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Efficacy of combined antioxidant therapy in male subfertility-A systematic review and meta-analysis

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ABSTRACT

Antioxidant therapy is a potentially promising approach to improve clinical outcomes for couples undergoing assisted reproduction techniques long-term. The review aims to (a) collate evidence for the effectiveness of combined oral antioxidant supplementation, including a head-to-head comparison in the treatment of male subfertility, and (b) investigate whether other intervention features, including duration, specific combinations, or dosage affect clinical outcomes in this population. Randomized controlled trials (RCTs) that examined the effectiveness of combined antioxidants on male subfertility, electronic databases including PubMed, Embase, CINAHL, PSYCHINFO, and Cochrane Central Register of Controlled Trials (CENTRAL) were searched. We also searched for unpublished data and references of identified articles. Two reviewers screened eligible studies according to pre-defined criteria and relevant data extracted. The Jadad scale assessed the quality of studies. The study used RevMan version 5.4.1 Meta-analysis software to analyze the effect of combination antioxidants for each outcome measure. Metadata was presented as relative risks for dichotomous outcomes and as standardized mean differences (or mean differences) for continuous outcomes. The systematic review and meta-analysis aimed to report available evidence of whether combination antioxidant is effective and safe in sub-fertile men undergoing assisted reproductive techniques. Also, this review highlighted whether any specific oral antioxidant combinations, dosage, or duration of therapy have a major influence on the clinical outcomes.

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Introduction

Infertility is now considered a global health problem. Male subfertility is described as the inability to achieve clinical pregnancy with frequent unprotected sexual intercourse after a year (1). It is defined as a deficiency of one's capacity to reproduce either with a corresponding partner. Globally, an estimation of 15% of couples experiences infertility in their marriage, leading to over 45 million people with such cases worldwide. This poor reproductive potential in such couples is mainly a result of the malefactor in 20%–30% of the time and contributing 50% of cases (2, 3). Additionally, meta-regression analyses comprising over 184 studies concerning semen quality in recent times disclosed shocking data that the sperm counts and sperm concentrations in patients had reduced by 59.3% and 52.4% from 1973 to 2011, in that order. Hence, this fact becomes generally known that semen quality is gaining heightened public attention (4). Male factor infertility is regarded as changes in sperm concentration and/or morphology and/or motility in a minimum of one sample of two sperm analyses, gathered within a 1 and 4 weeks period (5, 6). Reduced capacity of fertilising is associated with high concentrations of reactive oxygen species in semen, which may harm the cell membrane.

Poor fertility potential has different aetiologies, ranging from genetic mutations to medical conditions or medication lifestyle factors (7). Conventional semen analysis is observed as the basis for the early assessment of the infertile male (8). Yet, this test does not certainly equate to reproductive outcomes and cannot distinguish infertile from fertile males. Abnormal semen parameters cannot foretell fertility as these males may still father a child even with impaired sperm characteristics (9). Other studies have arisen that can identify the likely causes of male factor

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infertility, which cannot be noticed by a routine semen analysis (10).

Oxidative stress, which is caused by the unevenness between antioxidants and reactive oxygen species (ROS), is seen as one of the key causes of male infertility and result in a high rate of abnormal semen parameters and sperm deoxyribonucleic acid (DNA) fragmentation (SDF) (11). The sources of reactive oxygen species in semen can be intrinsic and extrinsic. Activated leukocytes (mostly polymorph nuclear leukocytes as well as macrophages) resulting from inflammation and infection are the noteworthy intrinsic producers of reactive oxygen species in semen. Another key source is immature spermatozoa with irregular head morphology and cytoplasmic retention (12). Impaired, abnormal or deficient spermatozoa due to impaired spermatogenesis can as well yield excessive ROS. Furthermore, semen contains Sertoli cells, which have also been revealed to have the ability to produce reactive oxygen species. Other intrinsic etiologies comprise varicocele (higher grade is linked with more amounts of reactive oxygen species production), testicular torsion, cryptorchidism, as well as ageing (13). Extrinsic sources like alcohol consumption (14), cigarette smoking (15), contact to radiation (16) as well as other environmental pollutants have been linked with high seminal and testicular reactive oxygen species levels. Reactive oxygen species levels can upset sperm functional and structural reliability comprising motility, count, morphology, and viability, thus making it one of the key medical causes of male factor infertility (17).

