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The effect of nutrients and dietary supplements on sperm quality parameters: a systematic review and meta-analysis of randomized clinical trials

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Abstract:	Infertility, which affects roughly 15% of the world's population, is a global public health issue recognized by the World Health Organization. Therefore, it is of major clinical and public health importance to investigate whether modifiable lifestyle factors -such as stress, drug use, smoking, alcohol intake and diet- may influence human fertility. A systematic review and meta-analysis of randomized clinical trials (RCTs), using the MEDLINE-Pubmed database, were conducted to assess the effect of nutrients, dietary supplements or food on sperm quality parameters. In total, 29 articles were included for qualitative analysis and 15 for quantitative meta-analysis. Total sperm concentrations [expressed as mean differences (MD), with 95% confidence intervals (CI)] were increased by selenium (3.91x106 spz/ml [3.08, 4.73]), zinc (1.48x106 spz/ml [0.69, 2.27]), omega-3 fatty acids (10.98x106 spz/ml [10.25, 11.72]), and coenzyme Q10 (CoQ10) (5.93x106 spz/ml [5.36, 6.51]). Sperm counts were increased by omega-3 fatty acids (18.70x106 spz [16.89, 20.51]) and CoQ10 supplementation (10.15x106 spz [8.34, 11.97]). Sperm total motility was increased by selenium (3.30% [2.95, 3.65]), zinc (7.03% [6.03, 8.03]), omega-3 fatty acids (7.55% [7.09, 8.01]), CoQ10 (5.30% [4.98, 5.62]), and carnitines (7.84% [6.54, 9.13]), whereas sperm progressive motility was increased only after supplementation with carnitines (7.45% [6.24, 8.67]). Finally, sperm morphology was enhanced by selenium (1.87% [1.50, 2.24]), omega-3 fatty acids (0.91% [0.69, 1.13]), CoQ10 (1.06% [0.72, 1.41]), and carnitines (4.91% [3.68, 6.15]) supplementation. This meta-analysis of RCTs suggests that some dietary supplements could beneficially modulate sperm quality parameters and affect male fertility. However, results must be cautiously interpreted due to the limited sample size the meta-analyzed studies and the considerable observed inter-study heterogeneity. Registration number: PROSPERO-2017: CRD42017058380.

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TITLE

The effect of nutrients and dietary supplements on sperm quality parameters: a systematic review and meta-analysis of randomized clinical trials

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Abbreviations: CI, confidence interval; CoQ10, coenzyme Q10; DHA, docosahexaenoic acid; EPA, eicosapentanoic acid; FSH, follicle-stimulating hormone; HOS, hypo-osmotic swelling; LAC, L-acetyl carnitine; LC, L-carnitine; LH, luteinizing hormone; MD, mean differences; MeSH, Medical Subject Headings; OS, oxidative stress; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; RCT, randomized clinical trials; ROB, Risk of Bias; ROS, reactive oxygen species; SCI, sperm chromatin integrity; SD, standard deviation; SDF, sperm DNA fragmentation; SE, standard error; T, testosterone.

ABSTRACT

Infertility, which affects roughly 15% of the world's population, is a global public health issue recognized by the World Health Organization. Therefore, it is of major clinical and public health importance to investigate whether modifiable lifestyle factors -such as stress, drug use, smoking, alcohol intake and diet- may influence human fertility. A systematic review and meta-analysis of randomized clinical trials (RCTs), using the MEDLINE-Pubmed database, were conducted to assess the effect of nutrients, dietary supplements or food on sperm quality parameters. In total, 29 articles were included for qualitative analysis and 15 for quantitative meta-analysis. Total sperm concentrations [expressed as mean differences (MD), with 95% confidence intervals (CI)] were increased by selenium ($3.91x10^6$ spz/ml [3.08, 4.73]), zinc ($1.48x10^6$ spz/ml [0.69, 2.27]), omega-3 fatty acids ($10.98x10^6$ spz/ml [10.25, 11.72]), and coenzyme Q10 (CoQ10) ($5.93x10^6$ spz/ml [5.36, 6.51]). Sperm counts were increased by omega-3 fatty acids ($18.70x10^6$ spz [16.89, 20.51]) and CoQ10 supplementation ($10.15x10^6$ spz [8.34, 11.97]). Sperm total motility was increased by selenium (3.30% [2.95, 3.65]), zinc (7.03% [6.03, 8.03]), omega-3 fatty acids (7.55% [7.09, 8.01]), CoQ10 (5.30% [4.98, 5.62]), and carnitines (7.84% [6.54, 9.13]), whereas sperm progressive motility was increased only after supplementation with carnitines (7.45% [6.24, 8.67]). Finally, sperm morphology was enhanced by selenium (1.87% [1.50, 2.24]), omega-3 fatty acids (0.91% [0.69, 1.13]), CoQ10 (1.06% [0.72, 1.41]), and carnitines (4.91% [3.68, 6.15]) supplementation. This meta-analysis of RCTs suggests that some dietary supplements could beneficially modulate sperm quality parameters and affect male fertility. However, results must be cautiously interpreted due to the limited sample size the meta-analyzed studies and the considerable observed inter-study heterogeneity.

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KEYWORDS: Diet, nutrition, nutrients, food, sperm quality, male infertility, RCT

INTRODUCTION

Infertility, which affects roughly 15% of the world's population, is a global public health issue recognized by the World Health Organization (1). In the case of male fertility, a recent meta-regression analysis reported a significant worldwide decline in total sperm counts between 1973 and 2011 (2). These data strongly suggest a significant decline in male reproductive health, with crucial implications for human reproduction and perpetuation of the species. Research aimed at revealing the causes and implications of this decline is therefore urgently needed.

