid TE, Eskenazi B, Marchetti F, Young S, Weldon RH, Baumgartner A, et al. Micronutrients intake is associated with improved sperm DNA quality in older men. *Fertility and Sterility* 2012;**98**(5):1130–7.

Sheweita 2005
Sheweita S, Tilmisany A, Al-Sawaf H. Mechanisms of male infertility: Role of antioxidants. *Current Drug Metabolism* 2005;**6**(5):495–501.

Shiva 2011
Shiva M, Gautam AK, Verma Y, Shivgotra V, Doshi H, Kumar S. Association between sperm quality, oxidative stress, and seminal antioxidant activity. *Clinical Biochemistry* 2011;**44**(4):319–24.

Showell 2013
Showell MG, Brown J, Clarke J, Hart RJ. Antioxidants for female subfertility. *Cochrane Database of Systematic Reviews* 2013, Issue 8. [DOI: 10.1002/14651858.CD007807.pub2.

Sikka 1995
Sikka S, Rajasekaran M, Hellstrom W. Role of oxidative stress and antioxidants in male infertility. *Journal of Andrology* 1995;**16**(6):464–8.

Stankiewicz 2003
Stankiewicz M, Norman R. Zinc for male infertility. *Cochrane Database of Systematic Reviews* 2003, Issue 4.

Talevi 2013
Talevi R, Barbato V, Fiorentino I, Braun S, Longobardi S, Gualtieri R. Protective effects of in vitro treatment with zinc, d-aspartate and coenzyme q10 on human sperm motility, lipid peroxidation and DNA fragmentation. Reproductive Biology and Endocrinology 2013; Vol. 11:81.

Tarozzi 2007
Tarozzi N, Bizzaro D, Flamigni C, Borini A. Clinical relevance of sperm DNA damage in assisted reproduction. *Reproductive Biomedicine Online* 2007;**14**(6):746–57.

Tournaye 2006
Tournaye H. Evidence-based management of male subfertility. *Current Opinion in Obstetrics & Gynecology* 2006;**18**:253–9.

Tremellen 2008
Tremellen K. Oxidative stress and male infertility - a clinical perspective. *Human Reproduction Update* 2008;**14**(3): 243–58.

Tsafrir 2010
Tsafrir A. Is a carnitine based food supplement (PorimoreTM) for infertile men superior to folate and zinc with regard to pregnancy rates in intrauterine insemination cycles?. Information obtained from ClinicalTrials.gov on 1 March 2010.

Walczak-Jedrzejowska 2013
Walczak-Jedrzejowska R, Wolski JK, Slowikowska-Hilczer J. The role of oxidative stress and antioxidants in male fertility. *Central European Journal of Urology* 2013;**66**(7):60–7.

Wathes 2007
Wathes DC, Abayasekara DR, Aitken RJ. Polyunsaturated fatty acids in male and female reproduction. *Biology of Reproduction* 2007;**77**(2):190–201.

Zareba 2013
Zareba P, Colaci DS, Afeiche M, Gaskins AJ, Jorgensen N, Mendiola J, et al. Semen quality in relation to antioxidant intake in a healthy male population. *Fertility and Sterility* 2013;**100**(6):1572–9.

Zhou 2007
Zhou X, Liu F, Zhai S. Effect of L-carnitine and/or L-acetyl-carnitine in nutrition treatment for male infertility: a systematic review. *Asia Pacific Journal of Clinical Nutrition* 2007;**16 Suppl 1**:383–90. MEDLINE: 17392136

Zini 2011
Zini A, Dohle G. Are varicoceles associated with increased deoxyribonucleic acid fragmentation? [Review]. *Fertility and Sterility* 2011;**96**(6):1283–7.

* *Indicates the major publication for the study*

CHARACTERISTICS OF STUDIES

Characteristics of included studies *[ordered by study ID]*

Akiyama 1999

Methods	Randomised crossover trial. Single centre
Participants	Country: Japan Male infertility Mean age: 36 years (treatment group age range 24 to 49, control 30 to 37 years) N = 10 recruited Inclusion criteria: male infertility (ROS > 5 x 10,000 counts/10,000,000 viable spermatozoa) Exclusion criteria: azoospermia, pyospermia Duration of study: 8 months
Interventions	Ethylcysteine 600 mg/day for 3 months (n = 5) versus Vitamin E 600 mg/day (n = 5) With a one month wash out, then crossover for another 3 months. Only data from the first phase were used in data analysis
Outcomes	Semen parameters
Notes	In Japanese. Data extraction translated by Ichiro, a colleague of Samantha Roberts, 29 January 2009 Author contacted 'no further information is available'

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	"Patients were divided randomly"
Allocation concealment (selection bias)	Unclear risk	No details
Blinding (performance bias and detection bias) All outcomes	Unclear risk	No details
Incomplete outcome data (attrition bias) All outcomes	Low risk	No incomplete outcome data
Selective reporting (reporting bias)	Low risk	Sperm parameters reported

Attallah 2013

Methods	Open label randomised controlled trial
Participants	Country: Egypt Isolated idiopathic athenozospermia Prior to intrauterine insemination (IUI) Mean age: unknown, "both treatment groups were homogenous at the time of randomization regarding the type and duration of infertility" N = 60 Inclusion criteria: only couples with idiopathic athenozospermia (progressive motility < 32%) with normal other seminal criteria and normal infertility workup for female partner Trial duration: unknown
Interventions	N-acetylcysteine (NAC) 600 mg (n = 30) versus No treatment (n = 30) Duration of treatment: 12 weeks prior to IUI
Outcomes	Mean sperm concentration Percentage of progressive sperm motility Clinical pregnancy rate
Notes	Conference abstract Couples were randomised - attempted to contact authors 4 February 2014, unable to find e-mail address. Letter posted 12 February 2014

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	"Couples were randomised" - does not describe methods
Allocation concealment (selection bias)	Unclear risk	No description of methods
Blinding (performance bias and detection bias) All outcomes	High risk	"Open-labelled"
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	No description of dropouts
Selective reporting (reporting bias)	Unclear risk	Unknown - conference abstract

Azizollahi 2013

Methods	Randomised study, double blind placebo-controlled - 4-arm trial
Participants	Country: Iran Infertile subjects (N = 160) with varicocelectomy only 112 completed the study Mean age: age ranges and duration of infertility of male partners were from 20 to 43 years (mean ± SD: 29.07 ± 6.8) and 1 to 10 years (mean ± SD: 3.32 ± 2.4) respectively Inclusion criteria: the presence of a grade III varicocele, on I to III scale, was the criteria to enter the study. It was assessed by clinical parameters and was confirmed by Doppler ultrasound scanning Exclusion criteria: patients with the evidence of leukocytospermia, low testicular volume < 15 mL, congenital urogenital abnormalities and urogenital infections were excluded from the study Duration of study: May 2008 to November 2010, 2.5 years Duration of treatment: 6 months
Interventions	Zinc (n = 32) versus Folic acid (n = 26) versus Zinc and folic acid (n = 29) versus Placebo (n = 25) Patients in each group took one capsule orally per day after dinner following varicocelectomy for 6 months. The dosage of the zinc sulfate (Alhavi pharmaceutical Co, Tehran, Iran) and folic acid (Iran Daru, Tehran, Iran) was 66 mg and 5 mg per capsule, respectively. Patients in placebo group received the same capsules without the effective drug
Outcomes	Sperm parameters; number, morphology, halo formation rate, motility, forward progressive motility
Notes	IRCT registration no: IRCT138802261910N1 Contact details: SN Nematollahi-mahani email: nematollahimahani@yahoo.com or nnematollahi@kmu.ac.ir E-mailed the author 3 March 2014. Author replied 6 March 2014 with information included in the ROB table. Author e-mailed again to ask about pregnancy data and dropouts from which group. The author informed us that Azizollahi 2011 was part of this trial and gave pregnancy and dropout data (there were originally 40 in each group) . "At that time we observed 2 pregnancies in zinc/folic acid group, 1 pregnancy in zinc group, and no pregnancy in placebo and folic acid group. These data were just 6 months after the start of the trial."

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	For randomisation we used a table with 200 numbers (1 to 200). Before the trial we gave each group a number between 1 and 4 and

Azizollahi 2013 (*Continued*)

		allocated each group into the table. By this method the first, fifth, ninth, 13th and .. . patients were allocated into the group 1 and the same manner was applied to the other groups
Allocation concealment (selection bias)	Low risk	"We used sealed containers with the randomization number on them. Drugs or placebo were in opaque capsules"
Blinding (performance bias and detection bias) All outcomes	Low risk	"Our study was double blind. Neither the urologist nor the patient or examiner in the lab were aware of the arrangement of the study"
Incomplete outcome data (attrition bias) All outcomes	Low risk	Information gained from communication with the author explained the dropouts numbers
Selective reporting (reporting bias)	Low risk	Clinical pregnancy rate data gained from email correspondence with the author

Balercia 2005

Methods	Randomised trial, double blind. No details of randomisation or concealment
Participants	Country: Italy Infertile men recruited from an andrology clinic Mean age 30 (range 24 to 38 years) N = 60 recruited Inclusion criteria: primary infertility > 2 years after regular intercourse with a fertile woman; 20 to 40 years of age; normal rheologic characteristics; sperm count > 20 x 10^6 / mL; sperm motility < 50%; normal sperm morphological features > 30%; seminal WBC < 1 x 10^6 /mL; negative sperm culture and chlamydia and mycoplasma urealyticum; normal serum gonadotropins; T, E^2 and PRL; absence of infectious or genital disease; no anatomic abnormalities of the genital tract; absence of systemic diseases or treatment with other drugs within the 3 months before enrolment in the study. Absence of smoking, alcohol or recreational drug use or of occupational chemical exposure Total duration of study 9 months
Interventions	L carnitine 3 g/day orally (n = 15) versus L acetyl carnitine 3 g/day orally (n = 15) versus L carnitine 2 g/day + L acetyl carnitine 1 g/day (n = 15) versus placebo (n = 15) Duration of treatment: 6 months

Balercia 2005 (*Continued*)

Outcomes	Semen parameters	
Notes		
Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	"Placebo controlled double blind randomised trial". No methods of randomisation mentioned
Allocation concealment (selection bias)	Unclear risk	No details
Blinding (performance bias and detection bias) All outcomes	Low risk	"double blind" study. Patients blinded but no details as to who else
Incomplete outcome data (attrition bias) All outcomes	Low risk	1 withdrawal from the L carnitine 2g/day + L acetyl carnitine 1 g/day group
Selective reporting (reporting bias)	Low risk	Outcomes reported

Balercia 2009

Methods	Double blind, placebo-controlled randomised trial
Participants	Country: Italy (University of Marche, Ancona) Infertile men recruited from andrology clinic Mean age: 32 years (range 27 to 32) N = 60 men recruited Inclusion criteria: age 20 to 40 years, infertility > 2 years, regular sexual intercourse with a potentially fertile female; normal rheologic characteristics (appearance, consistency and liquefaction) of semen and volume and pH in normal range, sperm count > 20 x 10^6 / mL, sperm motility < 50% (WHO 1999), normal morphology > 30%, seminal white blood cells < 1 x 10^6 /mL and a negative sperm culture and chlamydia and M.urealyticum detection. Normal levels of gonadotropins absence of genital disease and anatomical abnormalities of the genital tract including variocoele and antibodies. Absence of systemic disease or treatment with other drugs within 3 months of being enrolled in the study. Absence of smoking, alcohol and drug addiction and exposure to occupational chemicals Exclusion criteria: transient decrease in semen quality during run in and those who had sudden improvement in semen parameters during run in
Interventions	Coenzyme Q10 100 mg 2 times /day (n = 30) versus Placebo (n = 30) Duration of study: 9 months

Balercia 2009 (*Continued*)

Outcomes	Primary: semen parameters Secondary: pregnancy rate
Notes	

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	"randomised" - no details. At end of trial the paper mentions - "after opening randomisation list" page 1789
Allocation concealment (selection bias)	Unclear risk	No details
Blinding (performance bias and detection bias) All outcomes	Low risk	"Double blind" - placebo used therefore low risk for performance bias
Incomplete outcome data (attrition bias) All outcomes	Low risk	"5 patients dropped out of the study", 2 from the treatment group and 3 from the placebo group; this was discovered after opening the randomisation list at the end of the study. Intention to treat was carried out
Selective reporting (reporting bias)	Low risk	Outcomes reported

Biagiotti 2003

Methods	Randomised study - conference proceeding
Participants	Country: Italy, Andrology clinic in Bologna Population: severe idiopathic oligoasthenospermia (sperm concentration < 5000 /μl) Mean age: group a and b 35 (range 30 to 40 years), Group c 31 (range 24 to 34) N= 42 Inclusion criteria: severe idiopathic oligoasthenospermia (sperm concentration < 5000 / μl) Exclusion criteria: Genomic, hormonal or inflammatory diseases Duration of study: ?
Interventions	a. Acetyl-carnitine 1 g/day + L-carnitine 2 g/day + cinnoxicam (n = 14) versus b. ALC + LC (n = 14) versus c. No therapy (n = 14) Duration of treatment: ?

Biagiotti 2003 (*Continued*)

Outcomes	Semen parameters	
Notes	Conference abstract - no data given. Contacted authors but no reply re questions as yet	
Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	"randomised (1patient = 1 block) analysis of variance" Was this at the time of sequence generation or at data analysis?
Allocation concealment (selection bias)	Unclear risk	Not mentioned
Blinding (performance bias and detection bias) All outcomes	High risk	Not mentioned. Control is no treatment.
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Unclear
Selective reporting (reporting bias)	Unclear risk	Unclear conference abstract

Cavallini 2004

Methods	Randomised controlled trial. Allocation concealment: anonymous colour coded boxes
Participants	Country: Italy Population: idiopathic plus variocoele associated oligo-asthenospermia (OAT) Mean age: 34 years (range 27 to 40) N = 325 Inclusion criteria: OAT men with deficiencies in all sperm patterns whose chief complaint was primary couple infertility > 12 months with regular intercourse. Normal sperm appearance, consistency, liquefaction, volume, pH. Female partner without fertility problems. Varicoceles Exclusion criteria: azoospermia, seminal white blood cell concentration more than 1000, 000/mL, positive urethral chlamydia swab test, oligospermia < 5,000,000 /ml, hormonal alterations, age > 40 yrs, presence of anti-sperm antibodies, drug, tobacco or alcohol abuse, ongoing medical treatments, presence of hydrocoele, diabetes,hypertension, x-ray exposure in previous 8 months, peptic ulcer, unexplained gastric pain, previous hypersensitivity to NSAIDS or carnitines, carnitine metabolism deficiency, bilateral variocoele, prostate abnormalities, previous or current testicular pathology, testicle echographic abnormalities Duration of study: 9 months

Interventions	Group 1: placebo, starch tablets 2 times /day + glycerine suppository (1 every 4 days) (n = 47) Group 2: L-carnitine 1 x 2 g/day plus acetyl-L-carnitine 500 x 2 mg/day plus glycerine suppository (n = 39) Group 3: L-carnitine 1x 2 g/day plus acetyl-L-carnitine 500 x 2 mg/day plus glycerine suppository plus cinnoxicam suppository 1 x 30 mg (every 4 days) (n =44) Cinnoxicam is a non-steroidal ant-inflammatory therefore this arm (Group 3) was not included in meta-analysis as per protocol Duration of treatment: 6 months
Outcomes	Primary: sperm parameters Secondary: pregnancy, side effects
Notes	Continuous data taken from Cavallini 2004a 'excluded conference abstract' no data for placebo group Unit of analysis variocoele therefore cannot extract data that were presented as median (interquartile range) Author contacted regarding uneven numbers and missing placebo and continuous data Author replied that raw data were not available due to computer crash

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	"casual random tables"
Allocation concealment (selection bias)	Low risk	"drug placebos identical in appearance", "anonymized carnitine and cinnoxicam and glycerine suppository containers; and filled and sealed anonymous color coded boxes", "the color code was disclosed to physicians by pharmacists and by IRB at the end of the research"
Blinding (performance bias and detection bias) All outcomes	Low risk	"All study personnel and participants were blinded to treatment assignment for the duration of the study"
Incomplete outcome data (attrition bias) All outcomes	High risk	325 randomised but only 185 accounted for; 55 dropouts from 185 (42%), 53 reasons given for the dropouts
Selective reporting (reporting bias)	Low risk	Sperm parameters were primary outcome. Intention to collect biochemical pregnancy data as secondary outcome recorded in the methods

Ciftci 2009

Methods	Randomised controlled trial Allocation concealment: sealed envelopes
Participants	Country: Turkey Population: men attending fertility clinic with idiopathic infertility. Normal sperm parameters Mean age: treatment group 33.1 ± 4.5, control 32.8 ± 3.7 years N = 120 recruited Inclusion criteria: men attending fertility clinic with idiopathic infertility. Normal sperm parameters Exclusion criteria: cryptorchidism, vasectomy, abnormal liver functioning, smoking, alcohol consumption Duration of study: 3 months
Interventions	N-acetylcysteine 600 mg/day (n = 60) versus placebo (n = 60) Duration of treatment: 3 months
Outcomes	Primary outcomes: Total antioxidant capacity, peroxide levels, oxidative stress Secondary outcomes: Other semen parameters
Notes	Attempt made to contact author regarding data reported in SDs or SEs, e-mail sent 24 September 2010

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	"patients were randomly allocated to the study group (60 men) or control group (60 men)" No mention of how the randomisation was carried out
Allocation concealment (selection bias)	Low risk	"Using sealed envelopes, these patients were randomly allocated..."
Blinding (performance bias and detection bias) All outcomes	Low risk	"The patients in the study and control groups were unaware of whether they were receiving the drug or placebo." ? researchers blinded
Incomplete outcome data (attrition bias) All outcomes	Low risk	All men recruited were analysed. No withdrawals
Selective reporting (reporting bias)	Low risk	Report includes all expected outcomes Baseline data were similar for both groups

Conquer 2000

Methods	Randomised placebo controlled trial, 3 arms
Participants	Country: Canada Population: healthy astheno-zoospermic individuals who were patients of an infertility clinic Mean age: placebo = 35.2, DHA 400 mg = 38.3, DHA 800 mg = 34.4 N = 28 Inclusion criteria: Astheno-zoospermic, sperm motility < 50% of total sperm Exclusion criteria: Not stated Duration of study: Not stated
Interventions	Fatty acid omega 3 Docosahexaenoic acid (DHA) 400 mg/day (n = 9) versus DHA 800 mg/day (n = 10) versus placebo (n = 9) Duration of treatment: 3 months
Outcomes	Sperm parameters
Notes	Data with SEs converted to SDs Placebo arms split