Therefore, the use of antioxidants to reduce reactive oxygen species overproduction is a possible option to recover semen quality. Antioxidant therapy to reduce seminal OS levels may improve natural conception and the outcome of aided reproductive technologies. Antioxidants are the most vital protection against free radical-induced infertility (18). Antioxidants like folate, vitamin C and E, zinc, carnitine, carotenoids, and selenium are the scavengers of reactive oxygen species. Their utilisation has been considered a therapy to avert the adverse impact of high concentration on semen parameters (19).

Vitamin C (ascorbic acid) is a water-soluble reactive oxygen species scavenger with increased potency. It is found in concentrations ten-fold higher in seminal plasma as against serum, safeguarding human spermatozoa from endogenous oxidative impairment by neutralising superoxide, hydroxyl, and hydrogen peroxide radicals while avoiding sperm agglutination.

Vitamin E is also a key lipid-soluble antioxidant molecule within the cell membrane. It is believed to disturb lipid peroxidation and improve the activity of different antioxidants that scavenge free radicals produced during the univalent reduction of molecular oxygen and at the normal activity of oxidative enzymes. The outcome of in vitro experiments recommends that vitamin E may defend spermatozoa from oxidative damage, motility losses and augment the performance of sperm in the hamster egg penetration assay. Current randomised control trials have recounted vitamin E to be an effective therapy for infertile males with high- reactive oxygen species levels (20).

Carnitine is a water-soluble antioxidant generally derived from the diet of human might be responsible for sperm energy metabolism and offer the primary fuel for sperm motility. Carnitines increase the cellular energetics in mitochondria by aiding the entry and use of free fatty acids inside the mitochondria and also repair the phospholipid composition of mitochondrial membranes by reducing fatty acid oxidation.

Finally, Selenium as scavengers of reactive oxygen species may safeguard against oxidative sperm DNA damage and is needed for normal testicular development, spermatogenesis, motility and function. The specific means by which Se removes OS is not much established. Selenoenzymes, like the sperm capsular selenoprotein glutathione peroxidase and phospholipid hydroperoxide glutathione peroxidase (PHGPX), can facilitate its effects. A significant connection has been detected between sperm concentration and seminal plasma Se in patients with infertility. The efficacy of combined treatment with Se and vitamin E has been investigated as Vitamin E functions synergistically with Se as an antiper-oxidant (18). Several clinical trials have looked at the potential of antioxidant supplementation to treat male factor infertility (21, 22). The review aims to collate evidence for the effectiveness of combined oral antioxidant supplementation, including head-to-head comparison in the treatment of male subfertility, and to investigate whether other intervention features

including duration, specific combinations, or dosage affect clinical outcomes in this population.

Materials and methods

Search Strategy

The study's search strategy was carried out in accordance with modified Preferred Reporting Items for Systematic Review and Meta-Analysis (22). The literature search was carried out from PubMed, Embase, CINAHL, PSYCHINFO, MEDLINE, Cochrane Central Register of Controlled Trials (CENTRAL), and unpublished data and references list to find previous studies that have investigated the use of antioxidant therapy in male subfertility.

Inclusion criteria

In our search, accepted studies were to include the following characteristics: (a) antioxidant therapy analysed for patients after oral antioxidant; (b) Comparison of treatment of male subfertility c) available full text; d) parameters (sperm concentration, sperm motility and progressive sperm motility), and live birth rate; e) We included the most currently published articles only if studies show identical experiments; however, we included the individual study if different indicators were used.

Quality Assessment of Studies

We adopted the Jadad scale in evaluating the quality of studies (23). We also assessed the quality of studies with a focus on methods such as concealment of the allocation process, blinding method, and the allocating sequence generation. The quality of the individual study was categorized into threefold: Degree 1 quality (every quality standard is satisfied), Degree 2 quality (some ambiguous quality standard) and Degree 3 quality (satisfy few quality standards and there is some ambiguity).

Data extraction

The following were recorded from the Studies: (a) abbreviations associated with the authors (first names, last names and publication year; (b) the size of the sample (c) Method of Intervention; (d) Criteria for Inclusion (e) Sperm motility and combined antioxidants.

Meta-analysis

The study used RevMan version 5.4.1 Meta-analysis software to analyze the differences between the variables. The study summarized changes in sperm parameters focusing on sperm motility, and pregnancy rate to examine the efficacy of combined antioxidant therapy. Metadata was presented as relative risks for dichotomous outcomes and as standardized mean differences (or mean differences) for continuous outcomes. Dichotomous data were evaluated using the Peto odds ratio with a 95% confidence interval. Moreover, the study employed random effects, as more than 50% of the participants were more than 12 for each of the studies.