Investigating modifiable lifestyle factors that influence human fertility -such as stress, drug use, smoking, alcohol intake and diet- is of major clinical and public health importance for understanding the problem. Indeed, several observational studies that explored the associations between dietary patterns, food and nutrient consumption and sperm quality suggest that adhering to a healthy diet (e.g. the Mediterranean diet) may improve male sperm quality parameters (3). In addition to observational studies, which are important for creating new hypotheses, randomized clinical trials (RCTs) are also needed. Such trials are considered the gold standard in terms of scientific evidence if the quality of the design the interventions and the execution of the trial is high, as they enable strong conclusions to be drawn and can be used for future clinical and public health recommendations. Several RCTs have tested the effect of food and nutrients on male fertility parameters. Differences in supplements tested, study duration and design, as well as the different interventions, populations and measured outcomes make it extremely difficult to compare these trials.

One systematic review of clinical trials recently attempted to summarize knowledge in this field (4). Unfortunately, the authors of the review merged observational studies and RCTs, did not take into account certain relevant papers, and included others that were of low quality or that contained a high risk of bias, which made it difficult to draw strong conclusions.

The aims of the present systematic review of RCTs that have tested the effect of nutrients, dietary supplements or food on sperm quality parameters were: a) to update scientific evidence on the topic by assessing the risk of bias in all the articles selected, and b) to meta-analyze the effect of similar interventions on selected endpoints.

METHODS

Protocol and registration

The present study and the corresponding search protocol have been registered in the PROSPERO registry (http://www.crd.york.ac.uk/PROSPERO) as PROSPERO 2017: CRD42017058380.

Literature search strategy

A systematic, comprehensive search of the literature published between the earliest available online indexing year and October 2017 by searching the MEDLINE-Pubmed database (http://www.ncbi.nlm.nih.gov/pubmed) and hand-searching the references lists of the retrieved papers was carried out in accordance with the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (5,6).

Using Medical Subject Headings (MeSH) and keywords, two search subsets were used: a) one subset comprised male infertility-related keywords (fertility OR infertility OR male infertility OR male fertility OR sperm dysfunction(s) OR sperm DNA damage OR varicocele OR asthenozoospermia OR oligozoospermia OR oligoasthenozoospermia OR oligoasthenoteratozoospermia OR teratozoospermia); and, b) the second subset comprised nutrition and/or diet-related keywords (diet OR nutrients OR food OR food supplement OR dietary supplement OR probiotic OR nuts OR vitamin C OR vitamin E OR zinc OR antioxidants OR cereals OR meat OR vegetable OR fruit OR fishes OR legumes OR milk OR yogurt OR cheese OR seeds OR eggs OR dairy product(s) OR micronutrient(s) OR vitamins OR alcohol consumption OR l-carnitine OR n-acetylcysteine OR glutathione OR coenzyme q10 OR selenium OR fatty acids OR sugar). The following inclusion filters were applied in the search: Classical Article, Clinical Conference, Clinical Study, Clinical Trial, Clinical Trial-Phase I, Clinical Trial-Phase II, Clinical Trial-Phase III, Clinical Trial-Phase IV, Controlled Clinical Trial, English Abstract, Journal Article, Letter, Meta-Analysis, Multicenter Study, Pragmatic Clinical Trial,

Evaluation Studies, Case Reports, Congresses, Dataset, Introductory Journal Article, Abstract, Humans, English, and Male. The complete search strategy is available in Supplementary data (Supplemental Appendix).

Eligibility criteria and study selection

The titles and abstracts of all the pre-selected articles were screened for eligibility by two independent researchers (A.S-H. and N.R-E.), who are specialists in male (in)fertility and human nutrition, respectively. Any discrepancies were re-evaluated together with a third author (J.S-S.). After primary screening (to evaluate the scope of the study), the full texts of the selected articles were obtained. Only RCTs studies in which fertile/infertile men were well-defined (men with or without sperm disorders, sperm DNA damage or idiopathic infertility) were included for the qualitative analysis. The primary outcomes of the selected studies had to have referred to the following semen quality parameters: semen volume, ejaculate pH, total sperm count or concentration, sperm vitality, sperm motility (progressive or total motility), sperm morphology, acrosome resistance, sperm DNA fragmentation (SDF) or damage, sperm chromatin integrity (SCI), sperm reactive oxygen species (ROS), sperm aneuploidies, sperm function parameters, or hormonal levels. Exclusion criteria: case-control, cross-sectional, observational prospective or retrospective studies, animal or in vitro studies, review articles, studies conducted on individuals with varicocele or other fertility-related diseases, studies with drug interventions, studies with 15 or fewer participants per intervention, uncontrolled intervention studies, and studies with a high risk of bias (see the risk of bias section). Finally, RCTs testing the effect of food extracts, botanic extracts or drugs have also been excluded from the present review.

Data extraction

Using a standardized model, the following information from each study was extracted: author(s), year of publication, journal, title of the article, location of the study, age, population studied, sample size, study design (parallel or crossover), intervention(s),

primary outcomes, and main conclusions. Data were first extracted and further checked by the researchers for discrepancies in order to minimize the possibility of errors.