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	"The 28 subjects were randomly assigned to ..."
Allocation concealment (selection bias)	Unclear risk	Not stated
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Placebo capsules made with "corn oil/soy oil" however blinding not stated
Incomplete outcome data (attrition bias) All outcomes	Low risk	All men randomised were in the analysis, no dropouts. All specified outcomes were reported
Selective reporting (reporting bias)	Low risk	Outcomes reported

Dawson 1990

Methods	Randomised controlled trial
Participants	Country: USA Population: males with sperm agglutination Mean age: age range 25 to 45 years N = 30 Inclusion criteria: sperm agglutination over 25%, negative sperm antibodies, physically normal, no inflammatory disease Exclusion criteria: unclear Duration of study: 4 weeks
Interventions	Ascorbic acid (AA) 1000 mg (n = 10) versus AA 200 mg (n = 10) versus placebo (n = 10) Duration of treatment: 3 weeks
Outcomes	Seminal parameters
Notes	Placebo numbers split by 2 Data were given in SE converted to SD

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	"By random selection, three groups of 10 subjects each.. "
Allocation concealment (selection bias)	Unclear risk	Not stated
Blinding (performance bias and detection bias) All outcomes	Low risk	"Each subject was told he was receiving AA and expected improvement in sperm quality"
Incomplete outcome data (attrition bias) All outcomes	Low risk	No dropouts
Selective reporting (reporting bias)	Low risk	All specified outcomes were reported

Dimitriadis 2010

Methods	Randomised controlled trial
Participants	Country: Japan Population: infertile men with oligoasthenospermia Mean age: unclear N = 96

Dimitriadis 2010 (*Continued*)

	Inclusion criteria: unclear Exclusion criteria: unclear Duration of study: 12 weeks
Interventions	L-carnitine 1000 mg/day (n = 26) versus No treatment (n = 22) Other groups randomised but not appropriate for this review are: vardenafil (n = 23) and sildenafil (n = 25)
Outcomes	Seminal parameters
Notes	Tried multiple times to contact authors for randomisation details and methods. No response. Last contacted in Feburary 2014. E-mail addresses tried: saitomo@kochi-u.ac.jp, akrosnin@hotmail.com

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Details not given in paper and no response from authors
Allocation concealment (selection bias)	Unclear risk	Details not given in paper and no response from authors
Blinding (performance bias and detection bias) All outcomes	High risk	Control no treatment. Details not given in paper and no response from authors
Incomplete outcome data (attrition bias) All outcomes	Low risk	All data points accounted for
Selective reporting (reporting bias)	Low risk	All data points accounted for

Eslamian 2012

Methods	Randomised controlled trial - triple blind
Participants	Country: Iran Population: astheno-zoospermic infertile men Mean age: unclear N = 50 Inclusion criteria: patients interest in contribution aged 20-45 who have passed at least one year from the date they have decided to have a baby, not to using pregnancy protection methods, affected by idiopathic asthenozoospermia based on WHO criteria, normal serum gonadotropin, testosterone and prolactin values Exclusion criteria: affected by genital system infection or taking drug for the infection during past three months, affected by anatomical anomalies in genital system such as

Eslamian 2012 (*Continued*)

	varicocoele, surgical history on testicles and vasdeferane Duration of study: 12 weeks
Interventions	465 mg of DHA plus 600 IU of vitamin E (n = 25) versus Placebo (n = 25)
Outcomes	Seminal parameters - serum fatty acid concentration and sperm membrane fatty acid concentration
Notes	In Arabic - translated. Tried multiple times to contact authors for further study details with no response. Last tried to contact Feburary 2014: janati@avicenna.ac.ir

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Stratified blocked randomisation
Allocation concealment (selection bias)	Low risk	Cans containing capsules marked as A1, A2, B1, B2 and patients, researchers and physician were unaware of the types of drugs
Blinding (performance bias and detection bias) All outcomes	Low risk	Cans containing capsules marked as A1,A2, B1, B2 and patients, researchers and physician were unaware of the types of drugs
Incomplete outcome data (attrition bias) All outcomes	Low risk	Withdrawals and exclusions: **Intervention group (3 withdrawals)**: one man could not refer to the clinic in sixth week, the wife of the other one got pregnant, and another one was excluded because he have not taken more than 10% of the capsules **Control group (6 withdrawals)**: two men could not refer to the clinic in sixth week, one man could not refer to the clinic in 12^{th} week. One man used complementary Coenzyme Q_{10}, and another one was excluded because he have not taken more than 10% of the capsules
Selective reporting (reporting bias)	Low risk	Sperm parameters reported

Galatioto 2008

Methods	Randomised controlled, intention to treat, single centre study. Central allocation - pharmacy, blinded Power calculation performed
Participants	Country: Italy Population: men with persistent oligospermia (5 to 20 m/ml) Mean age: treatment group 32 years (27.5 to 35.5), control 33 (23 to 36) N = 42 Inclusion criteria: having performed a retrograde embolization with concomitant oligospermia, persistent oligospermia and infertility > 12 months Exclusion criteria: smoking, alcohol consumption, taking any fertility drugs within 3 months prior to the study, serious medical or psychiatric condition, abnormal hormonal profile, sperm infection
Interventions	N-acetylcysteine (NAC) 600 mg and vitamins-minerals (vitamin C, vitamin E, vitamin A, thiamine, riboflavin, piridoxin, nicotinamide, pantothenate, biotin, cyanocobalamin, ergocalciferol, calcium, magnesium, phosphate, iron, manganese, copper, zinc (n = 20) versus no treatment (n = 22) Duration: 12 months after end of study which ran for 90 days
Outcomes	Primary: seminal parameters Secondary: pregnancy (undefined) and adverse effects
Notes	Attempted to contact author regarding median data. No response as yet

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	"Subjects were randomly assigned to either antioxidant therapy or no medical therapy. Randomisation number was assigned by random allocation software using a block randomisation design"
Allocation concealment (selection bias)	Low risk	'All steps of randomisation process were performed blindly in the pharmacy of our hospital.'
Blinding (performance bias and detection bias) All outcomes	High risk	Control is no treatment
Incomplete outcome data (attrition bias) All outcomes	Low risk	"intention to treat"

Galatioto 2008 (*Continued*)

Selective reporting (reporting bias)	Low risk	Does not appear to be any selective reporting

Greco 2005

Methods	Double blind randomised controlled trial. Trialists and patients blinded. Methods were unclear
Participants	Country: France Population: infertile males Mean age: ? N = 64 Inclusion criteria: TUNEL assay showed a presence of fragmented DNA ≥ 15% of ejaculated spermatozoa Exclusion criteria: variocele, genitourinary inflammation, infection, smoking Duration of study: ?
Interventions	Vitamin C 500 mg 2 x /day + vitamin E 500 mg 2 x /day (n = 32) versus placebo (n = 32) Duration of treatment: 2 months
Outcomes	Sperm parameters
Notes	

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	"The study participants were randomised into 2 groups"
Allocation concealment (selection bias)	Unclear risk	No mention of allocation concealment
Blinding (performance bias and detection bias) All outcomes	Low risk	"The study was double-blinded with both the authors and the patients unaware of which of the patients was in the treatment or control arm of the study"
Incomplete outcome data (attrition bias) All outcomes	Low risk	All specified outcomes are assessed. No dropouts
Selective reporting (reporting bias)	Low risk	Does not appear to be any selective reporting

Keskes-Ammar 2003

Methods	Randomised controlled trial - open label
Participants	Country: Tunisia Population: infertile men Mean age: 35.5 ± 6.8 (SD) Recruited: N = 78 Randomised: N = 54 Inclusion criteria: infertile men who had been married one year Exclusion criteria: ? Duration of study: 10 months
Interventions	Vitamin E (400 mg/day) + selenium (225 mg/day) for 3 months (n = 12) versus vitamin B (4.5 g/day) (n = 8) Duration of treatment: 3 months
Outcomes	Semen parameters
Notes	Attempted to contact authors regarding high attrition rate > 50% ?78 men randomised or 78 recruited then only 54 (28 in intervention and 26 in control). Then only 20 analysed due to non-compliance

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	"Randomisation was performed with random numbers" and the numeric code was withheld from researchers and patients"
Allocation concealment (selection bias)	Low risk	"the numeric code was withheld from researchers and patients"
Blinding (performance bias and detection bias) All outcomes	High risk	"The trial was randomised and open"
Incomplete outcome data (attrition bias) All outcomes	Low risk	High attrition rate was due to non-compliance, no intention to treat
Selective reporting (reporting bias)	Low risk	Sperm parameters reported

Kessopoulou 1995

Methods	Double blinded randomised placebo crossover trial Power calculation performed
Participants	Country: UK, Sheffield Population: men with high levels of reactive oxygen species (ROS) attending Obstetrics and Gynecology department for infertility Couples were undergoing IVF Mean age: ? Median age: 32 years Recruited: N = 30 Inclusion criteria: attending fertility clinic, high levels of ROS in semen. Female partner has patent tubes and is ovulating Exclusion criteria: men with antisperm antibodies, > 20% spermatozoa with I g (immunoglobulin A) or IgG antibodies and sperm concentration < 5 x 0^6 mL Duration of study: 2 years
Interventions	Vitamin E 300 mg 2 x /day (n = 15) versus identical placebo (n = 15) Duration of treatment: 3 months
Outcomes	Primary outcomes: semen parameters Secondary outcomes: adverse effects. Live birth
Notes	Attempted to contact author regarding median data, no response as yet Only first phase data used in analysis

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	"The study was a randomised double blind placebo controlled trial". "The randomisation was performed by the manufacturer"
Allocation concealment (selection bias)	Unclear risk	"The randomisation was performed by the manufacturer"
Blinding (performance bias and detection bias) All outcomes	Low risk	"the code was blind for the researcher and patients. The code was broken at the end of the trial"
Incomplete outcome data (attrition bias) All outcomes	Low risk	All outcomes are reported
Selective reporting (reporting bias)	Low risk	Outcomes reported as stated in the methods section

Kumamoto 1988

Methods	Double-blind randomised parallel trial
Participants	Country: Japan, 25 centres Population: male patients with abnormal sperm count or motility Mean age: average 32.8 (SD 4.8) Recruited: 375 Inclusion criteria: 1. Average sperm count $\leq 40 \times 10^6$ /mL measured on $\geq$ 2 occasions OR 2. Average sperm count $\geq$ 40 count $\leq 40 \times 10^6$ /mL measured on $\geq$ 2 occasions AND sperm motility < 50% Exclusion criteria: 1. Sperm count only measured at 1 occasion 2. Average sperm count $\leq 2 \times 10^6$/mL 3. Sperm motility = 0% 4. Testicular size < 8 mL using orchidometer bilaterally 5. Use of hormone or anti-hormone drug within preceding 3 months before the study period 6. WBC > 5/HPF in the semen or the presence of possible genito-urinary infection 7. Presence of hypoganadism or endocrine disease Presence of undescended testes, genito-uninary tract obstruction, varicocele or any other serious associated condition also included concomitant use of anti-hormonal and hormonal treatment and the 2 patients with polypharmacy were excluded from the data analysis Duraton of trial: January 1985 to June 1986 Duration of treatment: 12 weeks
Interventions	1. Mecobalamin group 6,000 micrograms/day (n = 125) (vitamin B12) versus 2. Mecobalamin group 1,500 micrograms/day (n = 124) versus 3. Placebo (n = 126)
Outcomes	Sperm concentration Sperm motility
Notes	Paper translated by Dr Tomoko Kumaga and Tan Wantao. No contact details available for authors. No useable data available. Data in spreadsheet to be added to other tables No ITT completed

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	"The 396 patients were divided into 3 groups (6000ug/day, 1500ug/day, placebo) by randomization. The implementation of randomization and allocation concealment was carried out by two people (Doctor Yamamoto, Doctor Shimizu)

Kumamoto 1988 (*Continued*)

Allocation concealment (selection bias)	Unclear risk	See above
Blinding (performance bias and detection bias) All outcomes	Low risk	Double blind - placebo used
Incomplete outcome data (attrition bias) All outcomes	High risk	No ITT
Selective reporting (reporting bias)	High risk	Subgroup analysis performed as an addition post-treatment

Lenzi 2003

Methods	Randomised placebo-controlled, double blind crossover trial Power calculation performed
Participants	Country: Italy Population: male factor infertility - oligoasthenoteratozoospermia (OAT) Mean age: ? Range: 20 to 40 years N = 100 Inclusion criteria: men are aged between 20 to 40 years with infertility lasting longer than 2 years. Regular sexual intercourse with a gynaecologically normal female partner with no female infertility. Absence of endocrine disease, genital infections, obstructive cryptorchism, antisperm antibodies, normal sperm parameters with no significant differences after 3 tests. Mild oligospermia. Sperm concentration 10 to 20 x 10^6/mL and motility 10% to 30% Exclusion criteria: not mentioned Duration of study: 10 months
Interventions	L-carnitine 2 g/day (n = 43) versus placebo (n = 43) Duration of treatment: 6 months
Outcomes	Semen parameters and pregnancy rate
Notes	First phase data only used in analysis Attempted to contact author regarding standard deviations, how many were in each group for the first phase and how many of the 4 who went to assisted reproduction did so in the first phase and what do they mean by 172 cycles. No response yet

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**

Lenzi 2003 (*Continued*)

Random sequence generation (selection bias)	Unclear risk	"we report on a randomised placebo controlled cross over trial". No mention of method of randomisation
Allocation concealment (selection bias)	Unclear risk	No mention
Blinding (performance bias and detection bias) All outcomes	Low risk	Double blinded, "seemingly identical placebo"
Incomplete outcome data (attrition bias) All outcomes	Low risk	14 withdrew - 4 went onto assisted reproduction, 6 did not return for second period and 4 due to pregnancy in first phase. Therefore should only be ?4 at the most lost from first phase. No intention to treat All withdrawals accounted for for whole trial however how many were lost in the first phase in first phase
Selective reporting (reporting bias)	Low risk	All outcomes are reported

Lenzi 2004

Methods	Placebo-controlled, double blind randomised trial. No mention of method of randomisation or allocation concealment "When codes were broken at the end of the study" Power calculation performed
Participants	Country: Italy Population: infertile males with oligoasthenoteratozoospermia Mean age: not stated. Age range 20 to 40 years N = 60 Duration of study: 8 months Inclusion criteria: oligoasthenoteratospermia, age between 20 to 40 years, infertility > 2 years with regular intercourse. No endocrine disease, cryptorchidism, genital infections or obstructions, variocoele or testicular hypertrophy, antisperm antibodies Exclusion criteria: none Duration of study: 8 months
Interventions	L-carnitine 2 g/day + L-acetyl-carnitine 500 mg 2 x /day (n = 30) versus placebo (n = 26) Duration of treatment: 6 months
Outcomes	Semen parameters and pregnancy rate
Notes	Attempted to contact author regarding 8 month follow up data. No reply as yet

Lenzi 2004 (*Continued*)

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	"placebo controlled double blind randomised trial"
Allocation concealment (selection bias)	Unclear risk	Mentions coding - "When codes were broken at the end of the study"
Blinding (performance bias and detection bias) All outcomes	Low risk	"Double blind" - placebo used therefore low risk for performance bias
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	4 men withdrew from the placebo group. 60 randomised 56 analysed. No intention to treat
Selective reporting (reporting bias)	Low risk	Outcomes reported

Li 2005

Methods	Double blinded randomised parallel trial
Participants	Country: Eastern China Population: infertile men with oligoasthenospermia Mean Age: Treatment 30 ± 5.5 (23 to 45 years), Control 32 ± 3.5 (24 to 46 years) N = 150 Inclusion criteria: no smoking or alcohol. Any fertility medication needed to be stopped 2 weeks before Exclusion criteria: nil Duration: 3 months
Interventions	L-carnitine 2 g/day + acetyl-L-carnitine 1 g/day (n = 85) (90 with intention to treat) versus vitamin E (100 mg tid) + vitamin C (100 mg tid) (n = 53) (60 with intention to treat) Duration of treatment: 3 months
Outcomes	Seminal parameters and pregnancy rate per couple
Notes	Withdrawal: 5 from treatment group and 7 from control Contact author re methods of randomisation, concealment and whether SD or SEs used and query that this is the same trial as Li 2005a Translated by Shaofu Li 10 November 2008

Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**

Li 2005 (*Continued*)

Random sequence generation (selection bias)	Unclear risk	Randomised controlled trial. Methods not described
Allocation concealment (selection bias)	Unclear risk	Allocation concealment not described
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Double blind but unclear who is blinded as the control is another antioxidant i.e. not placebo
Incomplete outcome data (attrition bias) All outcomes	Low risk	Attrition explained
Selective reporting (reporting bias)	Unclear risk	Unclear whether data given in standard deviations or standard errors. Assumed to be SDs

Li 2005a

Methods	Randomised trial. No information on concealment
Participants	Country: Eastern China Population: infertile men with oligoasthenospermia Mean age: 29 ± 3.5 (23 to 40 years) N = 80 Inclusion criteria: no smoking or alcohol. Any fertility medication needed to be stopped 2 weeks before Exclusion criteria: nil Duration: not stated
Interventions	L-carnitine 2g/day (n = 40) versus vitamin E 100 mg + vitamin C 100 mg tid (n = 40) Duration of treatment: 3 months
Outcomes	Seminal parameters and pregnancy
Notes	Attempted to contact author re methods of randomisation, concealment and whether SD or SEs used and whether this is the same trial as Li 2005. Also asked whether there were any data on pregnancy rate. Translator replied 22.09.09 no pregnancy data were available in the text of the trial Translated by Shaofu Li 10/11/08

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	No mention of methods of randomisation

Li 2005a (*Continued*)

Allocation concealment (selection bias)	Unclear risk	No mention of allocation concealment
Blinding (performance bias and detection bias) All outcomes	Unclear risk	No mention of blinding
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Withdrawal: 8 from treatment (n = 32) and 9 from control (n = 31). 21% loss to follow up. No intention to treat
Selective reporting (reporting bias)	Unclear risk	Unclear whether data given in SDs or SEs