Results and discussion

Selection of studies

Review authors RS and RM-P did an initial screen of titles and abstracts retrieved by the search. The search was conducted by MGS and RS. We retrieved the full texts of all potentially eligible studies. Two review authors (RS and RM-P) independently examined these full-text articles for compliance with the inclusion criteria and selected eligible studies. We corresponded with study investigators as required, to clarify study eligibility. Disagreements were resolved by discussion. All included studies were randomised. Only the first phase data were used in the meta-analysis as all studies reported first and second phase data separately. The search for the study detected 73 studies and excluded 54 studies after a thorough review of different titles and abstracts. With the remaining 19 studies, 13 studies were excluded due to the absence of relevant data. Finally, we found six articles (24, 25, 26, 27) comparing antioxidant therapy against placebo. The summarised characteristics of the included six studies are presented in Tables 1 and 2 below.

Included studies

Three studies that involve 235 patients (that is 119 subfertile males (experimental group) and 116 men in the placebo group) contained meaningful data on sperm outcome after three months. The study made use of a random effect model to establish the changes occurring between the two groups, which disclosed a Mean Difference of 2.44 at a 95% confidence interval [-2.40-7.28, $p=0.34 > 0.05$; $I2=66\%$]. This explains

that no significant improvement was discovered for the experimental group (subfertile male) regarding sperm mobility (Figure 1).

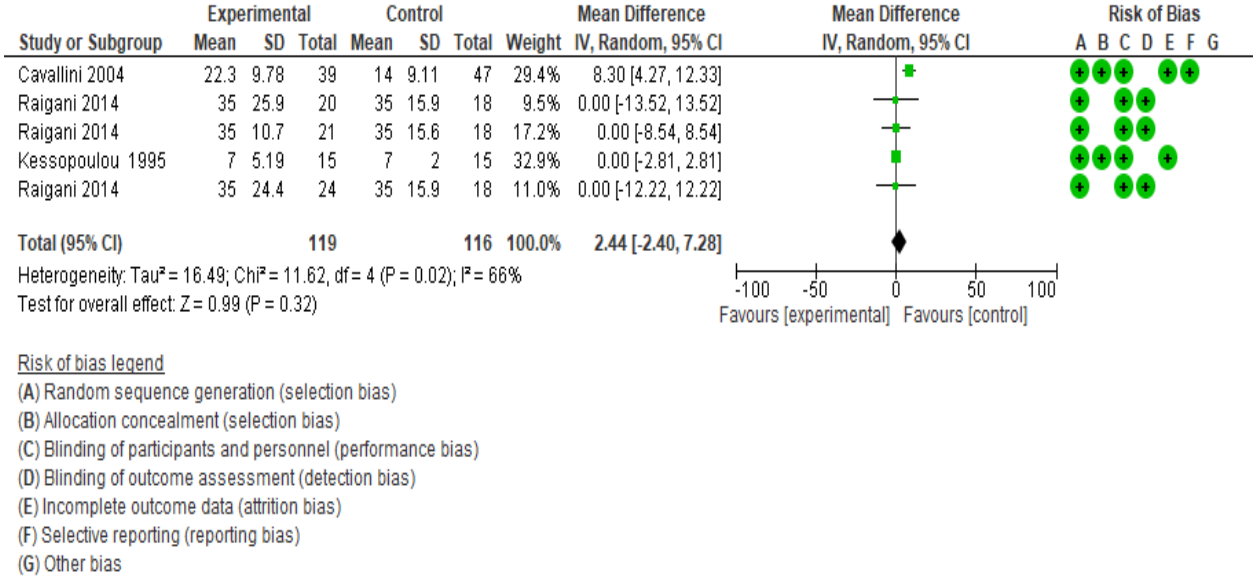

Figure 1. Analysis (meta-analysis). Total sperm motility at 3 months after Antioxidant Therapy. Mean Difference, Forest Plot and Risk of Bias

Table 1. Risk of Bias Table (Cochrane Risk tool)