Risk of bias

Using all the data extracted, the quality of the studies selected were evaluated through a Risk of Bias (ROB) index based on seven categories (7). ROB was assessed in parallel by two authors (A.S-H. and N.R-E.) and discrepancies were re-evaluated together with a third author (J.S-S.). Applying this system, the ROB of individual studies using the following criteria were assessed: 1) random sequence generation (due to inadequate generation of randomized sequence); 2) allocation concealment (due to inadequate concealment of allocations prior to assignment); 3) blinding of participants and personnel (due to knowledge of the allocated interventions by participants and personnel during the study); 4) blinding of outcome assessment (due to knowledge of the allocated interventions by outcome assessors); 5) incomplete outcome data (due to the amount, nature or handling of incomplete outcome data); 6) selective reporting (due to selective outcome reporting); and 7) other bias (due to problems not covered elsewhere). Studies whose mean ROB were high were considered to be of low quality and therefore excluded, while those whose mean ROB were low or unclear were accepted for the systematic qualitative review and quantitative analysis.

Statistical analysis

Meta-analysis was conducted using Review Manager (RevMan) software v.5.3 (http://community.cochrane.org/tools/review-production-tools/revman-5) in accordance with the COCHRANE guidelines (7). The difference in the change from the baseline values for the intervention and placebo/control arms was derived from each trial. However, if the change from the baseline values was not available, end-of-treatment values were used. When necessary, an imputed standard deviation (SD) or standard error (SE) for the between-treatment difference was calculated. In crossover trials, to impute SD for between-treatment differences, correlation coefficients between baseline

and end-of-treatment values within each trial was derived using a published equation (8). When multiple intervention arms were present in a single trial, intervention arms were pooled to obtain a single pairwise comparison, to mitigate unit-of-analysis error. To evaluate the differences in sperm quality parameters between the intervention and the control groups, the data were pooled using the inverse variance method with the fixed effects model and the results were expressed as mean differences (MD) with 95% confidence intervals (CI). Statistical significance was set at *P*-value<0.05. Heterogeneity between the studies was evaluated using a chi-square test and the I^2 index with a significance level set at *P*-value<0.10. I^2 values <50 were deemed moderate, ≥50 to <75 were deemed substantial and ≥75% were deemed of considerable heterogeneity (7).

RESULTS

Study characteristics

A total of 2,381 articles were identified after a primary search of MEDLINE-Pubmed and one study from other sources (Supplemental Figure 1). After analyzing every abstract (n=2,382), 2,240 articles were excluded because they were beyond the scope of the present study (does not assess the effect of nutrients, supplements or food on sperm quality parameters). A total of 142 articles were collected as full texts and their inclusion/exclusion criteria and ROB were assessed: of these articles, 110 were excluded because they did not meet the inclusion/exclusion criteria (no control group, n=34; small sample size, n=26; non-RCT, n=22; did not meet primary outcome, n=5; studies with animals or non-male subjects, n=3; *in-vitro* studies, n=2; participants with varicocele, n=5; other fertility-related diseases, n=8; using intervention with drugs, n=3; two interventions at the same time, n=1; and using a food extract n=1), and 4 were excluded because they were classified as having a high ROB. After applying all the eligibility parameters, 28 articles were included for qualitative analysis. When two or more studies had analyzed the same exposures and outcomes results were meta-analyzed. Therefore, 15 were quantitatively analyzed using a meta-analysis approach.

The articles included subjects (n=2,900) from 11 countries: Australia, England, Germany, Iran, Italy, Kuwait, Saudi Arabia, Scotland, Spain, The Netherlands, and The USA. The age of the participants ranged from 18 to 52 years old. There were 26 parallel-group RCTs and two crossover RCTs.

Qualitative analysis

Eight of the twenty-nine articles assessed antioxidant supplements (9–16). Four articles evaluated folic acid and/or zinc (17–20), two articles evaluated omega-3 fatty acid supplements (21,22), five articles assessed Coenzyme Q10 (CoQ10) supplements (23–27), three assessed carnitines (28–30), and six assessed some other dietary

supplements (31–36). All the studies evaluated had sperm parameters and quality as study outcomes **(Table 1)**.

Antioxidant supplements

The vast majority of the RCTs were conducted using antioxidants or cocktails of antioxidants. The studies included in the qualitative analysis are shown in Table 1 (9–16).

Selenium supplements were tested in three studies (9,10,12). While Hawkes and collaborators (9) reported that supplementation with 300 µg/day of selenium had no effect on conventional sperm parameters or serum hormones, Scott and collaborators (1998) (12) reported that 100 µg/day of selenium for 3 months improved sperm motility and increased the chance of conception, and Safarinejad (2009) (10) reported that 200 µg/day of selenium for 6 months improved semen volume, total sperm count and concentration, and morphology. In the study by Scott *et al.*, (12) adding vitamin C and vitamin E to the supplement had no synergic effect on these semen parameters. In the study by Safarinejad and collaborators (10), adding N-acetyl cysteine to the selenium supplement improved these parameters but also affected certain sex hormones, increasing testosterone (T), luteinizing hormone (LH) and inhibin B, and decreasing follicle-stimulating hormone (FSH).

No effect on conventional semen parameters and sex hormones has been demonstrated using vitamin E or vitamin C + E supplementation (11,13,15) except in one RCT (14), in which the administration of 300 mg of vitamin E significantly improved sperm motility after 6 months. However, an improvement in the fecundity capacity was reported using the zona binding test after 3 months of vitamin E supplementation (13), in sperm DNA fragmentation indexes after 2 months of vitamin C + E supplementation (15) and in pregnancy rates after 3 months of vitamin E supplementation (14).