Lombardo 2002

Methods	Randomised controlled crossover trial
Participants	Country: Italy Population: male infertility, oligoasthenospermia Mean age: not stated N = 100 Inclusion criteria: age 20 to 40 years. Infertility > 2 years. 3 baseline semen analysis demonstrating concentration 10 to 20 10^6/mL, 10% to 30% total motility, forward progression < 15%, abnormal morphological forms < 70%, curvilinear velocity 10 to 30 /second + linearity < 4 Exclusion criteria: not mentioned Duration: 10 months
Interventions	L-carnitine 2 g/day (n=?) versus placebo (n =?) Duration of treatment: 2 months
Outcomes	Semen parameters
Notes	Abstract only Attempted to contact author re first phase data, outcomes, randomisation, concealment and whether there was a full publication of the trial

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	randomised - "all patients had an initial 2 months run in period and then randomised to 2 months of carnitine or placebo."
Allocation concealment (selection bias)	Unclear risk	No mention of allocation concealment

Lombardo 2002 (*Continued*)

Blinding (performance bias and detection bias) All outcomes	Low risk	"double blind crossover trial" - placebo used therefore low risk for performance bias
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	86 patients completed the trial out of 100. Need to see full trial for the reasons for withdrawals and intention to treat
Selective reporting (reporting bias)	Unclear risk	Unclear (conference abstract)

Martinez-Soto 2010

Methods	Randomised double-blind study controlled trial
Participants	Country: Spain Population: infertile men Mean age: DHA group 35.23, placebo 36.10 - overall average age 35 N = 42 - abstract, n = 64 from author Inclusion criteria: men suffering from male factor infertility, according to the World Health Organization guidelines (WHO 1999), and who were undergoing infertility evaluation during the period 2009 to 2011 Exclusion criteria: oncological patients, those suffering from metabolic disease, chromosomal or genetic alterations, and patients on anticoagulant treatment Duration: 10 weeks
Interventions	Brudy Plus - enzymatic nutraceutical triglyceride oil - 1500 mg/day of DHA-enriched oil (the patients ingested 1000 mg of DHA and 135 mg of eicosapentaenoic acid (EPA) per day) (n = 35) versus placebo (n = 29) Duration of treatment: 10 weeks
Outcomes	Sperm DNA fragmentation, seminal parameters, lipid composition, antioxidant capacity
Notes	Pharmaceutical funding: Intervention Brudy plus and trial was supported by Brudy technology S.L Abstract from conference Contacted author multiple times via e-mail, JuanCarlos.Martinez@ivi.es, for further study details. Clarified that the abstract details were different from that in the final study, a copy of the unofficial manuscript was submitted to the review authors. Last contact was on 26 February 2014

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Random list with a computer program

Martinez-Soto 2010 (*Continued*)

Allocation concealment (selection bias)	Low risk	Closed and numerated envelopes with allocation group
Blinding (performance bias and detection bias) All outcomes	Low risk	Participants knew that they was included in group A or B but only Brudy technology knew the assignation to the control group or experimental group
Incomplete outcome data (attrition bias) All outcomes	Low risk	Unable to be ascertained from study
Selective reporting (reporting bias)	Low risk	Outcomes reported

Merino 1997

Methods	Randomised controlled trial
Participants	Country: Mexico Population: male idiopathic asthenozoospermia attending a fertility clinic Mean age: 30.8 ± 6 (20 to 40 years) N = 47 Inclusion criteria: free of urogenital symptoms, idiopathic asthenozoospermia, not received drugs in prior 6 months and healthy Exclusion criteria: variocoele, inflammatory diseases, endocrine disorders Duration of study: 6 months
Interventions	Pentoxifylline 1200 mg to 400 mg /3 x day (n = 25) versus placebo (n = 22) Duration of intervention: 6 months
Outcomes	Seminal parameters and hormonal assays
Notes	Attempted to contact author to ask if had data in means + SD and not medians + range as published. Letter sent 15 September 2009. Letter returned to sender

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	"The 47 men were divided at random"
Allocation concealment (selection bias)	Unclear risk	No mention of allocation concealment
Blinding (performance bias and detection bias) All outcomes	Unclear risk	No mention of blinding however the trial is placebo controlled

Merino 1997 (*Continued*)

Incomplete outcome data (attrition bias) All outcomes	Unclear risk	No withdrawals
Selective reporting (reporting bias)	Low risk	All outcomes reported

Micic 1988

Methods	Randomised controlled trial Methods not stated. No mention on methods of concealment
Participants	Country: Belgrade, Hungary Population: idiopathic oligoasthenospermia Mean age: 29 ± 2 in treatment group, 26 ± 3 in control group N = 90 Inclusion criteria: idiopathic oligoasthenospermia and no pregnancy for 2 years Exclusion criteria: variocoele, inflammatory diseases, endocrine disorders Duration of study: 3 months
Interventions	Pentoxifylline 1200 mg/day (n = 51) versus no treatment (n = 39) Duration of treatment: 3 months
Outcomes	Seminal parameters
Notes	Attempted to contact author regarding extractable data for pregnancy rate plus methods of of randomisation and concealment also don't know if adverse events are single or multiple events. No reply as yet

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	"A randomised group of 90 men"
Allocation concealment (selection bias)	Unclear risk	No mention of allocation concealment
Blinding (performance bias and detection bias) All outcomes	High risk	No mention of blinding. The control is no treatment
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Don't know if adverse events are single or multiple. Pregnancy data not extractable
Selective reporting (reporting bias)	Low risk	All outcomes reported

Morgante 2010

Methods	Randomised controlled trial
Participants	Country: Italy Population: Infertile men with with asthenospermia Mean age: Between 25 and 49 N = 180 Inclusion criteria: man age between 28 and 45, sperm concentration < 20 x 10^6 spermatozoa /mL, sperm progressive motility < 30%, normal morphology < 30%, leucocyte < 1 x 10^6 /mL, no infections Exclusion criteria: men younger than 28 and over 45, sperm concentration > 20 x 10^6 spermatozoa /mL, sperm progressive motility > 30%, normal morphology > 30%, leucocyte > 1 x 10^6 /mL, current infections, history of testicular pathology: cryptorchidism, varicocele, surgical operations, radiotherapy or chemotherapy, use of anabolic steroids, deficiency of hypothalamic-pituitary-gonadal axis, genital tract infections Duration: 3 months
Interventions	L-arginine 1660 mg, carnitine 150 mg, acetyl-carnitine 50 mg and ginseng 200 mg in one vial (n = 90) versus no treatment (n = 90) Duration of treatment: 3 months
Outcomes	Semen parameters Study also measured sexual satisfaction
Notes	Contacted author via email, giuseppe.morgante@unisi.it, to clarify study details, recruitment, randomisation, blinding, ethics approval, study population, withdrawals and to clarify progressive mortality. Last response was on 12.03.14 'Total motility and progressive motility are similar terms for the same definition: all the spermatozoa that have progressive or not linear motility.' Motility has been excluded from this analysis Initially translated from Italian by Roberto D'Amico

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	'Randomised' unable to ascertain risk
Allocation concealment (selection bias)	Unclear risk	Unable to ascertain risk
Blinding (performance bias and detection bias) All outcomes	High risk	Control is no treatment
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Unable to ascertain risk

Morgante 2010 (*Continued*)

Selective reporting (reporting bias)	Low risk	Outcomes reported

Nadjarzadeh 2011

Methods	Randomised controlled trial Power calculation performed
Participants	Country: Iran Population: infertile men with oligoasthenoteratozoospermia who have been trying for pregnancy for > 1 yr unprotected intercourse Mean age: 34 N = 60 Inclusion criteria: seminal white blood cells < 1,000,000 /mL, absence of anatomical abnormalities of the genital tract, absence of infectious genital diseases or systemic diseases, absence of treatment with other drugs and dietary supplement during the 3 months before enrolling in the study, at last absence of smoking, drug, and alcohol use or occupational chemical exposure Exclusion criteria: seminal white blood cells > 1,000,000 /mL, presence of anatomical abnormalities of the genital tract, presence of infectious genital diseases or systemic diseases, presence of treatment with other drugs and dietary supplement during the 3 months before enrolling in the study, currently smoking, using drug, or alcohol use or occupational chemical exposure Duration: 3 months
Interventions	CoQ10 - 200 mg/day orally (n = 23) versus placebo (n = 24) Duration of treatment: 3 months
Outcomes	Sperm motility and concentration Also measure progression, total antioxidant capacity (TAC)
Notes	Contacted regarding methods, randomisation, allocation concealment, recruitment, blinding and dropouts. Response from Azadeh Nadjarzadeh, azmm1383@yahoo.com, October 2013

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Participants were randomized using block randomization. It was done by Dr Motevallian who is epidemiologist and it has done before study
Allocation concealment (selection bias)	Low risk	Before the trial a colleague, that had not role in the study, coded the bottles of Coenzyme Q10 and placebo (that were similar)

		in A and B and give them to one of the staff of Avicenna Research centre. Only that person has a list of randomisation and give A or B bottles to the participants according to their code
Blinding (performance bias and detection bias) All outcomes	Low risk	Both participants and investigators blinded - The appearance and the bottles of capsules were similar and none of outcome assessors knew group, because everyone had a code after being allocated group A and B
Incomplete outcome data (attrition bias) All outcomes	Low risk	13 dropped out (22%) - 7 from treatment group and 6 from the control group
Selective reporting (reporting bias)	Low risk	Outcomes reported

Nozha 2001

Methods	Randomised comparative study No mention of allocation concealment Abstract only
Participants	Country: Tunisia Population: Infertile males with oligoasthenoteratozoospermia Mean age: not stated N=? Inclusion criteria: males with oligoasthenoteratozoospermia. Exclusion criteria: none mentioned Duration of study: not stated
Interventions	Vitamin E (400 mg) + selenium (200 μg) /day (n = 12) versus vitamin B (B_2, B_6 and B_{12}) (n = 8) Duration of treatment: 3 months
Outcomes	Seminal parameters
Notes	Attempted to contact authors regarding methods of randomisation and data - no extractable data from the abstract. No reply as yet

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	"In a prospective randomised comparative study"

Nozha 2001 (*Continued*)

Allocation concealment (selection bias)	Unclear risk	No mention of allocation concealment
Blinding (performance bias and detection bias) All outcomes	High risk	No mention of blinding. Control is no treatment
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Unclear
Selective reporting (reporting bias)	Low risk	Outcomes reported

Omu 1998

Methods	Randomised controlled trial Open trial - control is no treatment
Participants	Country: Kuwait Population: men with asthenozoospermia attending infertility and andrology clinic Mean age: 37.8 ± 7.9 in treatment group, 38.1 ± 8.2 in control N = 100 Inclusion criteria: men with asthenozoospermia. Spermatozoal motility impaired with >4 0% non-motile sperm. Have been trying to conceive for at least one year. Plus no obvious female factor Exclusion criteria: none mentioned Duration of study: 12 months
Interventions	Zinc 250 mg 2 x day (n = 49) versus no treatment (n = 48) Duration of treatment: 3 months
Outcomes	Seminal parameters
Notes	Attempted to contact authors regarding methods randomisation and concealment questioned. No reply as yet

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Randomised. "100 men with Asthenozoospermia were randomised into two groups"
Allocation concealment (selection bias)	Unclear risk	No mention of allocation concealment

Omu 1998 (*Continued*)

Blinding (performance bias and detection bias) All outcomes	High risk	Control is no treatment
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	100 men randomised, 97 analysed, drop-outs are accounted for
Selective reporting (reporting bias)	Low risk	Outcomes reported

Omu 2008

Methods	Randomised controlled 4-armed trial, open as one arm of the trial is no therapy
Participants	Country: Kuwait Population: men with asthenozoospermia attending infertility clinic in Kuwait Mean age: 35 ± 1 N = 45 Inclusion criteria: asthenozoospermia with normal sperm concentration (20 to 250 million/mL) but with 40% or more immotile sperm Exclusion criteria: asthenozoospermia but sperm concentration of < 20 million/mL Duration of study: No stated
Interventions	Zinc 200 mg 2 x /day (n = 11) versus zinc 200 mg + vitamin E 10 mg 2 x /day (n = 12) versus zinc 200 mg + vitamin E 10 mg + vitamin C 5 mg 2 x /day (n = 14) versus no therapy (n = 8) Duration of intervention: 3 months
Outcomes	Seminal parameters
Notes	Attempted to contact author re methods of randomisation - states that "8 men served as non- therapy control". No reply as yet

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	"the 45 asthenozoospermic men were randomised into four groups"
Allocation concealment (selection bias)	Unclear risk	No mention of allocation concealment
Blinding (performance bias and detection bias) All outcomes	High risk	No mention of blinding. Control is another antioxidant or no treatment

Omu 2008 (*Continued*)

Incomplete outcome data (attrition bias) All outcomes	Low risk	All outcomes are reported. No dropouts
Selective reporting (reporting bias)	Low risk	Outcomes reported

Peivandi 2010

Methods	Double blind randomised crossover trial
Participants	Country: Iran Population: infertile men with at least two abnormal spermiograms N = 30 Exclusion criteria: variocoele, testicular atrophy, ejaculatory disorders, use of medications, azoospermia, endocrinological disorders, ICSI candidacy or other causes of infertility Duration of first phase: 8 weeks
Interventions	L-carnitine (2 g/day) (n = 15) versus placebo (n = 15)
Outcomes	Sperm parameters
Notes	Abstract in English, full text in Arabic. Contacted the author and he is filling out the data extraction sheets. Author responded but data queries remain contacted again re SDs and pregnancies in first phase of crossover. Author responded saying that the data was given in SDs and there were 3 pregnancies in the first phase

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	"patients were randomly allocated to two groups of A and B"
Allocation concealment (selection bias)	Low risk	"sealed opaque envelopes"
Blinding (performance bias and detection bias) All outcomes	Low risk	"Double blind" "outcome assessor was blinded". Placebo controlled
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	'loss to follow up was not accounted for"
Selective reporting (reporting bias)	Low risk	Outcomes reported

Poveda 2013

Methods	A placebo-controlled double blind randomised trial
Participants	Country: Panama Infertile healthy men (N = 60) "60 patients completed the study -?how may were randomised. "There was no statistical differences in age, body mass index or initial sperm parameters between groups" Inclusion criteria: infertile healthy men without previous treatments, non smokers, no alcoholics or drug users Exclusion criteria: varicocele and leukocyte-spermia were excluded Duration of study: January 2012 to March 2013 Duration of treatment: 13 weeks No power calculation described
Interventions	Group I: L-carnitine (Cardispan, Gossman de C.V) 1 g pill each 12 hours (n = ?) Group II: Spermotrend (Catalysis) 1 pill each 8 hours (n = ?) Group III: Maca extract (NatureWay Products, Inc) 1 g pill each 12 hours (n = ?) Group IV: placebo 1 pill each 12 hours (n = ?)
Outcomes	Sperm motility Sperm concentration Normal sperm (morphology)
Notes	The study was approved by the National Bioethical Committee. Funded by a grant given by the Ministry of Economy and Finances. Panama, Republic of Panama Unknown withdrawal numbers, age Conference abstract. Letter written and posted regarding methods and data 12 February 2014

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	Unknown methods of randomisation
Allocation concealment (selection bias)	Unclear risk	Unknown methods of allocation concealment
Blinding (performance bias and detection bias) All outcomes	Low risk	Double blind and placebo-controlled
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Unknown
Selective reporting (reporting bias)	Low risk	Outcomes reported

Pryor 1978

Methods	Double blind randomised crossover trial
Participants	Country: UK (two centres) Population: men with severe oligozoospermia Mean age: ? Randomised: N = 64 Inclusion criteria: 1 sperm count of less than 10 million per ejaculate on each of 2 occasions immediately preceding the trial 2. No uncorrected varicoceles or testicular maldescent 3. Testicular biopsy already performed (Johnsen 1970) 4. No drugs taken in past 3 months which were known to affect spermatogenesis 5. No history of biliary disease owing to a suggestion that arginine might interfere with the metabolism of bile salts The wives of all these men had been fully investigated with regard to fertility Duration of study: unknown Duration of treatment: 12 weeks for 1st phase."Each treatment period lasted 12 weeks with no intervening wash-out period" Exclusion criteria: men with varicocoele
Interventions	Arginine 4 g/day (n = 35) versus placebo (n = 29)
Outcomes	Total sperm motility Hormone levels
Notes	No mention of funding sources. E Merck Ltd supplied the capsules of arginine and placebo. Unclear if consent sought. Dropouts explained but unclear from which group No data available for sperm parameters Pregnancy not stated in the methods section as an outcome of interest but reported in the results 10 withdrew reasons were given but unsure from which group, the paper stated that they used ITT but data not presented The study didn't report the outcomes for the different phases of the trial (i.e. not separated into phase 1 phase 2). Pregnancy data is separated into phase one data but probably biochemical and will be used in biochemical pregnancy table. Unable to contact author

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Methods of randomisation not explained
Allocation concealment (selection bias)	Unclear risk	Not mentioned
Blinding (performance bias and detection bias) All outcomes	Low risk	Double blind - placebo controlled

Pryor 1978 (*Continued*)

Incomplete outcome data (attrition bias) All outcomes	Unclear risk	10 withdrew but unsure from which group
Selective reporting (reporting bias)	Low risk	Outcomes reported

Rolf 1999

Methods	Randomised placebo-controlled double blind study No mention of allocation concealment Power calculation performed
Participants	Country: Munster Germany Population: men with infertility for over one year. Mean age: treatment 36.1 ± 5.0, placebo 35.2 ± 4.8 N = 33 Inclusion criteria: asthenozoospermia (< 50% motile) diagnosed after 2 examinations, normal or reduced sperm concentration (> 20 x 10^6 per ejaculate) and without infection of access glands Exclusion criteria: none mentioned Duration of study: 8 weeks
Interventions	Vitamin C 1000 mg + vitamin E 800 mg/day (n = 15) versus placebo (n = 16) Duration of treatment: 8 weeks
Outcomes	Primary: semen parameters Secondary: pregnancy rate and adverse effects
Notes	Contacted author about the allocation concealment and pregnancy and adverse effects were outcomes in their protocol. Rolf replied saying that pregnancy and adverse effects were stated in the protocol

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	"Randomisation was performed with random numbers without further stratification by the pharmacist and the code was withheld from researchers and patients"
Allocation concealment (selection bias)	Unclear risk	Pharmacist performing randomisation and code withheld from patients and researchers. However no mention of type of containers or envelopes

Rolf 1999 (*Continued*)

Blinding (performance bias and detection bias) All outcomes	Low risk	Double - patients and researchers
Incomplete outcome data (attrition bias) All outcomes	Low risk	All data reported, 2 patients withdrew from the trial - "results from two patients were rejected from analysis." 1 from the treatment group due to poor compliance and 1 from the placebo group due to genital tract infection. Intention to treat
Selective reporting (reporting bias)	Low risk	All semen outcomes reported and author states (e-mail 22.09.09) that pregnancy and adverse effects were set a priori in the protocol