Author	Selection of Bias		Performance Bias	Detection Bias	Attrition Bias	Reporting Bias	Other bias
	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)	Other bias
Cavallini 2004	Low risk	Low risk	Low risk	Unclear risk	Low risk	Low risk	Unclear risk
Raigani 2014	Low risk	Unclear risk	Low risk	Low risk	Unclear risk	Unclear risk	Unclear risk
Kessopoulou 1995	Low risk	Low risk	Low risk	Unclear risk	Low risk	Low risk	Unclear risk
Galatioto 2008	Low risk	Unclear risk	Low risk	Unclear risk	Low risk	Unclear risk	Unclear risk
Wong 2002	Low risk	Low risk	Unclear risk	Unclear risk	Low risk	Low risk	Unclear risk
Micic 2017	Low risk	Low risk	Unclear risk	Unclear risk	Unclear risk	Low risk	Unclear risk

This is because the individual assessment of the three studies shows there was no improvement for subfertile men in the two studies (25, 26). For instance, (25) the first study showed that using Folic acid as a treatment showed no mean difference (0.00 at 95% confidence interval [-13.52-13.52]. A similar no different outcome was discovered for Folic acid + Zinc on one hand and Vitamin E on the other hand. Nevertheless, L-carnitine + Acetyl-carnitine appeared to be efficient in improving sperm out in sub-fertile patients (mean difference of 8.30 at 95% confidence interval [4.27-12.33]. However, the combination of L-carnitine + Acetyl-carnitine with the other antioxidants (Folic acid, Folic acid + Zinc and Vitamin E proved not to be effective as the overall effect of the study were not significant.

Two studies that involve 230 patients (that is 108 subfertile males (experimental group) and 122 males in the placebo group) showed relevant data on sperm outcome after six months. The study likewise adopted the random effect model to establish the changes occurring between the two groups, which showed a

mean difference of 10.14 at 95% confidence interval [6.60-13.69, overall effect p=0.02< 0.05; I2=66%]. This demonstrates significant improvement for the experimental group (subfertile male) regarding sperm mobility (Figure 2). This means that the combination of L-carnitine + Acetyl-carnitine, Folic acid, Zinc, and Zinc + Folic acid is effective in improving sperm outcomes of male patients with subfertility problems. Furthermore, the individual study assessment of the two studies revealed that there will be a significant improvement in sperm outcome for subfertile men in case the antioxidants are taken individually without combining treatment (28). Other antioxidants (Folic acid, Zinc, and Zinc + Folic acid) are likely to bring improvement in the sperm outcome of sub-fertile patients. The outcome on L-carnitine + Acetyl-carnitine showed a mean difference of 10.40 at a 95% confidence interval [6.77-14.03], followed by the others which score a mean difference of 5 separately. This explains that when subfertile patients are given the oral treatment of L-carnitine + Acetyl-carnitine.

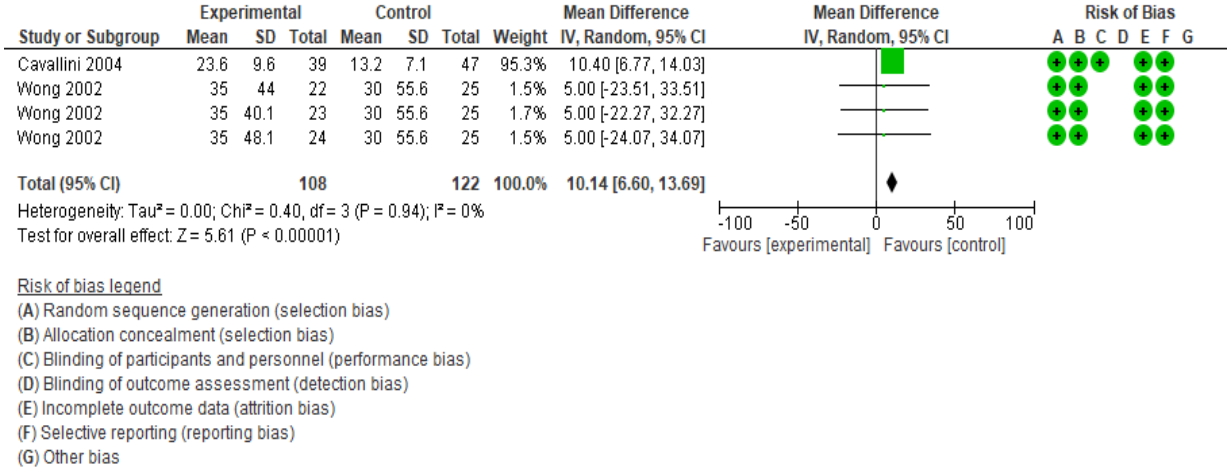

Figure 2. Analysis (meta-analysis). Total sperm motility at 6 months after Antioxidant Therapy. Mean Difference, Forest Plot and Risk of Bias