Finally, supplementation for 12 weeks with 600 mg of alpha-lipolic acid improved total sperm count, concentration and motility (progressive motility) but had no effect on semen volume, sperm vitality or morphology (16).

Folic acid and zinc

Four studies investigated the effects of folic acid and/or zinc supplements on different semen variables (Table 1). Although the main conclusions are controversial, some of the results are worth emphasizing. While the intake of folic acid + zinc sulfate led to improvements in sperm concentration (17,18) and morphology (18), isolated folic acid or zinc supplementation improved other sperm-related parameters. Specifically, improvements in sperm chromatin integrity (SCI) indexes (19) or sperm concentration, sperm motility, sperm integrity membrane through the hypo-osmotic swelling (HOS) test, fertilizing capacity, conception and pregnancy (20) were reported after supplementation with zinc sulfate in infertile patients with idiopathic oligoasthenoteratozoospermia and asthenozoospermia, respectively. Also, an improvement in sperm morphology was demonstrated after supplementation with folic acid (5 mg/d) in subfertile healthy patients (18).

Omega-3 fatty acids

Two parallel-group RCTs (Table 1) evaluated the effect of omega-3 fatty acid supplementation on sperm parameters (21,22).

Supplementation with docosahexaenoic acid (DHA) + Eicosapentanoic (EPA) (990 mg/d and 135 mg/d, respectively) for 10 weeks demonstrated no effect on sperm parameters but improved SDF (21). Although supplementation with higher amounts of DHA + EPA (0.72 g/d and 1.12 g/d, respectively) led to significant improvements in total sperm count and concentration, sperm motility and morphology, it had no effect on semen volume or serum sex hormone concentrations (22).

Coenzyme Q10

In terms of intervention (3–6 months in length and 200–300mg/d of supplementation), the most homogeneous group of studies are those that used coenzyme Q10 (CoQ10) (see Table 1) (23–27)**.** The two articles by Nadjarzadeh *et al.* (2011 and 2014) (23,25) are considered as one study.

Studies testing the effect of supplementation with CoQ10 for a moderate-to-short-term intervention period (≤3 months) reported no effect on conventional sperm parameters (23,25). On the other hand, RCTs that explored the effects after 6 months of intervention reported improvements in classical sperm parameters such as sperm motility (26), total sperm count and concentration (24), and morphology (27). The two studies by Safarinejad *et al.* (24,27) also described a peripheral increase in the inhibin-B hormone and a reduction in LH and FSH after CoQ10 supplementation. A reduction in acrosome-reacted spermatozoa in the ejaculated (an important parameter in the fecundation process) was also observed by Safarinejad in 2009 (27).

Carnitines

Three RCTs studies with carnitines are summarized in Table 1 (28–30).

The administration of all types of isolated carnitines, such as L-acetyl carnitine (LAC), L-carnitine (LC), or complexes of both carnitines (LAC and LC), has been shown to increase sperm motility (28–30). Supplementation with between 2 g/d and 3 g/d of LC improved sperm concentration (30) and morphology (28). Finally, in the aforementioned study (28), 1 g/d of LAC also improved sperm concentration, but no effect of carnitine intake on semen volume was reported in any of these studies.

Other dietary supplements

Table 1 summarizes the effects of several dietary supplements on sperm parameters (31–36).

In idiopathic oligoasthenotratozoospermic patients, an improvement in semen volume, total sperm count, concentration, progressive motility and morphology, and levels of

FSH, LH, and T were reported after 6 months' supplementation with one Flortec® capsule per day (*Lactobacillus paracasei* B21060 5x10^9 CFUs/d + arabinogalactan 1,243 mg/d + oligo-fructosaccharides 700 mg/d + L-glutamine 500 mg/d) (31).

After 3 months' supplementation with 4 g/d of Myoinositol, total sperm count and concentration, progressive motility, and T levels increased, and acrosome-reacted spermatozoa, LH and FSH levels decreased (32).

Supplementation with Menevit®, a complex enriched with many antioxidants (lycopene 6mg/d, vitamin E 400 IU/d, vitamin C 100 mg/d, zinc 25 mg/d, selenium 26 µg/d, folate 0.5 mg/d, garlic 1 g/d, and palm oil) had no effect on any conventional parameter (33).

Nigella sativa was tested (34). After the intervention with this herb, an improvement in several seminogram parameters in the ejaculate (including semen volume and pH, sperm concentration, motility, sperm morphology, and semen round cells) were reported.

One RCT that used saffron (*Crocus sativus*), an ancestral herbal remedy traditionally thought to improve semen parameters, as a supplement showed that consuming 60 mg/d of saffron for 26 weeks had no effect on conventional sperm parameters or serum hormones (36).

To the best of our knowledge, the only food that has been tested in a RCT were walnuts (35). This study showed that consuming 75 g/d of raw walnuts in the context of a western-style diet improved sperm motility, sperm vitality and morphology in healthy individuals but that supplementation with walnuts had no effect on semen volume, sperm concentration or aneuploidy index.

Quantitative analysis

The relatively high number of RCTs that have used selenium, zinc, folic acid, omega-3, CoQ10 and carnitines as supplements and the homogeneity between them led us to conduct a meta-analysis to test the effect of these supplements on various sperm outcomes.