Safarinejad 2009

Methods	Double blind placebo-controlled randomised study. Randomised using permuted blocks Allocation concealment: sealed envelopes Blinding: double, identical coating of tablets Power calculation performed
Participants	Country: Iran Population: idiopathic oligoasthenoteratospermia, asthenospermia or teratospermia of 2 years duration Mean age: 31 years (25 to 48 years) Recruited: N = 548 Randomised: N = 468 Inclusion criteria: sperm count > 5 x 10^6 /ml, over 2 years of failed conception, no female fertility problems, no history of possible cause for male infertility Exclusion criteria: abnormal testes, history of cancer or chemotherapy, testosterone or antiandrogen use, use of selenium or N-acetyl-cystine supplements, abnormal hormone levels, genital disease, genital inflammation or variocoele, history of genital surgery, major surgery, central nervous system injury, a known sperm defect or retrograde ejaculation. Y chromosome abnormalities, sexually transmitted disease, genitourinary infection, leukocytospermia, smoking, any environmental exposures to reproductive toxins. Medical, neurological or psychological problems. A history of drug or alcohol abuse, hepatobiliary disease or significant renal insufficiency. Any endocrine abnormality, a body mass index (BMI) of 30 kg/m^2 or over, participation in another investigational study and a likelihood of being unavailable for follow up Duration of study: 56 weeks
Interventions	Selenium 200 µg/day (n = 116) versus N-acetylcysteine 600 mg/day (n = 118) versus selenium 200 µg/day + N-acetylcysteine 600 mg/day (n = 116)

	versus placebo (n = 118) Duration of treatment: 26 weeks or 6.5 weeks Analysed: n = 105 in selenium group (loss 11), n = 106 in placebo group (loss 12), n = 105 in N-acetylcysteine group (loss 13) and n = 104 in selenium + N-acetylcysteine group (loss 12)
Outcomes	Primary outcome: semen parameters Secondary outcomes: adverse events
Notes	Attempted to contact authors regarding side effect data that had not yet been added to the review due to the query of multiple comparisons. Also to ask whether data is in SD (as reported in the text) or SE, as requested by statistician 24.09.10

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	"randomisation table generated by the method of random permuted blocks. Patient randomisation numbers were allocated to each site in ascending sequence in blocks."
Allocation concealment (selection bias)	Low risk	"Assignment to treatment groups was performed using a sealed envelope technique. "
Blinding (performance bias and detection bias) All outcomes	Low risk	"Eligible patients were randomly assigned to double blind.." "Placebo pills were coated with titanium oxide to ensure an identical appearance and smell."
Incomplete outcome data (attrition bias) All outcomes	Low risk	All withdrawals were accounted for in each treatment group. Withdrawal was mainly due to withdrawal of consent followed by lost to follow up and lastly for reasons of missing data. No intention to treat
Selective reporting (reporting bias)	Low risk	The published report includes all expected outcomes

Safarinejad 2009a

Methods	Randomised controlled trial, double blind Allocation concealment: Not mentioned Power calculation performed
Participants	Country: Tehran, Iran Population: infertile males between 21 and 42 years with idiopathic oligoasthenoteratospermia Mean age: treatment group 28 ± 9, placebo 28 ± 10 (range 21 to 42 years) Recruited: N = 268 Randomised: N = 212 Inclusion criteria: minimum 2 years unprotected intercourse with 2 years unwilling childlessness. male infertility diagnosed if 1 or more standard semen parameters were below cutoff levels accepted by World Health Organisation (WHO). A fertile female partner. No known medical condition that could account for infertility, testicular volume 12 ml or greater. No medical therapy for at least 12 weeks before the study begins. Only patients seeking medical attention for infertility were included Exclusion criteria: azoospermia or severe oligospermia (sperm count less than 5 million/ml. An history of epypidymo-orchitis, prostatitis, genital trauma, testicular torsion, inguinal or genital surgery. Any genital or central nervous system disease, endocrinopathy, cytotoxic drugs, immunosuppressants, anticonvulsives, androgens, antiandrogens, a recent history of Sexually transmitted disease. Psychological or physiological abnormalities that would impair sexual functioning or ability to produce sperm samples. Drug, alcohol or substance abuse. Liver disease, renal insufficiency or chromosome abnormalities. occupational and environmental exposures to reproductive toxins. A body mass index (BMI) of 30 kg/m^2 or over, participation in another investigational study and a likelihood of being unavailable for follow up Duration of study: 20 months, from February 2005 until October 2006
Interventions	Coenzyme Q10 (CoQ10) 300 mg/day (n = 106) versus placebo (n = 106) Duration of treatment: 26 weeks or 6.5 months Analysed n = 98, 8 withdrawals in treatment group, analysed n = 96, 10 withdrawals in the placebo group
Outcomes	Primary outcomes: semen parameters and testicular volume Secondary outcomes: adverse effects and hormone levels
Notes	Attempted to contact authors to ask whether data is in SD (as reported in the text) or SE, as requested by statistician 24.09.10

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	"Each eligible patient received a randomisation number, which was determined by a computer generated schedule. Therafter a randomisation table was generated by the

Safarinejad 2009a (*Continued*)

		method of random permuted blocks. Individuals who were geographically and operationally independent of the study investigator performed the study randomisation"
Allocation concealment (selection bias)	Unclear risk	Allocation concealment: not mentioned
Blinding (performance bias and detection bias) All outcomes	Low risk	"The clinician prescriber and the patients were blinded to the treatment condition. To maintain and guarantee blinding CoQ10 and placebo were identical in appearance. Participant data collected during this trial were kept confidential and locked in a secure office area. Randomisation codes were opened only after all patients had completed the whole study protocol."
Incomplete outcome data (attrition bias) All outcomes	Low risk	All patients who dropped out of the trial were accounted for - 8 from treatment group and 10 from placebo group for reasons such as withdrawal of consent, missing data and loss to follow up
Selective reporting (reporting bias)	Low risk	Outcomes reported

Safarinejad 2011

Methods	Randomised controlled trial Power calculation performed
Participants	Setting: Iran - fertility clinic Recruitment October 2006 to December 2008 N = 254 infertile men attending a fertility work-up (referred or self referred) having been infertile for at least 2 years Mean age PTX 32.1 ± 4.3 years, placebo 32.8 ± 4.6 years. Mean duration of infertility in PTX 5.1 ± 2.8 years, placebo 5.2 ± 2.6 years Inclusion criteria: ≤ 45 years old. unable to conceive after 2 years. Normal basal serum of testosterone, luteinising hormone (LH), follicle-stimulating hormone (FSH), thyroid-stimulating hormone (TSH), prolactin (PRL), and inhibin B (> 150 pg/ml); no known medical condition that could account for OAT, total testicular volume ≥ 12 mL, and a normal fertile female partner Exclusion criteria: history of cancer chemotherapy, androgens and antiandrogens, or history of the following: cryptorchidism, orchitis, testicular torsion or trauma, varicocele, relevant genitourinary infection, sexually transmitted disease, reproductive hormone levels outside of normal limits, hyperprolactinemia, alcohol or substance abuse, hepatobiliary disease, significant renal insufficiency, presence of antisperm antibodies, Y chromosome microdeletions, and karyotypic abnormalities. Men with severe oligo-

	zoospermia (< 5 million/mL), leukocytospermia (more than 10^6 white blood cells /mL) , tobacco use, and occupational and environmental exposures to potential reproductive toxins were also excluded. Any drugs that might be affecting the spermatogenesis courses should be discontinued 6 months before enrolment
Interventions	Pentoxyfiline (n = 127) 400mg PTX (Apo-Pentoxifylline, Apotex Inc. Toronto, Canada) , twice daily, orally for 24 weeks versus placebo (n = 127) components not stated, twice daily, orally for 24 weeks
Outcomes	Semen volume, sperm motility, sperm count, sperm morphology, sperm density, adverse events, seminal plasma antioxidant status SOD and CAT, % acrosome reaction reproductive hormones and genetic analyses
Notes	

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Randomised via a central computerised voice response system
Allocation concealment (selection bias)	Low risk	Central computerised voice response system
Blinding (performance bias and detection bias) All outcomes	Low risk	'Study medication was over-encapsulated so that they appeared the same. Patients appear to be blinded' and 'Posttreatment semen analyses were carried out in a blinded fashion with regard to treatment.'
Incomplete outcome data (attrition bias) All outcomes	Low risk	254 randomised, 127 to each group PTX 11 excluded during treatment protocol (protocol violation 4, withdrawn consent 2, lost to follow up 4, adverse events 1) after 12 week follow up 4 more excluded due to loss to follow up Placebo 10 excluded during treatment protocol (protocol violation 4, withdrawn consent 2, lost to follow up 4) after 12 week follow up 4 more excluded due to loss to follow up
Selective reporting (reporting bias)	Low risk	All outcomes reported appear to have been analysed

Safarinejad 2012

Methods	Randomised controlled trial Power calculation performed
Participants	Setting: single urology centre/private clinic in Iran Duration of trial from June 2010 to January 2011 N = 228 infertile men; mean age ubiquinol 31 years and placebo 32 years. Primary infertility for at least 2 years Inclusion criteria: history of primary infertility of more than 2 years, Abnormal sperm count and motility according to WHO criteria, Wife age between 20 and 40 years, Documentation of fertile female partner, No known medical or surgical condition which can result in infertility Exclusion criteria: history of cancer chemotherapy or radiotherapy; History of genital disease such as cryptorchidism and varicocoele; History of genital surgery; Body mass index 30 kg/m^2 or greater; Any endocrinopathy; Y chromosome microdeletion or karyotype abnormalities; Leukocytospermia (more than 106 white blood cells per mL); Drug, alcohol or substance abuse; tobacco use; use of anticonvulsants, androgens or antiandrogens; Significant liver (serum bilirubin greater than 2.0 mg/dl) or renal function (serum creatinine greater than 2.0 mg/dl) impairment; Occupational and environmental exposure to reproductive toxins Severe oligozoospermia (less than 5 x 10^6 /mL), azoospermia and testicular volume less than 12 mL
Interventions	Coenzyme Q10 (Ubiquinol) (n = 114) 200 mg ubiquinol (New Life CoEnz QH, Istanbu, Turkey), orally, once daily after a meal for 26 weeks versus placebo (n = 114) content not specified orally once daily after a meal for 26 weeks
Outcomes	Semen volume, sperm density, sperm motility, sperm morphology, seminal plasma antioxidant status
Notes	

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Computer generated random number table
Allocation concealment (selection bias)	Low risk	The randomisation codes were centrally assigned by the co-ordination centre after checking the main eligibility criteria
Blinding (performance bias and detection bias) All outcomes	Low risk	All investigators and study staff were blinded to treatment allocation during the whole study period, All of the participants were naive for treatment

Safarinejad 2012 (*Continued*)

Incomplete outcome data (attrition bias) All outcomes	Low risk	228 were randomised of 264 eligible Ubiquinol group - 13 excluded at end of treatment (3 protocol violations, 4 withdrawal of consent and 6 lost to follow up). At 12 weeks follow up a further 5 were lost to follow up Placebo group - 12 excluded at end of treatment (4 protocol violations, 4 withdrawal of consent, 6 lost to follow up. At 12 weeks follow up a further 7 were lost to follow up
Selective reporting (reporting bias)	High risk	The authors do not pre-specify which outcome measures will be reported. The primary outcome is a % change from baseline at the end of the treatment period

Scott 1998

Methods	Double blind randomised trial Allocation concealment: no mention
Participants	Country: Glasgow, UK Population: men attending subfertility clinic with low sperm motility Mean age: 33.3 years ± 0.64 Recruited: N = 69 Analysed: N = 64 Inclusion criteria: low sperm motility Exclusion criteria: not mentioned Duration of study: 3 months and two weeks
Interventions	Selenium 100 µg/day (n = 16) versus selenium 100 µg + vitamin A 1 mg + vitamin C 10 mg + vitamin E 15 mg/day (n = 30) versus placebo (n = 18) Duration of treatment: 3 months Analysed = 64, 5 withdrew 1 from selenium group, 4 from selenium + vitamin A, C and E group. All withdrawals were due to non-compliance
Outcomes	Primary outcome: semen parameters Secondary outcome: pregnancy rates
Notes	Uneven numbers, multivitamin numbers are double the other groups Need to ask author if they have separate numbers for pregnancy data. Currently have 5 pregnancies in the 2 treatment groups and none in placebo Who was blinded, was the placebo identical when group 2 contained so many different vitamins Was there any allocation concealment?

Scott 1998 (*Continued*)

	Author has retired and is not able to be contacted	
Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	"As the patients entered the trial they were randomly allocated to one of three treatments, which had in turn been randomised within each block of four numbers and 'blinded' using a numeric code." Unclear as to why the uneven nature of the numbers in the groups i.e. 30 in multivitamin group and 16 in selenium, 18 in placebo
Allocation concealment (selection bias)	Unclear risk	Not mentioned
Blinding (performance bias and detection bias) All outcomes	Low risk	Double blind, placebo controlled
Incomplete outcome data (attrition bias) All outcomes	Low risk	Numbers of withdrawals and reasons (non compliance) were reported
Selective reporting (reporting bias)	Low risk	Outcomes reported

Sigman 2006

Methods	Randomised double blind trial Allocation concealment: adequate
Participants	Country: Minneapolis, USA Population: males aged 18 to 65 years IVF Mean age: 36.2 ± 5.8 (SD), 35.3 ± 7.5 (SD) Recruited: N = 26 Analysed: N = 21, 5 withdrawals Inclusion criteria: males 18 to 65 years with infertility of at least six months duration, sperm concentration of at least 5 million sperm/mL, motility of 10% to 50%, absent pyospermia and normal FSH and testosterone levels Exclusion criteria: history of post-pubertal mumps, cryptorchism, vasal or epididymal surgery, history of medication or chemotherapy. recent alcohol, chronic marijuana. Use of testosterone or steroids. Exposure to environmental toxins. Recent history of fever or diabetes, liver failure, renal failure, endocrine disorder, untreated variocoele, urogenital infection, or prior vasectomy reversal Duration of study: 24 weeks

Sigman 2006 (*Continued*)

Interventions	Carnitine (L-carnitine 2000 mg +L-acetylcarnitine 1000 mg/day) (n = 12) versus placebo (n = 9)
Outcomes	Primary outcome: semen parameters Secondary outcomes: pregnancy - 2 pregnancies, 1 in the treatment arm after IVF and one in placebo through intercourse
Notes	Author replied 21.09.09 saying: 'The published 2006 trial is the published version of the 2003 abstract (Pryor 2003)' and giving details of randomisation and concealment. Author says he will try and find out about the 5 patients that dropped out Why did - "5 additional patients entered the study but dropped out before completion" - when did these patients enter and were they randomised? "One of these 5 dropped out because of pregnancy three months after starting carnitine" Pryor paper excluded as it is the same study as Sigman, author also gave details of randomisation and allocation concealment, author will try to find info on 5 patients who dropped out

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	"Patients were randomised to receive carnitine or placebo" "The randomisation was done by a third party a company that oversaw the trial. We sent the patient number of new recruited patients in to them, they assigned them a study number that was associated with a collection of medication/placebo." The author replied to randomisation query 23.09.09 saying that the protocol stated that - "treatments will be assigned randomly to a subject number. The numbers will range from 1-84 for study centre 1 and 85-168 for study centre 2. Randomisation of treatments for each centre will be done independently. One half of subject numbers will be placebo, the other half, active ingredient."
Allocation concealment (selection bias)	Low risk	"The investigators and study sites had the study medication/placebo packets identified by number only. They were blinded to what was in the medication/placebo packets. We were sent the code at the conclusion of the trial." The author replied to a query on allocation concealment on 23.09.09 saying that the protocol stated that - "

Sigman 2006 (*Continued*)

		Integrated Data Solutions, Inc. will keep the randomisation code in a separate sealed envelope for each site until the end of the study. The randomisation lists will be provided to the packaging company for packaging of the packets into patient medication boxes."
Blinding (performance bias and detection bias) All outcomes	Low risk	"Both the investigators and the patient were blinded to the treatment arm assignment."
Incomplete outcome data (attrition bias) All outcomes	Low risk	"5 additional patients entered the study but dropped out before completion. One of these dropped out because of pregnancy three months after starting carnitine." Author replied to query re drop outs - "I have data on one drop out at my site - the drop out occurred after randomisation to carnitine. The drop out occurred before the first follow-up study visit. The other four drop outs were from the other study site - I am trying to get that data for you" (23.09.09)
Selective reporting (reporting bias)	Low risk	All outcomes of interest were reported

Sivkov 2011

Methods	Randomised controlled study - open label
Participants	Population: men with chronic prostatitis and abnormal fertility, for more than 6 months Age: 18 to 40 years Randomised: N = 30 Duration of treatment: 3 months
Interventions	Selznic (selenium + zinc and vitamins) (n = 15) versus placebo (n = 15)
Outcomes	Sperm motility Sperm concentration
Notes	No standard deviations available. Need to contact authors regarding methods, standard deviations, type of control and any pregnancy data. Paper translated from Russian by Vasya Vlassov. Vasya 17.02.14 saying that the control was placebo and SD's not given. Emailed the institution 18.02.14 regarding methods and data, no reply as of 7.03.13

Risk of bias

Sivkov 2011 (*Continued*)

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Randomisation methods not explained
Allocation concealment (selection bias)	High risk	No allocation concealment
Blinding (performance bias and detection bias) All outcomes	Unclear risk	'Open labelled' however placebo used maybe a translation problem
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	Unknown
Selective reporting (reporting bias)	Low risk	Outcomes reported

Suleiman 1996

Methods	Double blind randomised controlled trial No mention of allocation concealment
Participants	Country: Saudi Arabia Population: men attending a fertility centre Mean age: 34.8 (27 to 52 years), placebo 33.2 (22 to 45 years) N = 110 Inclusion criteria: asthenospermic ($\geq$ 20 x 10^6 /mL). sperm motility $\leq$ 40%, normal sperm count, leucocyte concentration < 5%, normal fructose concentration Normal female Exclusion criteria: none mentioned Duration of study: 6 months
Interventions	Vitamin E 100 mg 3 x /day (n = 52) versus placebo 3 x /day (n = 35) Duration of treatment: 6 months
Outcomes	Primary outcome: motility and MDA concentration Secondary outcome: live birth, pregnancy, miscarriage
Notes	Method of randomisation not stated. Large imbalance between the treatment (n = 52) and placebo group (n = 35) at analysis. Dropouts were accounted for in text