Antioxidants were established to be efficient to improve the quality of spermatozoa (29). Antioxidants such as vitamins C and E, melatonin, glutathione, and pantothenic acid improve sperm outcomes in subfertile men (30). The results of our review are in agreement with those of other published systematic reviews. Two other reviews described the effects of L-carnitine and L-acetylcarnitine on subfertile men. The systematic review and meta-analysis by (31) compared L-carnitine and L-acetylcarnitine therapy versus placebo treatment and found improvements in pregnancy rate and total sperm motility. Our review was unable to pool the results of the carnitine studies due to inconsistencies between the studies. The descriptive review by (32) discusses the improvement in pregnancy rates with oral intake of antioxidants, but (32) states that randomised controlled trials (RCTs) have not shown any effect on sperm motility and that there is a necessity for more RCTs in men with oxidative stress. Furthermore, (30) discusses in a review the effect of antioxidants in men with varicocele. They concluded that antioxidant therapy is a potential option as a primary treatment or adjunct after surgical repair of varicocele.

Another review (20) 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 more recent systematic review with meta-analysis studied the effectiveness of folate and folate plus zinc on sperm parameters in subfertile men (33). They concluded that folate alone was only effective on sperm concentration, and folate plus zinc only on sperm concentration and morphology. Both interventions did not have any effect on sperm motility. The effect of zinc plus folate or folate alone was confirmed in our review.

Conclusions

The evidence as discovered was low quality regarding the studies indicating that antioxidant supplementation in subfertile males will facilitate sperm quality and hence produce more live birth rates for couples visiting fertility clinics. It also demonstrates that clinical pregnancy rates will also surge. The main concentration of our meta-analysis was on the efficacy of antioxidant therapy in Sub fertile males, and the results established that antioxidant therapies are more effective after six months; More specifically, if subfertile men combine oral therapies such as L-carnitine + Acetyl-carnitine, Folic acid, Zinc, and Zinc + Folic acid. The results of our study support the administration of antioxidants as an ideal therapy for male subfertility. The subfertile male should be advised in this regard, but generally, the current evidence is inconclusive because of the failure of Sub fertile males to report on the clinical outcomes, poor reporting strategy on randomisation, and the small rate of sample size normally used for studies. This means more is required regarding the design of enhanced reporting on pregnancies and births associated with randomised trials (placebo-controlled) needed to clarify the precise role of antioxidants.

To conclude, our study has much significance when compared with other prior studies (29, 34-37). The study included studies in expanded and recent years and had detailed data. Moreover, the study was grounded on changes in sperm outcomes, enabling accurate inference on the efficacy of combined antioxidants. The RCT included in the study was also of high quality, which boosted the reliability of the study outcome.

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Conflicts of interest

The authors declare no conflict of interest, financial or otherwise.

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Table 2. Characteristics of the Study

Authors	Sample Size		Therapy	Duration of Therapy	Method of Administration	Randomization/inclusion of population	Outcome
	Experiment	Placebo					
Cavallini 2004	39	47	L-carnitine + Acetyl-carnitine	6months	Oral Capsule	Multiple arms, placebo Idiopathic OAT men with varicocele	More effective in improving sperm parameters and pregnancy than those of carnitines alone
Raigani 2014	65	18	Folic acid and zinc sulphate vs placebo	Four Months	Oral Capsule	Subfertile men were selected from infertile couples referred to Avicenna infertility clinic due to prove male factor infertility.	Antioxidant therapy did not ameliorate sperm quality in infertile men
Kessopoulou 1995	15	15	Vitamin E vs placebo	Three Months	Oral Capsule	Male infertility	No differences in sperm outcomes between the groups
Galatioto 2008	20	22	N-acetylcysteine (NAC) 600 mg + vitamins-minerals	Three Months	Oral Capsule	Men with persistent oligospermia after embolisation of varicocele	NAC does not improve the pregnancy rate
Wong 2002	103	108	Zinc sulphate and folic acid	Six months	Oral Capsule		Sperm count improved for Subfertile men
Micic 2017	125	50	Proxeed Plus	Six months	Oral Capsule	Men with OAT	Improved progressive sperm motility in infertile men

NAC: N-acetylcysteine; OAT: oligoasthenoteratozoospermi