Selenium

Data from three studies have been meta-analyzed. Supplementation of 100–300 µg/d of selenium for between 3 and 11 months improved (MD [95% CI]) sperm concentration ($3.91x10^6$ spz/ml [3.08 to 4.73], *P*-value<0.001), total motility (3.30% [2.95 to 3.65], *P*-value<0.001) and morphology (1.87% [1.50 to 2.24], *P*-value<0.001) **(Figure 1)**. Inter-study heterogeneity was not significant (I^2≤20, *P*-value>0.1).

Zinc

Using data from three studies, the present study found that 66-500 mg/d of zinc supplementation for 3 to 6 months improved sperm concentration ($1.48x10^6$ spz/ml [0.69 to 2.27], *P*-value<0.001) and total motility (7.03% [6.03 to 8.03], *P*-value<0.001) **(Figure 2)**. Inter-study heterogeneity was not significant (I^2≤1, *P*-value>0.1).

Folic acid

Data from two studies with folic acid have been meta-analyzed (Supplemental Figure 2). The supplementation with 5 mg/d of folic acid for 3 to 6 months did not improve sperm concentration, total motility or morphology in fertile and subfertile participants (I^2=0, *P*-value>0.1).

Omega-3 fatty acids

Administration of a supplement containing 1 g/d DHA and 1 g/d EPA for 10 to 32 weeks improved total sperm count ($18.70x10^6$ spz [16.89 to 20.51], *P*-value<0.001), sperm concentration ($10.98x10^6$ spz/ml [10.25 to 11.72], *P*-value<0.001), total motility (7.55% [7.09 to 8.01], *P*-value<0.001), and morphology (0.91% [0.69 to 1.13], *P*-value<0.001) **(Figure 3)**. There was evidence of considerable and significant heterogeneity between the two meta-analyzed studies (I^2>90, *P*-value<0.001).

Coenzyme Q10

Using data from four RCTs the present study found that supplementation with 200-300 mg/d CoQ10 for 3 to 6 months improved total sperm count ($10.15x10^6$ spz [8.34 to 11.97],

P-value<0.001), sperm concentration (5.93x10^6 spz/ml [5.36 to 6.51], *P*-value<0.001), sperm total motility (5.30% [4.98 to 5.62], *P*-value<0.001) and morphology (1.06% [0.72 to 1.41], *P*-value<0.001) **(Figure 4)**. While the effect on sperm progressive motility had substantial inter-study heterogeneity (I^2=65%, *P*-value=0.09), there was considerable inter-study heterogeneity for other sperm parameters (I^2≥89%, *P*-value<0.001). The two articles by Nadjarzadeh *et al.* (2011 and 2014) (23,25) were computed as one study.

Carnitines

Data from three studies have been meta-analyzed. Supplementation with 3 g/d of LC and 1 g/d of LAC for 2 to 6 months significantly improved sperm progressive motility (4.80% [3.32 to 6.28], *P*-value<0.001), total motility (4.82% [3.30 to 6.34], *P*-value<0.001), and morphology (2.98% [1.10 to 4.87], *P*-value=0.002) **(Figure 5)**. Except for sperm concentration, where homogeneity was very high (I^2=9%, *P*-value=0.33), there was evidence of considerable and significant heterogeneity between the studies for the motility and morphology parameters (I^2≥90, *P*-value<0.001).

DISCUSSION

This systematic review of RCTs provides the most wide-ranging analysis to date for the effects of nutrients, supplements or foods on sperm quality parameters. The meta-analysis included in the review revealed a significant beneficial effect on total sperm count from supplementation with omega-3 and CoQ10, on sperm concentration from supplementation with selenium, zinc, omega-3 and CoQ10, on sperm motility from supplementation with selenium, zinc, omega-3, CoQ10 and carnitines, and on sperm morphology from supplementation with selenium, omega-3, CoQ10 and carnitines. The review suggests that some dietary supplements may help to modulate male fertility.

Different underlying mechanisms could explain these results and therefore deserve to be commented. Oxidative stress (OS) is identified as one of the main mediators of male infertility. It causes sperm dysfunctions and is related to increased cellular damage triggered by ROS. This occurs naturally in sperm cells because high levels of sperm motility, in the case of the hyperactivation required in zona-pellucida binding, induce ROS (37). However, high levels of ROS were also strongly correlated with sperm DNA damage and low percentages of sperm motility (38), among other sperm-related outcomes. The ROS-DNA-damage sperm motility pathway may also act in the opposite direction, i.e. DNA damage induces ROS through the H2AX-Nox1/Rac1 pathway (39). In this scenario, the equilibrium between anti-oxidants and ROS may be key for achieving better sperm quality (mainly in terms of sperm motility, vitality and DNA damage). This is why most of the RCTs in the literature tested antioxidant supplements in order to balance OS. Some supplements (vitamin E and zinc) proved beneficial for increasing the live birth rate in couples with male or unexplained subfertility and some (certain carnitine supplements) proved beneficial for increasing the pregnancy rate. Other supplements had no beneficial effects in this regard (40).

The main antioxidants tested as supplements with a positive effect on sperm quality parameters were selenium and zinc. On one hand, selenium is essential for the normal

spermatogenesis of mammals and plays a pivotal role in increasing glutathione peroxidase-1 expression and activity, which in turn destroys hydrogen peroxide molecules (41). On the other hand, zinc is also an antioxidant element with a membrane-stabilizing activity by inhibiting membrane-bound oxidative enzymes such as NADP oxidase (42). A recent meta-analysis showed that the zinc content in the seminal plasma of infertile males were significantly lower than those of normal males, which indicates that zinc supplementation may significantly increase the sperm quality of infertile males (43). The present meta-analysis of RCTs in humans using zinc and selenium as supplements reinforces this hypothesis. However, no consistent beneficial effects of other antioxidants, including folic acid, have been demonstrated.