Risk of bias

Bias	Authors' judgement	Support for judgement

Suleiman 1996 (*Continued*)

Random sequence generation (selection bias)	Unclear risk	" Either 100mg vitamin E or a placebo was prescribed in a random double blind fashion". Method of randomisation not stated
Allocation concealment (selection bias)	Unclear risk	Not mentioned
Blinding (performance bias and detection bias) All outcomes	Low risk	"Double blinded". Does not report on who was blinded, however placebo-controlled
Incomplete outcome data (attrition bias) All outcomes	High risk	The exact dropout figures for each group is unclear - "A total of 110 patients were enrolled in the study, but some of the patients dropped out and some left the region and failed to continue. When the experiment was terminated, 52 patients were found to have taken vitamin E and 35 patients to have taken the placebo." Assuming the groups were equal initially then the placebo group lost 20 men and the intervention lost 3
Selective reporting (reporting bias)	Low risk	All outcomes stated in the methods were reported in results

Tremellen 2007

Methods	Randomised double blind controlled trial. randomisation by computer generated blocks. Using a 2:1 ratio (treatment 2: placebo 1) Allocation concealment: numbered bottles delivered to the site with all members of the trial blinded to sequence, identical placebo Power calculation performed
Participants	Country: Australia Population:male factor infertility undergoing IVF Mean age: treatment group - 37.1 ± 5.1, placebo group - 35.5 ± 4.3 Recruited: N = 82 Randomised: N = 60 Inclusion criteria: men with sperm samples showing oxidative stress and a significant level of DNA fragmentation (> 25% TUNEL positive) Exclusion criteria: female partner with diminished ovarian reserve or if the female partner is aged over 39 years Duration of study: 1.5 years
Interventions	Menevit (folate 0.5 mg, garlic 1000 mg, lycopene 6 mg, vitamin E 400 IU, vitamin C 100 mg, zinc 25 mg, selenium 26 μg, palm oil). One capsule per day (n = 40) versus placebo (identical in appearance and taste - containing palm oil) (n = 20)

Tremellen 2007 (*Continued*)

	Duraton of treatment: 3 months prior to IVF cycle
Outcomes	Primary outcome: embryo quality Secondary outcomes: pregnancy, multiple pregnancy, fertilisation rate, side effects
Notes	Associate Professor Tremellen provided live birth data in December 2014 "Only one pregnancy failed in the Menevit arm after 13 weeks (late miscarriage 19 weeks of male infant). All other pregnancies, including the twin pregnancies went on to live birth and all babies appear to be doing well from the records". There were three sets of twins in the combined antioxidants group and nil in the placebo group. Each twin pregnancy and live birth was counted as one event in the data analyses due to the protocol specifications of the review

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	"The randomisation schedule was computer generated in blocks of six by Bayer Consumer Care Australia". Using a 2:1 ratio "There were no significant differences between the active and the placebo group in terms of important baseline prognostic characteristics..."
Allocation concealment (selection bias)	Low risk	"the appropriately numbered bottles of capsules delivered to the clinical site without any participant knowing the treatment sequence. Patients were allocated the next numerical treatment package (one to sixty as they became eligible for enrolment"
Blinding (performance bias and detection bias) All outcomes	Low risk	"double blind placebo controlled trial"
Incomplete outcome data (attrition bias) All outcomes	Low risk	All withdrawals were accounted for, 2 from the intervention group, 4 from placebo all due to the couples not going through to embryo transfer
Selective reporting (reporting bias)	Low risk	All specified outcomes are reported

Wang 1983

Methods	Randomised placebo controlled trial
Participants	Country: Hong Kong Population: new patients attending infertility clinic Mean age: 33.7 ± 4.4 years (28 to 45 years) N = 46 Inclusion criteria: men with idiopathic oligospermia attending fertility clinic with normal hormones. They had not received treatment with pharmacologic agents for 1 year prior to entry into the trial. None of the female partners had irreversible causes of female infertility Exclusion criteria: not mentioned Duration of study: 6 to 9 months
Interventions	Pentoxifylline 400 mg 3 x /day (n = 11) versus placebo (n = 7) Duration of treatment: 6 months Other interventions not included in meta analysis were clomiphene citrate, mesterolone and testosterone enanthate
Outcomes	Primary outcome: semen parameters and pregnancy
Notes	Pentoxifylline supplied by Hoechst Aktiengesellshaft Attempted to contact the author re motility data as it was stated in the paper that this was an outcome. Also asked about control data for post-treatment period and methods of randomisation + allocation concealment. Author replied saying she no longer has the data

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	"the subjects were randomly assigned to one of the therapy groups.." No mention of methods of randomisation
Allocation concealment (selection bias)	Unclear risk	No mention of allocation concealment
Blinding (performance bias and detection bias) All outcomes	High risk	No blinding - one of the interventions was an intra muscular injection while the others were oral
Incomplete outcome data (attrition bias) All outcomes	High risk	Sperm motility data not given when motility was discussed as an outcome of interest
Selective reporting (reporting bias)	High risk	No post-treatment control data given however there is post-treatment given for treatment group No intention to treat reported Side effects reported in the results but this was not stated as an initial outcome

Wang 2010

Methods	Random controlled trial
Participants	Country: China Population: male infertility - asthenozoospermia Age: 23 to 26 years old. "Balanced in age, course of disease, and Semen parameters" Recruited: all male asthenozoospermia outpatients in the Second Hospital of HeBei Medical University from August 2007 to August 2009 Randomised: N = 135 Inclusion criteria: male asthenozoospermia patients, aged 23 to 26 years old, with a history of infertility for about 1 to 10 years, and with no contraception measures after marriage at least 12 months, has normal sex life, the wife's fertility is normal Semen analysis for at least twice based on WHO criteria: Forward mobile sperm (a + b level) < 50%, and fast forward movement sperm (a level) < 25% Sperm density > 20 x 10^6 /mL Tests for peripheral blood chromosome and reproductive hormones (FSH, LH, PRL, T) were normal The tests for semen ureaplasma mycoplasma and chlamydia trachomatis were negative Semen WBC < 1 x 10^6 /mL Exclusion criteria: cryptorchidism, testicular dysplasia, varicoceles, reproductive tract infection Duration of trial: August 2007 to August 2009 Duration of treatment: 3 months
Interventions	L-carnitine 2g/day plus vitamin E (n = 68) versus vitamin E (n = 67)
Outcomes	Pregnancy rates Adverse effects % forward motile sperm Sperm density % sperm normal morphology
Notes	Funding source: population and family planning commission of HeBei Province, China. Paper translated by Liu Qin 22 patients lost during the study - no reasons given for dropouts, 7 from the intervention and 15 from the control, the trial did not use ITT. E-mailed Qin (translator) regarding pregnancy and adverse event data, then may need to write to the authors

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	"A total of 135 patients with asthenozoospermia were randomly divided into Groups". Methods are unknown

Wang 2010 (*Continued*)

Allocation concealment (selection bias)	Unclear risk	Unknown
Blinding (performance bias and detection bias) All outcomes	Unclear risk	Unknown
Incomplete outcome data (attrition bias) All outcomes	High risk	22 dropouts. Numbers from each group are given but no reasons are provided for the withdrawals. Intention to treat not used in the trial analysis
Selective reporting (reporting bias)	Low risk	Outcomes reported

Wong 2002

Methods	Double blind randomised placebo-controlled interventional study. Computer generated randomisation Allocation concealment: central pharmacy coding	
Participants	Country: Netherlands Population: Fertile and subfertile men Mean age: 34.3 ± 3.9 years Recruited: N = 258 subfertile Randomised: N = 103 Inclusion criteria for subfertile group: failure to conceive after 1 year regular unprotected intercourse and sperm concentration of 5 to 20 million/mL Exclusion criteria for subfertile group: chromosomal disorders, cryptorchidism, vasectomy, use of folic acid or zinc supplements in the previous 3 months, vitamin B deficiency Duration of study: 1 year	
Interventions	Folic acid 5 mg/day (n = 22) versus zinc sulphate 66 mg/day (n = 23) versus zinc sulphate 66 mg/day + folic acid 5 mg/day (n = 24) versus placebo (n = 25) Duration of treatment: 26 weeks	
Outcomes	Semen parameters	
Notes	Data in median and range. Attempted to contact authors regarding means and standard deviations. Letter returned to sender	
Risk of bias		
Bias	**Authors' judgement**	**Support for judgement**

Wong 2002 (*Continued*)

Random sequence generation (selection bias)	Low risk	"eligible fertile and subfertile men were randomly assigned according to a simple computer-generated randomisation schedule in four blocks to receive folic acid and placebo, zinc sulphate and placebo, zinc sulphate and folic acid, or placebo and placebo, which resulted in eight subgroups. " "At the end of the trial, the research fellow received the randomisation list that matched the codes from the hospital pharmacy."
Allocation concealment (selection bias)	Low risk	"capsules were coded by the hospital pharmacy according to the randomisation list."
Blinding (performance bias and detection bias) All outcomes	Low risk	Double blind placebo-controlled "Neither the research fellow and the participants knew whether the participants received folic acid, zinc sulphate or placebo capsules" "Folic acid and placebo capsules were yellow and identical in appearance. Zinc sulphate and placebo capsules were white and identical in appearance"
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	9 men withdrew from the subfertile arm of the trial, 1 due to side effects (gastrointestinal) and 8 due to lack of motivation. It is unclear which treatment groups these men were randomised to
Selective reporting (reporting bias)	Low risk	Outcomes reported

Zalata 1998

Methods	A randomised pilot study Allocation concealment not mentioned
Participants	Country: Belgium Population: men attending andrology clinic Mean age: not given N = 22 Inclusion criteria: none given Exclusion criteria: none given Duration of study: ?4 to 6 months

Zalata 1998 (*Continued*)

Interventions	Acetylcysteine 600 mg/day (n = 5) versus EFA (DHA 1 g, y-linolenic acid, arachidonic acid 100 mg) 100 mg/day + antioxidant oil mixture and tocopherol + B-carotene (n = 12) versus acetylcysteine + EFA + antioxidants (n = 5) Duration of treatment: 4 to 6 months
Outcomes	Sperm parameters
Notes	Abstract only. No extractable data. Attempted to contact authors re availability of data as means, if published?, methods of randomisation and allocation concealment

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	"A prospective randomised pilot study" No details of randomisation given
Allocation concealment (selection bias)	Unclear risk	No details of allocation concealment
Blinding (performance bias and detection bias) All outcomes	Unclear risk	No details
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	No details
Selective reporting (reporting bias)	Unclear risk	Unclear, abstract only

Zavaczki 2003

Methods	Randomised placebo controlled clinical study Allocation concealment not mentioned
Participants	Country: Hungary Population: subfertile men attending andrology Department of Obstetrics and Gynaecology, University of Szeged Mean age: treatment group 29.6, placebo group 28.3 years Recruited: N = 26 Randomised: N = 20 Inclusion criteria: unsuccessful attempt at pregnancy for over one year. A healthy female partner examined by a gynaecologist. Sperm volume < 2 mL and/or sperm concentration < 20 million/mL and/or morphology ratio < 30% and/or motility < 50%. No genital tract infection, no bacteria or fungi in urine or semen. Hormones are within physiological range. Intact renal function. No excessive magnesium intake Exclusion criteria: none mentioned

	Duration of study: 3 months
Interventions	Magnesium 3000 mg/day (n = 10) versus placebo (n = 10) Number analysed: N = 14 Duration of treatment: 90 days
Outcomes	Primary: semen parameters Secondary: pregnancy and side effects
Notes	Attempted to contact authors regarding methods of randomisation and allocation concealment

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	"Patients were randomised and divided into Magnesium or Placebo groups" Methods of randomisation are not mentioned
Allocation concealment (selection bias)	Unclear risk	Not mentioned
Blinding (performance bias and detection bias) All outcomes	Unclear risk	"The members of Group P received the same number of placebo tablets which closely resembled the Magnerot tablets." No mention of blinding
Incomplete outcome data (attrition bias) All outcomes	Low risk	20 were randomised and 14 were analysed. "To date 26 patients have participated in the study and 20 men (10 in both groups) have completed the program of treatment. Six patients (2 in group M and 4 in group P were excluded from the program, including five cases for poor compliance, since they did not attend the control meeting at the end of treatment. One patient from Group M experienced severe diarrhoea and so his treatment was halted."
Selective reporting (reporting bias)	Low risk	All sperm data for outcomes in the trial were given, however clinical pregnancy only reported in the results section and not mentioned in methods

Characteristics of excluded studies *[ordered by study ID]*

Study	Reason for exclusion
Anarte 2012	Study population normozoospermic men and donors
Anarte 2013	Study population normo-zoospermic men and donors
Cai 2012	Study population not subfertile men
Chen 2012	Protocol exclusion criteria regarding exclusion of interventions this includes fertility drugs like tamoxifen. Group a tamoxifen + vitamin E, Group b tamoxifen
Comhaire 2005	Used non-randomised controls recruited from another unrelated trial
Ebisch 2003	Inappropriate population, polymorphisms
Elgindy 2008	Antioxidant given to the women and not to the men
Ghanem 2010	Intervention clomiphene + vitamin E versus placebo. Protocol exclusion criteria - "trials that included men taking other fertility enhancing drugs"
Hafeez 2011	Excluded due to incorrect intervention - plant extracts, herbal formulation
Jawad 2013	Not randomised "men were classified into groups". Numbers of men in the groups were uneven
Kim 2010	Female participants not men
Kumar 2011	Used a herbo-mineral supplement
Lenzi 1993	Route of supplementation was intramuscular not oral
Lu 2010	Population was women, not men
Micic 2001	Not randomised, 105 men in the treatment group and 35 in control. Abstract only
Niederberger 2011	A commentary on Ghanem 2010
Nikolova 2007	Trial is not randomised, allocation method is by alternation. Translated from Bulgarian by Ivan Sola. "50 of them were randomly invited to participate depending on their order of attendance to the clinic"
Pawlowicz 2001	Not a randomised controlled trial
Polak 2013	The population is women not men
Safarinejad 2011a	Incorrect intervention - saffron, herbal not a supplement
Soylemez 2012	Population is not subfertile men

(*Continued*)

Stanislavov 2009	Trial design is not random. The trial uses alternate allocation, odd and even numbers. Appears to be a report of the trial Nikolova 2007
Tang 2011	Protocol exclusion criteria (tamoxifen + Q10 versus tsamoxifen) "trials that included men taking other fertility enhancing drugs"
Verzeletti 2012	Spirulina platensis (4 g) and Resveratrol (500 mg) are plant extracts not antioxidant supplements
Vicari 2001	Inappropriate control (anti-inflammatory). Treatment is not compared to placebo or another antioxidant
Vicari 2001a	Inappropriate comparison. The same antioxidant is compared at different times - L-carnitine + acetyl-carnitine versus L-carnitine + acetyl-carnitine
Vicari 2002	Inappropriate control (anti-inflammatory). Treatment is not compared to placebo or another antioxidant
Wang 2010a	Protocol exclusion criteria regarding exclusion of interventions this includes fertility drugs like tamoxifen. Group a L carnitine and tamoxifen, Group b L carnitine, Group c tamoxifen
Wu 2012	Probably not randomised, no mention of randomisation in the abstract and uneven numbers between the groups, attempted to contact authors with no reply

Characteristics of studies awaiting assessment *[ordered by study ID]*

Anarte 2013a

Methods	Randomised
Participants	Male infertility
Interventions	DHA
Outcomes	Sperm parameters
Notes	Definitely same trial as excluded Anarte 2013 (checked 04.09.14)

Gopinath 2013

Methods	Randomised
Participants	Idiopathic oligoasthenozoospermia
Interventions	Combination antioxidants
Outcomes	Sperm parameters and pregnancy

Gopinath 2013 (*Continued*)

Notes	

Iacono 2014

Methods	Randomised
Participants	male idiopathic infertility
Interventions	Combination therapy of antiestrogen and a natural composite containing tribulus terrestris, alga ecklonia bicyclis, biovis and myo-inositol
Outcomes	Sperm parameters and pregnancy
Notes	Probably exclude due to incorrect intervention

Nadjarzadeh 2014

Methods	Randomised
Participants	Male subfertility
Interventions	Coenzyme Q10
Outcomes	Sperm parameters
Notes	

Nashivochnikova 2014

Methods	Unknown
Participants	Pathospermia
Interventions	Spematon (L-carnitine, zinc and vitamin E)
Outcomes	Acrosome reaction
Notes	Maybe a combination antioxidant. Needs translation (Russian)

Nematollahi-Mahani 2014

Methods	Randomised
Participants	Varicocelectomy
Interventions	Folic acid and zinc
Outcomes	Sperm parameters
Notes	

Characteristics of ongoing studies *[ordered by study ID]*

AGUNCO 2012

Trial name or title	Effect of Treatment With Myo-inositol on Human Semen Parameters in Patients Undergoing In Vitro Fertilization Cycles
Methods	Interventional, Phase 4
Participants	18 years to 40 years male
Interventions	Dietary Supplement: myo-inositol + folic acid
Outcomes	Primary outcome measures: Semen volume Spermatozoa count Spermatozoa progressive motility Number of spermatozoa after density gradient separation method
Starting date	March 2012
Contact information	Italy, University of Catania - Department of Surgery - Section of Obstetrics and Gynecology -Center of Physiopathology of Reproduction, AGUNCO Obstetrics and Gynecology Centre
Notes	ClinicalTrials.gov identifier: NCT01560065

Gonzalez 2009

Trial name or title	Assessment of the efficacy of dietary supplement Spermotrend in the treatment of male infertility
Methods	Randomised, double blind (subject, caregiver, investigator), placebo control, parallel assignment, efficacy study
Participants	Subfertile men
Interventions	Spermotrend (vitamins plus other antioxidants) versus placebo

Gonzalez 2009 (*Continued*)

Outcomes	Parameters of seminal analysis at weeks 24 Fertilisation achievement Presence of mild or severe adverse effects
Starting date	September 2009
Contact information	Rogelio Gonzalez Sanchez, MD 53 7 838 2626 ext 277 Gynecologic and Obstetric Hospital Havana, Cuba, 10400
Notes	Havana, Cuba, 10400 NCT00975117