Omega-3 PUFA are fatty acids with anti-inflammatory and antioxidant properties potentially modifying cell membrane composition and functionality. The mechanism by which omega-3 (and omega-6) PUFAs can affect spermatogenesis is their incorporation into the spermatozoa cell membrane. It has been demonstrated that the successful fertilization of spermatozoa depends on the lipid composition of the spermatozoa membrane (44). In line with this finding, the present RCT meta-analysis shows positive effects on sperm concentration after omega-3 PUFAs supplementation. However, other RCTs conducted in large samples of participants are needed in order to definitively endorse the beneficial effect of omega-3 supplementation on sperm motility and pregnancy indicators.

Coenzyme Q10 is also an antioxidant molecule with a central role in the electron-transport system. As Balercia *et al.* 2009 and Safarinefad 2009 (26,27) pointed out, CoQ10 inhibits organic peroxide formation in both seminal fluid and may therefore reduce sperm-cell OS. In the last two decades, interest in this molecule as a supplement for treating infertile men and fecundability has grown. In a meta-analysis conducted in 2013 by Lafuente *et al.* (46) and in the present review, an overall improvement was shown in sperm parameters but not in live birth or pregnancy rates (45). However, high

heterogeneity between the studies was reported, which indicates that more and larger studies are needed before supportive recommendations can be made.

The lack of clear effects of antioxidant supplements on sperm parameters in some of the studies included in our systematic review can be explained by the amount/dose of antioxidant used, as long-term treatments with larger amounts of phenolic or other antioxidant compounds have proven to have pro-oxidant effects. Besides, the low amount/dose of antioxidants used in these studies may have been unable to beneficially affect sperm parameters (46,47).

Majzoub and Agarwal (48) conducted a narrative review in relation to studies using antioxidant in idiopathic oligoasthenoteratozoospermia and concluded that additional randomized controlled studies are required to confirm the efficacy and safety of antioxidant supplementation in the medical treatment of idiopathic male infertility, as well as the dosage required to improve semen parameters, fertilization rates, and pregnancy outcomes in iOAT.

The present study shows that carnitine supplementation also has certain beneficial effects on spermatozoa motility and morphology, though there was also considerable heterogeneity between the three studies meta-analyzed. LC and LAC play important roles in sperm metabolism by providing immediate available energy for use by spermatozoa, which positively affects sperm motility, the spermatogenic process and maturation (49). In addition, carnitines are involved in the transportation of long-chain fatty acids into the mitochondrial matrix for beta-oxidation and exert anti-oxidant activity by increasing the expression of antioxidant enzymes (28). Finally, although studies with food extracts or botanic extracts may be of potential interest for fertility modulation, this type of supplements are out of the scope of the present review and meta-analysis, and therefore these RCTs have not been included.

Strengths and limitations

Certain limitations of the present study should be acknowledged. The search strategy was limited to the MEDLINE-Pubmed database or hand-searching and did not include other databases (e.g. EMBASE). Although our search strategy included a broad number of search terms, and the use of most relevant scientific database combined with hand-searched reference lists, it is possible (though also improbable) that not all relevant publications were identified. It is also important to point out that, because few studies were included in the meta-analysis (<10 articles meta-analyzed per group), we were unable to assess the across-studies risk of bias with a post-hoc analysis. Also, in the present meta-analysis considerable inter-study heterogeneity was observed for most outcomes but this could not be explored with subgroup analysis because of the few studies included. It is therefore difficult to draw strong conclusions or to make evidence-based recommendations. Unfortunately, in our analyses we didn't control the background diet, and/or any dietary changes occurred during the intervention in most of the included RCTs. Indeed, whether the changes observed in some fertility parameters can be explained by changes in the intervention diet remain to be elucidated, thus decreasing the level of scientific evidence derived from these studies. Furthermore, a potential source of bias could be derived from the fact that the studies included in this review did not report information about background medication, and/or changes in medication during the trial. However, this is probably irrelevant because most of the studies have been conducted in healthy young population, rarely using medication. The subfertile populations were heterogeneous and had different phenotypes (e.g. asthenozoospermic, oligoasthenozoospermic or oligoasthenoteratozoospermic participants, or patients with idiopathic infertility who attended infertility clinics, etc.). It is difficult, therefore, to generalize the results to other phenotype of populations. Another limitation is to judge the biological significance of the improvements observed in some sperm parameters because their effect on fertility need to be confirmed with other studies. Finally, in some studies it is not clearly explained the manufacture reliability of

the supplements used nor its bioavailability, making difficult the interpretation of the findings.

The main strengths of the present study include its multi-stage design with multi-author validation, the evaluation of ROB, and the possibility of replicating the systematic review and meta-analysis with the same system. Finally, the age range of the populations studied is quite low (18 to 52 years) and corresponds to the main male reproductive age.

CONCLUSIONS

This systematic review and meta-analysis of RCTs provides the most wide-ranging analysis to date of the effects of nutrients, supplements and food on sperm quality parameters. The present study concludes that diet supplementation with certain antioxidants, especially selenium and zinc, omega-3 fatty acids, CoQ10, carnitines and certain foods rich in these supplements can beneficially modulate sperm quality parameters and affect male fertility. The small number of studies that have tested similar supplements, the small sample sizes included in those studies, and the high degree of inter-study heterogeneity across outcomes mean that further research may lead to a change in the estimates of the effects outlined in this meta-analysis. More RCTs with larger samples and clear inclusion/exclusion criteria are needed in future to test how these types of supplements affect not only sperm parameters but also fecundability.