Jensen 2011

Trial name or title	Vitamin D Supplementation and Male Infertility: The CBG-study a Randomized Clinical Trial
Methods	Double blinded randomised clinical trial
Participants	Male 18 years and over low sperm concentration and motility
Interventions	Drug: Cholecalciferol and calcium Other: Placebo
Outcomes	• semen quality [Time Frame: 150 days] [Designated as safety issue: No] difference in semen quality (semen variables total sperm count, sperm concentration, sperm motility, sperm morphology and semen volume) between VD and placebo treated men after 150 days of treatment • sperm motility [Time Frame: 150 days] [Designated as safety issue: No] Differences in sperm motility (ABC) and progressive sperm motility (AB) between placebo and VD group, supported by other motility measures such as length of penetration in egg media and difference in motility over time (3-5 hours from ejaculation)between VD and placebo treated men • sperm morphology [Time Frame: 150 days] [Designated as safety issue: No] Differences in percentage of spermatozoa with normal morphology assessed according to strict criteria between placebo and VD group • sperm concentration [Time Frame: 150 days] [Designated as safety issue: No] Differences in sperm concentration between placebo and VD group • total sperm count [Time Frame: 150 days] [Designated as safety issue: No] Differences in total sperm count between placebo and VD group • semen volume [Time Frame: 150 days] [Designated as safety issue: No] Differences in semen volume between placebo and VD group
Starting date	2011 recruiting 2014
Contact information	Further study details as provided by Rigshospitalet, Denmark
Notes	NCT01304927

Kamath 2014

Trial name or title	To compare the effectiveness of antioxidants versus no treatment for male partner for improving pregnancy rates in couples undergoing In vitro fertilization (IVF) for abnormal semen analysis
Methods	Randomised: Permuted block randomisation, variable method of allocation concealment: sequentially numbered, sealed, opaque envelopes. Blinding and masking: Open label
Participants	Inclusion criteria: Couples undergoing ART due to male factor infertility with the following parameters Mild Oligozoospermia 1 to 15 million/mL AND/OR Asthenozoospermia < 32% progressive motility AND/OR Teratozoospermia < 4% normal morphology Exclusion criteria: Severe oligozoospermia < 1 million/mL Taken treatment in past 3 months for male infertility Female age > 37 years Female partner - moderate or severe endometriosis
Interventions	Intervention1: Tablet Vitamin C 500 mg, Capsule Vitamin E 400 mg and Tablet Zinc 140 mg OD for 3 months versus no treatment
Outcomes	Clinical pregnancy rate. Timepoint: February 2015 Ongoing pregnancy rate Miscarriage rate Fertilisation rate Live birth rate Changes in sperm parametersTimepoint: February 2015
Starting date	01.02.13
Contact information	Mohan S Kamath Address: Dr Mohan S Kamath, MS,DNB, Fellow (Reproductive Medicine) Associate Professor, Reproductive Medicine Unit, Christian Medical College and Hospital, Vellore ï¿½?? 632004 632004 Vellore, TAMIL NADU India Telephone: 04162283301 Email: dockamz@gmail.com Affiliation: Christian Medical College and Hospital
Notes	CTRI/2013/02/003431 Email sent 26.03.14. Dr Kamath replied 3.04.14 saying that they were still in the recruitment phase and were hoping to finish the trial in 2015

Palumbo 2012

Trial name or title	Effect of Treatment With Myo-inositol on Human Semen Parameters in Patients Undergoing IVF Cycles
Methods	Allocation: Randomized Endpoint classification: Safety/Efficacy study Intervention model: Parallel assignment Masking: Open label Primary purpose: Screening
Participants	Asthenozoospermia Oligospermia Male 25 years to 65 years
Interventions	Dietary Supplement: Myo-inositol OAT
Outcomes	Samples of seminal fluid were obtained from two groups of patients undergoing to an IVF cycle: healthy normospermic subjects and subjects with oligoasthenoteratospermia (OAT, < 15 mil/ml) Semen volume, spermatozoa number and motility were evaluated during the initial semen analysis and after density gradient separation method. These parameters were evaluated before and after the administration of 4000mg/die of myo-inositol associated to 400 µg of folic acid (Inofolic lolipharma Rome) for three months A third group of healthy normospermic subject were traded with 400 µg of folic acid for three months and was consider a control group
Starting date	March 2013
Contact information	Sponsor: AGUNCO Obstetrics and Gynecology Centre Information provided by (Responsible Party): AGUNCO Obstetrics and Gynecology Centre. Italy
Notes	Marco Palumbo, M.D.University of Catania - Department of Surgery - Section of Obstetrics and Gynecology - Centre of Physiopathology of Reproduction Gianfranco Carlomagno, A.G.Un.Co. Obstetrics and Gynaecology Center NCT01828710

Rigshospitalet 2011

Trial name or title	Vitamin D supplementation and male infertility: a randomized double blinded clinical trial
Methods	Interventional clinical trial of medicinal product Study design: Controlled: yes Randomised: yes Open: no Single blind: no Double blind: yes Parallel group: yes Cross over: no Other: no If controlled, specify comparator, Other Medicinial Product: no Placebo: yes

Rigshospitalet 2011 (*Continued*)

	Other: no
Participants	semen concentration < 20 mio./ml or < 40 progressive motility, or < 7% morphologically normal sperm. VD serum level < 50 nM Are the trial subjects under 18? no Number of subjects for this age range: F.1.2 Adults (18 - 64 years) yes F.1.2.1 Number of subjects for this age range F.1.3 Elderly (≥ 65 years) no F.1.3.1 Number of subjects for this age range Exclusion criteria: hypercalcaemia Spontaneous intake 15 µg daily disease with granuloma indication of testicular biopsy
Interventions	Cholecalciferol
Outcomes	Main objective: To investigate whether vitamin D supplementation to vitamin D deficient or insufficient men improves semen quality and male fertility Primary end point(s): change in semen quality and fertility potential Secondary objective: Reproductive or sex hormones hormones Bone markers such as FGF23, osteocalcin, osteopontin, alkaline phophatase, calcitonin, PTH, all circulating VD forms, calcium, klotho, procollagen and other bone markers ART or spontaneous pregnancy Percentage spermatozoa expressing cyp24a1 Composition of pH, HCO3, calcium, zinc, phosphate, vitamin D, FGF23, Klotho, osteocalcin, osteopontin Fat free mass, muscle, BMI, weight Serum levels of Hb1Ac, cholesterol, lipids, indulin Frequency of infectious disease, acute phase reactants leucocytes, thrombocytes, reticulocytes, complement, immunoglobulins, autoantibodies, ANA, antiphospholipid, factor 2,7,10. Change in liver enzymes (ALAT, ASAT, GGT, LDH).
Starting date	11/02/2011
Contact information	Not Known
Notes	Denmark EUCTR2010-024588-42-DK

Sadeghi 2008

Trial name or title	Sadeghi M. Effects of coenzymeQ10 (CoQ10) supplementation on semen quality and seminal oxidative stress of idiopathic oligoasthenoteratozoospermic (iOAT) infertile men. World Health Organization International Clinical Trials Registry Platform Search Portal 2008. [ISRCTN: ISRCTN29954277]
Methods	Randomised double blind placebo controlled trial

Sadeghi 2008 (*Continued*)

Participants	Men with idiopathic oligoasthenoteratozoospermia with at least one year infertility
Interventions	Coenzyme10 (ubiquinone) 200mg 2x/day for 3 months
Outcomes	Sperm morphology, motility and sperm DNA fragmentation
Starting date	1.10.08
Contact information	sadeghi@avicenna.ac.ir
Notes	letter sent to author regarding data 17.09.09. Email sent 26.03.14 [ISRCTN: ISRCTN29954277]

Sadeghi 2009

Trial name or title	Effect of Ubiquinone Supplementation on Semen Quality, Antioxidant Enzyme, Oxidative Stress and DNA Fragmentation in infertile men
Methods	Randomisation: randomised. Blinding: Double blind. Placebo: used. Assignment: Parallel. Purpose: Treatment
Participants	Inclusion criteria: Inclusion : idiopathic oligoasthenoteratospermia, normal profile for hormones, normal genital anatomy Exclusion: major infection in genital organ, treatment for systemic disease during the last three months of inclusion, surgery background on genitalia, exposure to chemicals ,solvents or heavy metals, supplementation therapy in last three months of inclusion Exclusion criteria: Age minimum: 20 Age maximum: 50 Gender: male
Interventions	Co Q10 supplement: 2 (100 mg) per day (200 mg), 3 month. Intervention 2: Placebo (lactose): 2 capsule per day, 3 month
Outcomes	activity of seminal plasma catalase. Timepoint: 45 days , 90 days. Method of measurement: spectrophotometry activity of seminal plasma superoxide dysmutase. Time point: 45 days , 90days. Method of measurement: spectrophotometry DNA fregmentation . Timepoint: 45 days , 90 days. Method of measurement: Flow cytometry semen analysis. Timepoint: 45 days , 90 days. Method of measurement: HPLC, flourometry , spectrophotometry, ELIZA seminal plasma ubiquinone concentration. Timepoint: 45 days, 90 days. Method of measurement: HPLC sperm Isoprostan concentration. Timepoint: 45 days, 90 days. Method of measurement: ELIZA
Starting date	09.08.2011

Contact information	Mohammad Reza Sadeghi Address: Avicenna Research Institute, Shahid Beheshti University , Evin 1983969411 Tehran Iran, Islamic Republic Of Telephone: 00982122432024 Email: sadeghi@avicenna.ac.ir Affiliation: Avicenna Research Institute
Notes	Is this the same trial as Sadeghi 2008? NB the trials have different IRCT numbers [ISRCTN: IRCT138706031079N1] Email sent 26.03.14

DATA AND ANALYSES

Comparison 1. Antioxidant(s) versus placebo or no treatment

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Live birth; type of antioxidant	4	277	Peto Odds Ratio (Peto, Fixed, 95% CI)	4.21 [2.08, 8.51]
1.1 Vitamin E versus placebo	2	117	Peto Odds Ratio (Peto, Fixed, 95% CI)	6.44 [1.72, 24.04]
1.2 Zinc versus no treatment	1	100	Peto Odds Ratio (Peto, Fixed, 95% CI)	3.74 [1.02, 13.74]
1.3 Combined antioxidants versus placebo	1	60	Peto Odds Ratio (Peto, Fixed, 95% CI)	3.42 [1.15, 10.13]
2 Live birth; IVF/ICSI	2	90	Peto Odds Ratio (Peto, Fixed, 95% CI)	3.61 [1.27, 10.29]
3 Clinical pregnancy; type of antioxidant	7	522	Peto Odds Ratio (Peto, Fixed, 95% CI)	3.43 [1.92, 6.11]
3.1 Combined antioxidants versus placebo	1	60	Peto Odds Ratio (Peto, Fixed, 95% CI)	2.44 [0.84, 7.13]
3.2 Magnesium versus placebo	1	26	Peto Odds Ratio (Peto, Fixed, 95% CI)	8.73 [0.17, 445.08]
3.3 Vitamin E versus placebo	2	117	Peto Odds Ratio (Peto, Fixed, 95% CI)	6.71 [1.98, 22.69]
3.4 Zinc versus placebo or no treatment	2	153	Peto Odds Ratio (Peto, Fixed, 95% CI)	4.43 [1.39, 14.14]
3.5 N-acetylcysteine versus no treatment	1	60	Peto Odds Ratio (Peto, Fixed, 95% CI)	1.60 [0.42, 6.16]
3.6 Zinc plus folic acid versus placebo	1	53	Peto Odds Ratio (Peto, Fixed, 95% CI)	3.86 [0.15, 99.84]
3.7 Folic acid versus placebo	1	53	Peto Odds Ratio (Peto, Fixed, 95% CI)	0.0 [0.0, 0.0]
4 Clinical pregnancy; IVF/ICSI	2	90	Peto Odds Ratio (Peto, Fixed, 95% CI)	2.64 [0.94, 7.41]
5 Adverse events	8		Peto Odds Ratio (Peto, Fixed, 95% CI)	Subtotals only
5.1 Miscarriage	3	247	Peto Odds Ratio (Peto, Fixed, 95% CI)	1.74 [0.40, 7.60]
5.2 Gastrointestinal	6	429	Peto Odds Ratio (Peto, Fixed, 95% CI)	1.60 [0.47, 5.50]
5.3 Euphoria	1	86	Peto Odds Ratio (Peto, Fixed, 95% CI)	1.21 [0.16, 9.01]
5.4 Ectopic pregnancy	1	60	Peto Odds Ratio (Peto, Fixed, 95% CI)	4.48 [0.07, 286.49]
6 Sperm DNA fragmentation; type of antioxidant	2	100	Mean Difference (IV, Fixed, 95% CI)	-13.85 [-17.28, -10.41]
6.1 Vitamin C + vitamin E versus placebo at 2 months	1	64	Mean Difference (IV, Fixed, 95% CI)	-13.80 [-17.50, -10.10]
6.2 Docosahexaenoic acid (DHA) 1000mg/day versus placebo at 10 weeks	1	36	Mean Difference (IV, Fixed, 95% CI)	-14.12 [-23.23, -5.01]
7 Total sperm motility at 3 months or less; type of antioxidant	16	1039	Mean Difference (IV, Random, 95% CI)	10.02 [3.79, 16.25]
7.1 Docosahexaenoic acid (DHA) 400mg/day versus placebo	1	18	Mean Difference (IV, Random, 95% CI)	-7.80 [-27.79, 12.19]
7.2 Docosahexaenoic acid (DHA) 800mg/day vs placebo	1	19	Mean Difference (IV, Random, 95% CI)	-15.20 [-30.92, 0.52]
7.3 Docosahexaenoic acid (DHA) 1000mg/day versus placebo at 10 weeks	1	36	Mean Difference (IV, Random, 95% CI)	-6.45 [-17.64, 4.74]

7.4 Vitamin C acid 200mg/day versus placebo	1	20	Mean Difference (IV, Random, 95% CI)	2.0 [-18.82, 22.82]
7.5 Vitamin C 1000mg/day versus placebo	1	20	Mean Difference (IV, Random, 95% CI)	45.0 [19.72, 70.28]
7.6 Vitamin C + Vitamin E versus placebo at 2 months	2	95	Mean Difference (IV, Random, 95% CI)	1.46 [-5.82, 8.74]
7.7 Carnitines versus placebo or no treatment at 3 months	3	99	Mean Difference (IV, Random, 95% CI)	15.32 [-3.70, 34.35]
7.8 Selenium versus placebo at 3 months	1	34	Mean Difference (IV, Random, 95% CI)	14.9 [1.14, 28.66]
7.9 Combined antioxidants versus placebo or no treatment at 3 months	2	228	Mean Difference (IV, Random, 95% CI)	15.13 [13.56, 16.69]
7.10 N-acetylcysteine versus placebo/no treatment at 3 months	2	180	Mean Difference (IV, Random, 95% CI)	7.65 [0.68, 14.62]
7.11 Magnesium versus placebo at 90 days	1	20	Mean Difference (IV, Random, 95% CI)	14.5 [-6.01, 35.01]
7.12 Zinc versus no treatment or placebo at 3 months	2	76	Mean Difference (IV, Random, 95% CI)	14.66 [-5.91, 35.24]
7.13 Zinc + Vitamin E versus no treatment at 3 months	1	20	Mean Difference (IV, Random, 95% CI)	26.0 [12.85, 39.15]
7.14 Zinc + Vitamin E + Vitamin C versus no treatment at 3 months	1	22	Mean Difference (IV, Random, 95% CI)	26.0 [12.62, 39.38]
7.15 Coenzyme Q10 versus placebo	1	47	Mean Difference (IV, Random, 95% CI)	3.58 [-6.16, 13.32]
7.16 Zinc plus folic acid versus placebo	1	54	Mean Difference (IV, Random, 95% CI)	6.80 [-2.84, 16.44]
7.17 Folic acid versus placebo	1	51	Mean Difference (IV, Random, 95% CI)	8.40 [-0.99, 17.79]
8 Total sperm motility at 3 months or less (data not suitable for meta analysis)			Other data	No numeric data
8.1 L-carnitine + Acetyl-carnitine versus placebo (median and interquartile range)			Other data	No numeric data
8.2 Combined antioxidants versus no treatment			Other data	No numeric data
8.3 Vitamin E versus placebo			Other data	No numeric data
8.4 L-carnitine versus placebo			Other data	No numeric data
8.5 Selenium + Zinc versus placebo			Other data	No numeric data
9 Total sperm motility at 6 months; type of antioxidant	9		Mean Difference (IV, Random, 95% CI)	Subtotals only
9.1 Carnitines versus placebo at 6 months	3	107	Mean Difference (IV, Random, 95% CI)	7.28 [-9.47, 24.02]
9.2 Selenium versus placebo at 26 weeks (6 months)	1	140	Mean Difference (IV, Random, 95% CI)	3.20 [2.28, 4.12]
9.3 N-acetyl-cysteine versus placebo at 26 weeks (6months)	1	140	Mean Difference (IV, Random, 95% CI)	1.90 [0.98, 2.82]

9.4 Selenium plus N-acetyl-cysteine versus placebo at 26 weeks (6months)	1	139	Mean Difference (IV, Random, 95% CI)	6.30 [5.38, 7.22]
9.5 Coenzyme Q10 versus placebo at 6 months	3	479	Mean Difference (IV, Random, 95% CI)	6.58 [1.80, 11.37]
9.6 Vitamin E versus placebo at 6 months	1	87	Mean Difference (IV, Random, 95% CI)	13.0 [7.02, 18.98]
9.7 Zinc versus placebo	1	40	Mean Difference (IV, Random, 95% CI)	0.0 [-10.19, 10.19]
9.8 Zinc plus folic acid versus placebo	1	37	Mean Difference (IV, Random, 95% CI)	2.60 [-8.82, 14.02]
9.9 Folic acid versus placebo	1	34	Mean Difference (IV, Random, 95% CI)	1.70 [-8.49, 11.89]
10 Total sperm motility at 6 months(data not suitable for meta analysis)			Other data	No numeric data
10.1 L-carnitine + Acetyl-carnitine versus placebo (median and interquartile range)			Other data	No numeric data
10.2 Folic acid versus placebo			Other data	No numeric data
10.3 Zinc versus placebo			Other data	No numeric data
10.4 Zinc + folic acid versus placebo			Other data	No numeric data
11 Total sperm motility at 9 months or more; type of antioxidant	4		Mean Difference (IV, Random, 95% CI)	Subtotals only
11.1 L-carnitine versus placebo at 9 months	1	20	Mean Difference (IV, Random, 95% CI)	11.54 [1.66, 21.42]
11.2 L-acetyl carnitine versus placebo at 9 months	1	20	Mean Difference (IV, Random, 95% CI)	7.84 [-1.41, 17.09]
11.3 L-carnitine + L-acetyl carnitine versus placebo at 9 months	1	20	Mean Difference (IV, Random, 95% CI)	6.27 [-3.36, 15.90]
11.4 Coenzyme Q10 versus placebo at 9 months	3	479	Mean Difference (IV, Random, 95% CI)	1.88 [-1.58, 5.34]
12 Total sperm motility over time	23		Mean Difference (IV, Random, 95% CI)	Subtotals only
12.1 Total sperm motility at 3 months or less	16	832	Mean Difference (IV, Random, 95% CI)	9.55 [2.12, 16.97]
12.2 Total sperm motility at 6 months	9	964	Mean Difference (IV, Random, 95% CI)	6.86 [3.78, 9.94]
12.3 Total sperm motility at 9 months or more	4	509	Mean Difference (IV, Random, 95% CI)	3.17 [-0.10, 6.45]
13 Sperm concentration at 3 months or less; type of antioxidant	13		Mean Difference (IV, Random, 95% CI)	Subtotals only
13.1 Docosahexaenoic acid (DHA) 400g/day versus placebo	1	18	Mean Difference (IV, Random, 95% CI)	-5.30 [-41.09, 30. 49]
13.2 Docosahexaenoic acid (DHA) 800g/day versus placebo	1	19	Mean Difference (IV, Random, 95% CI)	1.50 [-35.23, 38.23]