AUTHORS' ROLES

A.S-H.: Initiated the idea of this review, designed it, collected and selected the data, assessed the articles, performed the meta-analysis and wrote the manuscript. N.R-E.: Selected the data, assessed the articles and critically reviewed the article for important intellectual content. N.B-T.: Reviewed the meta-analysis process and critically reviewed the article for important intellectual content. B.V.: Critically reviewed the article for important intellectual content. M.B.: Critically reviewed the article for important intellectual content. J.S-S.: Initiated the idea of this review, designed it, assessed the articles, and helped to draft and critically review the article for important intellectual content. All authors approved the final manuscript.

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REFERENCES

1. World Health Organization. WHO laboratory manual for the examination and processing of human semen. Geneva: World Health Organization; 2010.
2. Levine H, Jørgensen N, Martino-Andrade A, Mendiola J, Weksler-Derri D, Mindlis I, Pinotti R, Swan SH. Temporal trends in sperm count: A systematic review and meta-regression analysis. Hum Reprod Update. 2017;23:646–59.
3. Salas-Huetos A, Bulló M, Salas-Salvadó J. Dietary patterns, foods and nutrients in male fertility parameters and fecundability: a systematic review of observational studies. Hum Reprod Update. 2017;23:371–89.
4. Giahi L, Mohammadmoradi S, Javidan A, Sadeghi MR. Nutritional modifications in male infertility: A systematic review covering 2 decades. Nutr Rev. 2016;74:118–30.
5. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Goetzsche PC, Ioannidis JP, Clarke M, Devereaux P, Kleijnen J, Moher D. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. J Clin Epidemiol. 2009;62:e1-34.
6. Moher D, Liberati A, Tetzlaff J, Altman DG, Altman D, Antes G, Atkins D, Barbour V, Barrowman N, Berlin JA, et al. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. PLoS Med. 2009;6:e1000097.
7. O'Connor D, Green S, Higgins JP. Cochrane Handbook for Systematic Reviews of Interventions. Version 5. Wiley-Blackwell; 2008. 674 p.
8. Elbourne DR, Altman DG, Higgins PT, Curtin F, Worthington V, Vail A. Meta-analyses involving cross-over trials: methodological issues. Int J Epidemiol. 2002;31:140–9.
9. Hawkes WC, Alkan Z, Wong K. Selenium supplementation does not affect testicular selenium status or semen quality in North American men. J Androl. 2009;30:525–33.

10. 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. J Urol. 2009;181:741–51.
11. 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. Hum Reprod. 1999;14:1028–33.
12. Scott R, MacPherson A, Yatest RWS, Hussain B, Dixon J, Yates R, Hussain B, Dixon J, Yatest RWS, Hussain B, et al. The effect of oral selenium supplementation on human sperm motility. Br J Urol. 1998;82:76–80.
13. Kessopoulou E, Powers HJ, Sharma KK, Pearson MJ, Russell JM, Cooke ID, Barratt CLRR. A double-blind randomized placebo cross-over controlled trial using the antioxidant vitamin E to treat reactive oxygen species associated male infertility. Fertil Steril. 1995;64:825–31.
14. Suleiman S, Eamin Ali M, Zaki Z, El-Malik E, Nasr M. Lipid peroxidation and human sperm motility: Protective role of vitamin E. J Androl. 1996;17:530–7.
15. Greco E, Iacobelli M, Rienzi L, Ubaldi F, Ferrero S, Tesarik J. Reduction of the incidence of sperm DNA fragmentation by oral antioxidant treatment. J Androl. 2005;26:349–53.
16. Haghighian HK, Haidari F, Mohammadi-Asl J, Dadfar M. Randomized, triple-blind, placebo-controlled clinical trial examining the effects of alpha-lipoic acid supplement on the spermatogram and seminal oxidative stress in infertile men. Fertil Steril. 2015;104:318–24.
17. Ebisch IMW, Pierik FH, De Jong FH, Thomas CMG, Steegers-Theunissen RPM. Does folic acid and zinc sulphate intervention affect endocrine parameters and sperm characteristics in men? Int J Androl. 2006;29:339–45.
18. Wong WWY, Merkus HHMW., Thomas CM. C, Menkveld R, Zielhuis G a G, Steegers-Theunissen RP. R. Effects of folic acid and zinc sulfate on male factor

subfertility: a double-blind, randomized, placebo-controlled trial. Fertil Steril. 2002;77:491–8.

19. Raigani M, Yaghmaei B, Amirjannti N, Lakpour N, Akhondi MM, Zeraati H, Hajihosseinal M, Sadeghi MR. The micronutrient supplements, zinc sulphate and folic acid, did not ameliorate sperm functional parameters in oligoasthenoteratozoospermic men. Andrologia. 2014;46:956–62.

20. Omu AE, Dashti H, Al-Othman S. Treatment of asthenozoospermia with zinc sulphate: Andrological, immunological and obstetric outcome. Eur J Obstet Gynecol Reprod Biol. 1998;79:179–84.

21. Martínez-Soto JC, Domingo JC, Cordobilla B, Nicolás M, Fernández L, Albero P, Gadea J, Landeras J. Dietary supplementation with docosahexaenoic acid (DHA) improves seminal antioxidant status and decreases sperm DNA fragmentation. Syst Biol Reprod Med. 2016;62:387–95.