13.3 Docosahexaenoic acid (DHA) 1000mg/day versus placebo at 10 weeks	1	36	Mean Difference (IV, Random, 95% CI)	-1.38 [-18.78, 16.02]
13.4 Magnesium versus placebo at 90 days	1	20	Mean Difference (IV, Random, 95% CI)	5.20 [-2.61, 13.01]
13.5 Vitamin C + Vitamin E versus placebo at 2 months	2	95	Mean Difference (IV, Random, 95% CI)	1.36 [-10.01, 12.72]
13.6 N-acetylcysteine versus placebo at 3 months	1	120	Mean Difference (IV, Random, 95% CI)	-0.47 [-6.70, 5.76]
13.7 Carnitines versus placebo	2	78	Mean Difference (IV, Random, 95% CI)	14.29 [-15.50, 44.08]
13.8 Coenzyme Q10 versus placebo	1	47	Mean Difference (IV, Random, 95% CI)	-0.12 [-12.39, 12.15]
13.9 N-acetylcysteine versus no treatment	1	60	Mean Difference (IV, Random, 95% CI)	4.72 [-0.31, 9.75]
13.10 Combined antioxidants versus placebo or no treatment	2	219	Mean Difference (IV, Random, 95% CI)	-0.89 [-1.84, 0.06]
13.11 Zinc plus folic acid versus placebo	1	37	Mean Difference (IV, Random, 95% CI)	18.0 [1.13, 34.87]
13.12 Folic acid versus placebo	1	34	Mean Difference (IV, Random, 95% CI)	22.20 [3.79, 40.61]
13.13 Zinc versus placebo	1	40	Mean Difference (IV, Random, 95% CI)	16.9 [0.53, 33.27]
13.14 Selenium versus placebo	1	25	Mean Difference (IV, Random, 95% CI)	21.20 [-11.45, 53.85]
14 Sperm concentration at 3 months or less (data not suitable for meta analysis)			Other data	No numeric data
14.1 L-carnitine + Acetyl-carnitine versus placebo (median and interquartile range)			Other data	No numeric data
14.2 Vitamin E versus placebo			Other data	No numeric data
14.3 L-carnitine versus placebo			Other data	No numeric data
15 Sperm concentration at 6 months; type of antioxidant	7		Mean Difference (IV, Random, 95% CI)	Subtotals only
15.1 Carnitines versus placebo at 6 months	2	116	Mean Difference (IV, Random, 95% CI)	2.59 [-3.11, 8.30]
15.2 Selenium versus placebo at 26 weeks (6 months)	1	140	Mean Difference (IV, Random, 95% CI)	4.10 [1.82, 6.38]
15.3 N-acetyl-cysteine versus placebo at 26 weeks (6months)	1	140	Mean Difference (IV, Random, 95% CI)	3.30 [1.13, 5.47]
15.4 Selenium plus N-acetyl-cysteine versus placebo at 26 weeks (6months)	1	139	Mean Difference (IV, Random, 95% CI)	8.60 [6.28, 10.92]
15.5 Coenzyme Q10 versus placebo at 6 months	3	479	Mean Difference (IV, Random, 95% CI)	6.88 [1.20, 12.56]
15.6 Zinc plus folic acid versus placebo	1	37	Mean Difference (IV, Random, 95% CI)	17.70 [-1.88, 37.28]
15.7 Folic acid versus placebo	1	34	Mean Difference (IV, Random, 95% CI)	19.20 [4.74, 33.66]
15.8 Zinc versus placebo	1	40	Mean Difference (IV, Random, 95% CI)	9.70 [-7.01, 26.41]

16 Sperm concentration at 6 months(data not suitable for meta analysis)			Other data	No numeric data
16.1 L-carnitine + acetyl-carnitine versus placebo			Other data	No numeric data
16.2 Folic acid versus Placebo			Other data	No numeric data
16.3 Zinc versus Placebo			Other data	No numeric data
16.4 Zinc + folic acid versus placebo			Other data	No numeric data
17 Sperm concentration at 9 months; type of antioxidant	4		Mean Difference (IV, Random, 95% CI)	Subtotals only
17.1 Carnitines versus placebo at 9 months	1	60	Mean Difference (IV, Random, 95% CI)	4.12 [-1.74, 9.99]
17.2 Coenzyme Q10 versus placebo at 9 months or more	3	479	Mean Difference (IV, Random, 95% CI)	2.74 [-1.56, 7.05]
18 Sperm concentration over time	20		Mean Difference (IV, Random, 95% CI)	Subtotals only
18.1 Sperm concentration at 3 months or less	13	746	Mean Difference (IV, Random, 95% CI)	5.32 [-0.62, 11.26]
18.2 Sperm concentration 6 months	8	851	Mean Difference (IV, Random, 95% CI)	5.46 [1.81, 9.11]
18.3 Sperm concentration at 9 months or more	4	509	Mean Difference (IV, Random, 95% CI)	3.66 [-0.31, 7.64]

Comparison 2. Head to head antioxidant(s)

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Total sperm motility at 3 months or less; type of antioxidant	8		Mean Difference (IV, Fixed, 95% CI)	Subtotals only
1.1 Ethylcysteine 600mg/day vs Vitamin E	1	10	Mean Difference (IV, Fixed, 95% CI)	-1.90 [-41.97, 38.17]
1.2 Docosahexaenoic acid (DHA) 400g/day vs Docosahexaenoic acid 800mg/day	1	19	Mean Difference (IV, Fixed, 95% CI)	7.40 [-11.35, 26.15]
1.3 Vitamin C 200mg/day versus vitamin C 1000mg/day	1	20	Mean Difference (IV, Fixed, 95% CI)	-43.0 [-67.10, -18.90]
1.4 Vitamin E + Selenium versus Vitamin B at 3 months	1	54	Mean Difference (IV, Fixed, 95% CI)	0.0 [-10.71, 10.71]
1.5 Zinc versus Zinc + Vitamin E at 3 months	1	18	Mean Difference (IV, Fixed, 95% CI)	-1.0 [-13.00, 13.00]
1.6 Zinc versus Zinc + Vitamin E + Vitamin C at 3 months	1	12	Mean Difference (IV, Fixed, 95% CI)	-1.0 [-19.66, 17.66]
1.7 Zinc + Vitamin E versus Zinc + Vitamin E + Vitamin C at 3 months	1	18	Mean Difference (IV, Fixed, 95% CI)	0.0 [-18.97, 18.97]

1.8 Selenium versus combined antioxidants	1	46	Mean Difference (IV, Fixed, 95% CI)	3.20 [-10.13, 16.53]
1.9 L acetyl carnitine + L carnitine versus Vitamin E + Vitamin C	1	138	Mean Difference (IV, Fixed, 95% CI)	23.05 [20.09, 26.01]
1.10 Zinc + folic acid versus folic acid	1	80	Mean Difference (IV, Fixed, 95% CI)	-0.60 [-7.74, 6.54]
1.11 Zinc versus zinc + folic acid	1	80	Mean Difference (IV, Fixed, 95% CI)	-2.80 [-12.91, 7.31]
1.12 Zinc versus folic acid	1	80	Mean Difference (IV, Fixed, 95% CI)	-4.40 [-14.21, 5.41]
2 Total sperm motility at 6 months; type of antioxidant	3		Mean Difference (IV, Fixed, 95% CI)	Subtotals only
2.1 L-acetyl carnitine + L-carnitine versus L-carnitine	1	30	Mean Difference (IV, Fixed, 95% CI)	-3.46 [-9.72, 2.80]
2.2 L-acetyl carnitine + L-carnitine versus L-acetyl carnitine	1	30	Mean Difference (IV, Fixed, 95% CI)	0.64 [-6.37, 7.65]
2.3 Selenium versus N-acetyl-cysteine	1	234	Mean Difference (IV, Fixed, 95% CI)	1.30 [0.56, 2.04]
2.4 Selenium versus selenium plus N-acetyl-cysteine	1	232	Mean Difference (IV, Fixed, 95% CI)	-3.10 [-3.85, -2.35]
2.5 N-acetyl-cysteine vs selenium plus N-acetyl-cysteine	1	234	Mean Difference (IV, Fixed, 95% CI)	-4.40 [-5.14, -3.66]
2.6 Zinc + folic acid versus folic acid	1	80	Mean Difference (IV, Fixed, 95% CI)	0.90 [-5.45, 7.25]
2.7 Zinc versus zinc + folic acid	1	80	Mean Difference (IV, Fixed, 95% CI)	-2.60 [-9.13, 3.93]
2.8 Zinc versus folic acid	1	80	Mean Difference (IV, Fixed, 95% CI)	-1.70 [-6.42, 3.02]
3 Total sperm motility at 6 months			Other data	No numeric data
3.1 Zinc versus Folic acid			Other data	No numeric data
3.2 Zinc versus Zinc + folic acid			Other data	No numeric data
3.3 Folic acid versus Zinc + folic acid			Other data	No numeric data
4 Total sperm motility at 9 months or more; type of antioxidant	1		Mean Difference (IV, Fixed, 95% CI)	Subtotals only
4.1 L-aceytl carnitine + L-carnitine versus L-carnitine	1	30	Mean Difference (IV, Fixed, 95% CI)	-5.27 [-11.28, 0.74]
4.2 L-acetyl carnitine + L-carnitine versus L-acetyl carnitine	1	30	Mean Difference (IV, Fixed, 95% CI)	-1.57 [-6.46, 3.32]
5 Sperm concentration at 3 months or less; type of antioxidant	6		Mean Difference (IV, Fixed, 95% CI)	Subtotals only
5.1 Ethylcysteine 600mg/day vs Vitamin E	1	10	Mean Difference (IV, Fixed, 95% CI)	2.20 [-16.65, 21.05]
5.2 Docosahexaenoic acid (DHA) 400g/day versus Docosahexaenoic acid (DHA) 800g/day	1	19	Mean Difference (IV, Fixed, 95% CI)	-6.80 [-41.87, 28.27]

5.3 L-carnitine versus Vitamin E + Vitamin C	1	63	Mean Difference (IV, Fixed, 95% CI)	15.5 [12.49, 18.51]
5.4 L-carnitine plus vitamin E versus vitamin E	1	113	Mean Difference (IV, Fixed, 95% CI)	1.90 [-10.52, 14.32]
5.5 Selenium versus combined antioxidants	1	46	Mean Difference (IV, Fixed, 95% CI)	14.70 [-6.51, 35.91]
5.6 Zinc + folic acid versus folic acid	1	80	Mean Difference (IV, Fixed, 95% CI)	-4.20 [-22.21, 13.81]
5.7 Zinc versus zinc + folic acid	1	80	Mean Difference (IV, Fixed, 95% CI)	-1.10 [-18.63, 16.43]
5.8 Zinc versus folic acid	1	80	Mean Difference (IV, Fixed, 95% CI)	-5.30 [-23.38, 12.78]
6 Sperm concentration at 6 months; type of antioxidant	3		Mean Difference (IV, Fixed, 95% CI)	Subtotals only
6.1 L-aceytl carnitine +L-carnitine versus L-carnitine at 6 months	1	30	Mean Difference (IV, Fixed, 95% CI)	-8.13 [-21.79, 5.53]
6.2 L-acetyl carnitine + L-carnitine versus L-acetyl carnitine at 6 months	1	30	Mean Difference (IV, Fixed, 95% CI)	-2.17 [-15.26, 10.92]
6.3 Selenium versus N-acetyl-cysteine at 26 weeks (6 months)	1	234	Mean Difference (IV, Fixed, 95% CI)	0.80 [-0.71, 2.31]
6.4 Selenium versus selenium plus N-acetyl-cysteine at 26 weeks (6 months)	1	232	Mean Difference (IV, Fixed, 95% CI)	-4.5 [-6.20, -2.80]
6.5 N-acetyl-cysteine vs selenium plus N-acetyl-cysteine at 26 weeks	1	234	Mean Difference (IV, Fixed, 95% CI)	-5.30 [-6.86, -3.74]
6.6 Zinc + folic acid versus folic acid	1	80	Mean Difference (IV, Fixed, 95% CI)	-1.5 [-15.06, 12.06]
6.7 Zinc versus zinc + folic acid	1	80	Mean Difference (IV, Fixed, 95% CI)	-8.0 [-23.69, 7.69]
6.8 Zinc versus folic acid	1	80	Mean Difference (IV, Fixed, 95% CI)	-9.5 [-20.31, 1.31]
7 Sperm concentration at 6 months			Other data	No numeric data
7.1 Zinc versus Folic acid			Other data	No numeric data
7.2 Zinc versus Zinc + Folic acid			Other data	No numeric data
7.3 Folic acid versus Zinc + folic acid			Other data	No numeric data
8 Sperm concentration at 9 months or more; type of antioxidant	1		Mean Difference (IV, Fixed, 95% CI)	Subtotals only
8.1 L-acetyl carnitine + L-carnitine versus L-carnitine at 9 months	1	30	Mean Difference (IV, Fixed, 95% CI)	-6.13 [-15.99, 3.73]
8.2 L-acetyl carnitine + L-carnitine versus L-acetyl carnitine at 9 months	1	30	Mean Difference (IV, Fixed, 95% CI)	2.06 [-6.09, 10.21]

Comparison 3. Pentoxifylline versus placebo or no treatment

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Total sperm motility at 3 months or less; pentoxifylline versus placebo or no treatment	1	90	Mean Difference (IV, Fixed, 95% CI)	12.77 [9.23, 16.31]
2 Total sperm motility at 3 months or less (data not suitable for meta analysis)			Other data	No numeric data
2.1 Pentoxifylline versus placebo			Other data	No numeric data
3 Total sperm motility at 6 months; pentoxifylline versus placebo or no treatment	1	229	Mean Difference (IV, Fixed, 95% CI)	10.10 [9.09, 11.11]
4 Total sperm motility at 6 months (data not suitable for meta analysis)			Other data	No numeric data
4.1 Pentoxifylline versus placebo			Other data	No numeric data
5 Total sperm motility at 9 months; pentoxifylline versus placebo or no treatment	1	221	Mean Difference (IV, Random, 95% CI)	3.10 [1.93, 4.27]
6 Total sperm motility over time	2		Mean Difference (IV, Fixed, 95% CI)	Subtotals only
6.1 Total sperm motility at 3 months or less	1	90	Mean Difference (IV, Fixed, 95% CI)	12.77 [9.23, 16.31]
6.2 Total sperm motility at 6 months	1	229	Mean Difference (IV, Fixed, 95% CI)	10.10 [9.09, 11.11]
6.3 Total sperm motility at 9 months or more	1	221	Mean Difference (IV, Fixed, 95% CI)	3.10 [1.93, 4.27]
7 Sperm concentration at 3 months or less; pentoxifylline versus placebo or no treatment	1	18	Mean Difference (IV, Fixed, 95% CI)	4.30 [-0.69, 9.29]
8 Sperm concentration at 6 months; pentoxifylline versus placebo	2		Mean Difference (IV, Random, 95% CI)	Subtotals only
9 Sperm concentration at 9 months; pentoxifylline versus placebo	1	221	Mean Difference (IV, Fixed, 95% CI)	1.70 [0.62, 2.78]
10 Sperm concentration over time	2		Mean Difference (IV, Random, 95% CI)	Subtotals only
10.1 Sperm concentration at 3 months or less	1	18	Mean Difference (IV, Random, 95% CI)	4.30 [-0.69, 9.29]
10.2 Sperm concentration at 6 months	2	247	Mean Difference (IV, Random, 95% CI)	6.90 [-0.09, 13.89]
10.3 Sperm concentration at 9 months	1	221	Mean Difference (IV, Random, 95% CI)	1.70 [0.62, 2.78]
11 Adverse events	1		Peto Odds Ratio (Peto, Fixed, 95% CI)	Subtotals only
11.1 Vomiting	1	254	Peto Odds Ratio (Peto, Fixed, 95% CI)	4.98 [1.32, 18.81]
11.2 Dyspepsia	1	254	Peto Odds Ratio (Peto, Fixed, 95% CI)	4.68 [1.15, 19.07]
11.3 Headache	1	254	Peto Odds Ratio (Peto, Fixed, 95% CI)	2.41 [0.54, 10.78]

11.4 Diarrhoea	1	254	Peto Odds Ratio (Peto, Fixed, 95% CI)	7.63 [1.30, 44.67]
11.5 Tremor	1	254	Peto Odds Ratio (Peto, Fixed, 95% CI)	7.45 [0.46, 119.73]
11.6 Dizziness	1	254	Peto Odds Ratio (Peto, Fixed, 95% CI)	7.45 [0.46, 119.73]
11.7 Vertigo	1	254	Peto Odds Ratio (Peto, Fixed, 95% CI)	1.96 [0.20, 18.99]

ADDITIONAL TABLES

Table 1. Data for undefined or biochemical pregnancy

Undefined or biochemical pregnancy	Events Intervention	Total 1	Events Control	Total 2	Effect Estimate	CI	
Antioxidant(s) versus placebo or no treatment							
Combined antioxidants							
Galatioto 2008	1	20	0	22			
Arginine versus placebo							
Pryor 1978	2	35	2	29	0.82	0.82 [0.11, 6.16]	
Carnitines versus placebo or no treatment	25	154	3	145	5.33		
Sigman 2006	1	12	1	9	0.74	0.74 [0.04, 13.02]	
Peivandi 2010	3	15	0	15	8.57	8.57 [0.82, 89.45]	
Lenzi 2004	4	30	0	26	7.20	7.20 [0.95, 54.34]	
Lenzi 2003	6	43	0	43	8.37	8.37 [1.61, 43.58]	
Cavallini 2004	9	39	1	47	7.50	7.50 [2.01, 27.98]	
Balercia 2005	2	15	1	5	0.61	0.61 [0.04, 9.64]	
Coenzyme Q10 versus placebo	6	136	3	136	2.16		
Safarinejad 2009a	0	106	0	106	0	Not estimable	

Table 1. Data for undefined or biochemical pregnancy (*Continued*)

Balercia 2009	6	30	3	30	2.16	2.16 [0.53, 8.82]	
Pentoxifylline versus placebo							
Wang 1983	0	11	0	7	0	Not estimable	
Vitamin C plus vitamin E versus placebo							
Rolf 1999	0	15	0	16	0	Not estimable	
Head to head antioxidant(s)						CI Start	CI End
L-acetyl carnitine + L-carnitine versus L-acetyl carnitine							
Balercia 2005	2	7	2	15	2.66	0.27	25.80
L-acetyl carnitine + L-carnitine versus L-carnitine							
Balercia 2005	3	8	2	15	3.89	0.51	29.76
L-acetyl carnitine + L-carnitine versus vitamin E + vitamin C							
Li 2005	10	85	2	53	2.72	0.81	9.14
L-carnitine plus vitamin E versus vitamin E							
Wang 2010	21	68	3	67	6.01	2.49	14.47

Table 2. Outcomes and conclusions from all included trials

Study ID	Population, design	Outcomes described in methods section	Outcomes reported on in results	In meta-analysis Y or N	Results	Conclusions + = positive effect - = negative or no effect
Akiyama 1999	Infertile men - high ROS levels N = 10 Crossover Head to head Japanese	Sperm parameters	Sperm parameters	Y	Sperm density and motility did not improve but "sperm function" increased and ROS levels decreased	+ Ethylcysteine shown to be effective when compared to vitamin E for ROS associated infertility
Attallah 2013	Idiopathic athenozospermia IUI N = 30 parallel, no treatment conference abstract	Chemical and clinical pregnancy sperm parameters	Clinical pregnancy Sperm parameters	Y	NAC increased sperm concentration and motility Clinical pregnancy was not significantly different between the groups	+ NAC improves semen quality and improves pregnancy rates prior to IUI
Azizollahi 2013	Men post-varicocelectomy N = 160 4-armed trial	Sperm parameters	Sperm parameters	Y sperm parameters reported but clinical pregnancy from correspondence with author - men were asked at their last semen assessment session about pregnancy if yes ultrasound was used to confirm	Mild improvement in sperm parameters with the use of antioxidants	+ Co-administration of zinc and folic acid improved sperm parameters and increased varicocelectomy outcomes
Balercia 2005	Infertile men or unexplained infertility N = 60 Placebo and head to head	Sperm parameters	Sperm parameters Spontaneous pregnancies 5 LC + LAC 2 LC 2 LAC 3 placebo	Y - sperm N - pregnancy	Improvement in motility in LAC group. 12 spontaneous pregnancies (unknown if biochemical or clinical)	+ Long term carnitine is effective in increasing sperm motility

Table 2. Outcomes and conclusions from all included trials (*Continued*)

Balercia 2009	Infertile and unexplained N = 60	Sperm parameters	Sperm parameters Spontaneous pregnancies 6 Q10 3 placebo	Y - sperm N - pregnancy	Co enzyme Q10 increased sperm motility. 9 spontaneous pregnancies (unknown if biochemical or clinical)	+ Q10 effective in improving sperm kinetic features in asthenospermia
Biagiotti 2003	Severe idiopathic oligoasthenospermia conference abstract N = 42	Sperm parameters	Sperm	N - no data available	A significant improvement in morphology concentration, motility in the carnitine group No side effects	+ Quality of semen is positively associated with fertilisation and implantation rates in assisted reproduction
Cavallini 2004	Idiopathic and varicocoele associated infertility N = 325	Sperm parameters pregnancies side effects	Sperm parameters pregnancies at 6 months post-treatment and assumed to be clinical	N Medians only given for sperm parameters in full paper Analysis 1.8 , .Analysis 1.10; Analysis 1.13; Analysis 1.14; Analysis 1.16. Means in conference abstract but no data given for placebo group and data for group 3 (carnitine + cinoxacin) versus group 2 (carnitines) unable to be used as 3 includes cinoxacin an antiinflammatory drug Y clinical pregnancy	Significant increase in sperm parameters for carnitines when compared to placebo Carnitine groups had a significantly higher pregnancy rate than placebo group	+ The antioxidant plus antiinflammatory group was more effective in improving sperm parameters and pregnancy than those of carnitines alone or placebo however carnitines alone were more effective than placebo
Ciftci 2009	Idiopathic infertility N = 120	Sperm parameters	Sperm parameters	Y sperm parameters	NAC showed significant improvement in sperm parameters when compared with	+ Sperm parameters improved after the use of NAC

Table 2. Outcomes and conclusions from all included trials (*Continued*)

					placebo	
Conquer 2000	Asthenozoospermic men N = 28	Sperm parameters	Sperm parameters	Y sperm parameters (SEs converted to SDs)	DHA showed no effect on sperm motility or concentration	± DHA supplementation increased DHA levels in the sperm but not motility or concentration
Dawson 1990	Agglutination associated infertility N = 30	Sperm parameters	Sperm parameters	Y sperm parameters (SEs converted to SDs)	The group receiving 1000 mg of AA showed more improvement in parameters than the 200mg group and the placebo	+
Dimitriadis 2010	Oligoasthenospermia N = 75 4 arm trial only 2 arms able to be used	Sperm parameters	Sperm parameters	Y sperm parameters	An improvement in sperm concentration with carnitine versus no treatment	+ Enhancement of Leydig cell secretory function may increase sperm concentration and motility
Eslamian 2012	Asthenoszoospermic men N = 50	Sperm parameters	sperm parameters - sperm membrane and serum fatty acids	N outcomes not included in this review	Sperm parameters improved with DHA + vitamin E supplementation	+
Galatioto 2008	Oligospermia post-embolisation of varicocoele N = 42	Sperm parameters Pregnancy Adverse events	Sperm parameters Pregnancy Adverse events	N medians only given for sperm parameters Analysis 1.8 Y pregnancy at 12 months post-treatment assumed to be clinical Adverse events	Significant difference in sperm count in combined antioxidant group but not in motility One pregnancy in the NAC group No significant adverse effects	±

Table 2. Outcomes and conclusions from all included trials (*Continued*)

Greco 2005	Male infertility - high DNA fragmentation N = 64	Sperm parameters	Sperm parameters	Y sperm parameters	No significant difference in concentration or motility however DNA fragmentation was significantly reduced in the vitamin C + E when compared to placebo	+ A short oral treatment of VitC + E can reduce DNA fragmentation
Keskes-Ammar 2003	Men with high levels of ROS in semen N = 78 Head to head	Sperm parameters	Sperm parameters	Y sperm parameters	Treatment with Vit E and selenium increased sperm motility when compared to vitamin B	+
Kessopoulou 1995	Male infertility Crossover N = 30	Sperm parameters	Sperm parameters Live birth Clinical pregnancy	N medians only given for sperm parameters Analysis 1.8; Analysis 1.14 Y Pregnancy	No differences in sperm outcomes were seen between the groups. 1 pregnancy in the vitamin E group and nil in the placebo (first phase data)	- No difference in semen parameters
Kumamoto 1988	Male patients with abnormal sperm count and motility 3-armed trial N = 396	Sperm parameters	Sperm parameters	N scales given	No statistical difference in sperm outcomes in vitamin B 12 groups or placebo	-
Lenzi 2003	Male factor infertility N = 100 Crossover	Sperm parameters Pregnancy rates	Sperm parameters Pregnancy rates	N no SDs given for sperm parameters Analysis 1.8; Analysis 1.14 N no definition of pregnancy given see Table 1	The patient groups showed no differences in sperm outcomes between therapy (carnitine) and placebo groups Six pregnancies in the carnitine group and nil in the placebo (first phase)	+ The pregnancies obtained during the carnitine therapy period could suggest that carnitines may also lead to improvement in sperm function and fertilisation

Table 2. Outcomes and conclusions from all included trials (*Continued*)

Lenzi 2004	Infertile males - oligoasthenoteratozoospermia N = 60	Sperm parameters Adverse events	Sperm parameters Pregnancy rates	Y sperm parameters N no definition of pregnancy given see analysis for biochemical pregnancy Table 1 N adverse events	Four participants taking carnitine induced a pregnancy in their partner and nil in the placebo	+
Li 2005	Infertile males - oligoasthenoteratozoospermia (150) Head to head	Sperm parameters Pregnancy rates Adverse events	Sperm parameters Pregnancy rates	Y sperm parameters N no definition of pregnancy given see analysis for biochemical pregnancy Table 1	Y 10 pregnancies in the carnitine group and 2 in the vitamin E + C group	+ Lcarnitine and acetyl carnitine more effective than vitamin E + vitamin C for pregnancy, sperm parameters and no evidence of adverse events
Li 2005a	Infertile males - oligoasthenoteratozoospermia (80) Head to head	Sperm parameters	Sperm parameters	Y		+ Staistical significance for carnitines over vitamin E + C
Lombardo 2002	Infertile males Conference abstract Crossover (N = 100)	Sperm parameters	Sperm parameters	N no data		+ Sperm parameters (concentration, motility) carnitines versus placebo
Martinez-Soto 2010	Infertile males (N = 50) Conference abstract + communication with author	Sperm parameters	Sperm parameters	Y		+ DNA fragmentation
Merino 1997	Idiopathic asthenospermia (N = 47)	Sperm parameters		N medians only given for sperm parameters Analysis 3.2; Analysis 3.4		+

Table 2. Outcomes and conclusions from all included trials (*Continued*)

Micic 1988	Idiopathic asthenospermia (N = 90)	Sperm parameters		Y		+ Significant improvement in sperm motility in pentoxifylline versus no treatment
Morgante 2010	Idiopathic asthenospermia (N = 180)	Sperm parameters		Y		+ Sexual satisfaction Significant improvement in sperm motility
Nadjarzadeh 2011	Idiopathic oligoasthenospermia (N = 60)	Sperm parameters		Y		-
Nozha 2001	Oligoasthenospermia head to head (N = 20)	Sperm parameters		N no data available		+ Vitamin E + selenium associated with improved sperm motility when compared with vitamin B
Omu 1998	Asthenospermia (N = 100)	Sperm parameters	Sperm parameters pregnancy and live birth	Y pregnancy and live birth only N sperm parameters not appropriate for review		+ Pregnancy or live birth and sperm parameters
Omu 2008	Asthenospermia (N = 100)	Sperm parameters		Y		+
Peivandi 2010	Infertile men (N = 30) (crossover)	Sperm parameters		Y Y biochemical pregnancies Table 1		+ Sperm outcomes + biochemical pregnancies
Poveda 2013	Infertile men (N = 60) conference abstract	Sperm parameters		N		+ Sperm concentration and motility with L-carnitine and spermotrend

Table 2. Outcomes and conclusions from all included trials (*Continued*)

Pryor 1978	Oligozoospermia (N = 64) crossover	Sperm parameters Pregnancy rates		N bar graph of % patients showing an increase in motility and density Y pregnancy data included in biochemical analysis Table 1		- Arginine was no more effective than placebo for sperm parameters and biochemical pregnancy rates
Rolf 1999	Asthenospermia (N = 33)	Sperm parameters Pregnancy rates		Y	No adverse events or pregnancies in either group	- No difference vitamin E + C versus placebo
Safarinejad 2009	Idiopathic oligozoospermia (N = 468)	Sperm parameters		Y		+ N acetylcysteine, selenium
Safarinejad 2009a	Idiopathic oligozoospermia (N = 212)	Sperm parameters		Y		+ Coenzyme Q10
Safarinejad 2011	Idiopathic infertility (N = 254)	Sperm parameters		Y	Adverse events, sperm concentration and motility	+ Pentoxifylline
Safarinejad 2012	Idiopathic infertility (N=228)	Sperm parameters		Y		+ Coenzyme Q10
Scott 1998	Reduced sperm motility (N = 69)	Sperm parameters Pregnancy		Y N due to pregnancy data pooled in the two intervention groups		+ Sperm motility and pregnancy, combined antioxidants and selenium
Sigman 2006	Low sperm motility (N = 26)	Sperm parameters Pregnancy		Y		- Carnitine
Sivkov 2011	Subnormal spermatogenesis - prostatitis (N = 30) Russian	Sperm parameters		N no sd given see Analysis 1.8		+ Selenium + zinc

Table 2. Outcomes and conclusions from all included trials (*Continued*)

Suleiman 1996	Asthenospermia (N = 110)	Sperm parameters	Sperm parameters Pregnancy Live birth Miscarriage	Y Y Y Y		+ Vit E
Tremellen 2007	Male factor infertility (N = 60)	Pregnancy Side effects	Pregnancy Side effects	Y		+ Menevit
Wang 1983	Idiopathic oligozoospermia (N = 46)	Sperm parameters Pregnancy		Y no data on motility available		- Pentoxifylline
Wang 2010	Asthenospermia (N = 135) Chinese	Sperm parameters Pregnancy	Sperm parameters pregnancy	Y		+ Sperm motility, pregnancy - Sperm density and normal morphology
Wong 2002	Subfertile males (N = 103)	Sperm parameters		N Medians only see Analysis 1.10;and Analysis 1.16		+ Folic acid + zinc
Zalata 1998	Men attending andrology clinic (N = 22) conference abstract	Sperm parameters including DNA fragmentation		N only before and after median data given		+ DNA fragmentation but - Other sperm parameters Combined antioxidants and fatty acids (DHA)
Zavaczki 2003	Idiopathic infertility (N = 20)	Sperm parameters Clinical pregnancy		Y		- Magnesium

WHAT'S NEW

Date	Event	Description
10 February 2015	Amended	Correction of some analysis graph labels.

HISTORY

Date	Event	Description
28 November 2014	New search has been performed	14 new studies were added in this update (Attallah 2013, Azizollahi 2013, Dimitriadis 2010, Eslamian 2012, Kumamoto 1988, Martinez-Soto 2010, Morgante 2010, Nadjarzadeh 2011, Poveda 2013, Pryor 1978, Safarinejad 2011, Safarinejad 2012, Sivkov 2011, Wang 2010). The search was updated in August 2014 and six studies were placed in awaiting classification (Anarte 2013a; Gopinath 2013; Iacono 2014; Nadjarzadeh 2014; Nashivochnikova 2014; Nematollahi-Mahani 2014).
28 November 2014	New citation required and conclusions have changed	Comparisions were restructured into a more logical framework Clinical pregnancy rate data were used in this update rather than the undefined pregnancy rate data of the original review as this is more clinically meaningful when considering the evidence for use of antioxidants
7 December 2011	Feedback has been incorporated	Change of emphasis to conclusions, additional sensitivity analysis performed, Risk of Bias, Summary of Findings Table and Discussion sections edited to increase this review's focus on clinical outcomes of pregnancy and live birth
3 May 2011	Amended	2.1 Analysis edited to fixed effect Peto. The conclusions remain the same
8 March 2011	Amended	Changed summary of findings table to reflect quality of studies
21 December 2010	Amended	Minor edits made - no changes to conclusions
4 May 2007	New citation required and major changes	Substantive amendment

CONTRIBUTIONS OF AUTHORS

MGS: initiated, conceptualised and wrote the protocol; performed the searches, selected trials for inclusion, assessed quality, performed data extraction, entered data and wrote the final review and the update.

RMP: selected trials for inclusion in the 2014 update, assessed quality, entered text into tables of characteristics, performed data extraction and assisted with background text.

JB: co-drafted the protocol; selected trials for inclusion, assessed quality and performed data extraction. JB also provided advice on the data analysis and helped with incorporating the editorial comments into the original review and commented on the updated version.

AY: co-drafted the protocol and wrote the section concerning sperm DNA fragmentation for the background and provided technical advice on all versions.

MS: co-drafted the protocol and provided technical advice on semen parameters, and commented on all versions.

RH: advised and supervised the protocol, review and update, helped select trials for inclusion and wrote the implications for practice and research of the original review and assisted with the abstract. Professor Hart also provided clinical expertise.

DECLARATIONS OF INTEREST

None known

SOURCES OF SUPPORT

Internal sources

- Cochrane Menstrual Disorders and Subfertility Group, Other.

External sources

- None, Other.

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

In the 2011 full review sperm outcomes of concentration and motility were added as these two sperm outcomes are thought to reflect the oxidative process. A study by El-Taieb (El-Taieb 2009) states that “increased ROS generation and reduced antioxidant capacity is negatively correlated with sperm concentration and motility in infertile men”.

The comparisons ’antioxidant versus placebo’ and ’antioxidants versus no treatment’ were combined as the one comparison ’antioxidants versus control’, and then it was stated in the sensitivity analysis whether exclusion of those that failed to use placebo would have altered the conclusions - as per statistical advice in the editorial comments.

Subgrouping and sensitivity analysis were performed on the outcomes of live birth and pregnancy in order to assess the potential of overestimation of benefit and reporting bias.

Subgroup analysis was performed on trials that enrolled couples undergoing IVF or ICSI and a sensitivity analysis was performed on those studies enrolling men undergoing IUI.

A post hoc sensitivity analysis was conducted to examine the effect of excluding from the analysis those studies which reported remarkably low standard deviations as the review authors considered that these data were potentially erroneous.

In the 2014 update of the review ’pregnancy rate per couple’ was redefined to be ’clinical pregnancy rate’. Stillbirth as an outcome was removed; this will be reported as an adverse outcome, as reported by the trials. The outcome ’level of sperm DNA damage after treatment’ was reworded as ’level of sperm fragmentation’.

INDEX TERMS

Medical Subject Headings (MeSH)

Abortion, Spontaneous [epidemiology]; Antioxidants [*therapeutic use]; DNA Damage; DNA Fragmentation; Infertility, Male [*drug therapy; etiology]; Live Birth; Oxidative Stress [*drug effects]; Pregnancy Rate; Randomized Controlled Trials as Topic; Sperm Count; Sperm Motility [drug effects]; Spermatozoa [drug effects]

MeSH check words

Female; Humans; Male; Pregnancy