22. Safarinejad MR. Effect of omega-3 polyunsaturated fatty acid supplementation on semen profile and enzymatic anti-oxidant capacity of seminal plasma in infertile men with idiopathic oligoasthenoteratospermia: A double-blind, placebo-controlled, randomised study. Andrologia. 2011;43:38–47.

23. Nadjarzadeh A, Shidfar F, Amirjannati N, Vafa MR, Motevalian SA, Gohari MR, Nazeri Kakhki SA, Akhondi MM, Sadeghi MR. Effect of Coenzyme Q10 supplementation on antioxidant enzymes activity and oxidative stress of seminal plasma: A double-blind randomised clinical trial. Andrologia. 2014;46:177–83.

24. Safarinejad MR, Safarinejad SS, Shafiei N, Safarinejad SS. Effects of the reduced form of coenzyme Q10 (ubiquinol) on semen parameters in men with idiopathic infertility: A double-blind, placebo controlled, randomized study. J Urol. 2012;188:526–31.

25. Nadjarzadeh A, Sadeghi MR, Amirjannati N, Vafa MR, Motevalian SA, Gohari MR, Akhondi MA, Yavari P, Shidfar F. Coenzyme Q 10 improves seminal oxidative defense but does not affect on semen parameters in idiopathic

oligoasthenoteratozoospermia: A randomized double-blind, placebo controlled trial. J Endocrinol Invest. 2011;34:224–8.

26. Balercia G, Buldreghini E, Vignini A, Tiano L, Paggi F, Amoroso S, Ricciardo-Lamonica G, Boscaro M, Lenzi A, Littarru GP. Coenzyme Q10 treatment in infertile men with idiopathic asthenozoospermia: a placebo-controlled, double-blind randomized trial. Fertil Steril. 2009;91:1785–92.

27. Safarinejad MR. Efficacy of coenzyme Q10 on semen parameters, sperm function and reproductive hormones in infertile men. J Urol. 2009;182:237–48.

28. 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. Fertil Steril. 2005;84:662–71.

29. Lenzi A, Sgrò P, Salacone P, Paoli D, Gilio B, Lombardo F, Santulli M, Agarwal A, Gandini L. A placebo-controlled double-blind randomized trial of the use of combined L-carnitine and L-acetyl-carnitine treatment in men with asthenozoospermia. Fertil Steril. 2004;81:1578–84.

30. Lenzi A, Lombardo F, Sgrò P, Salacone P, Caponecchia L, Dondero F, Gandini L. Use of carnitine therapy in selected cases of male factor infertility: A double-blind crossover trial. Fertil Steril. 2003;79:292–300.

31. Maretti C, Cavallini G. The association of a probiotic with a prebiotic (Flortec, Bracco) to improve the quality/quantity of spermatozoa in infertile patients with idiopathic oligoasthenoteratospermia: a pilot study. Andrology. 2017;5:439–44.

32. Calogero AE, Gullo G, La Vignera S, Condorelli RA, Vaiarelli A. Myoinositol improves sperm parameters and serum reproductive hormones in patients with idiopathic infertility: A prospective double-blind randomized placebo-controlled study. Andrology. 2015;3:491–5.

33. 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. Aust New Zeal J Obstet Gynaecol. 2007;47:216–21.

34. Kolahdooz M, Nasri S, Modarres SZ, Kianbakht S, Huseini HF. Effects of Nigella sativa L. seed oil on abnormal semen quality in infertile men: A randomized, double-blind, placebo-controlled clinical trial. Phytomedicine. 2014;21:901–5.

35. Robbins WA, Xun L, FitzGerald LZ, Esguerra S, Henning SM, Carpenter CL. Walnuts improve semen quality in men consuming a Western-style diet: randomized control dietary intervention trial. Biol Reprod. 2012;87:1–8.

36. 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. Phytother Res. 2011;25:508–16.

37. Aitken RJ, Gibb Z, Baker MA, Drevet J, Gharagozloo P. Causes and consequences of oxidative stress in spermatozoa. Reprod Fertil Dev. 2016;28:1–10.

38. Aitken RJ. Oxidative stress and the etiology of male infertility. J Assist Reprod Genet. 2016;33:1691–1692.

39. Kang MA, So EY, Simons AL, Spitz DR, Ouchi T. DNA damage induces reactive oxygen species generation through the H2AX-Nox1/Rac1 pathway. Cell Death Dis. 2012;3:e249.

40. Showell M, Mackenzie-Proctor R, Brown J, Yazdani A, Stankiewicz M, Hart R. Antioxidants for male subfertility. Cochrane Database Syst Rev Antioxidants. 2014;1:CD007411.

41. Schnabel R, Lubos E, Messow CM, Sinning CR, Zeller T, Wild PS, Peetz D, Handy DE, Munzel T, Loscalzo J, et al. Selenium supplementation improves antioxidant capacity in vitro and in vivo in patients with coronary artery disease. The SElenium Therapy in Coronary Artery disease Patients (SETCAP) Study. Am Heart J. 2008;156:1201.e1-11.

42. Prasad AS. Clinical, immunological, anti-inflammatory and antioxidant roles of zinc. Exp Gerontol. 2008;43:370–7.
43. Zhao J, Dong X, Hu X, Long Z, Wang L, Liu Q, Sun B, Wang Q, Wu Q, Li L. Zinc levels in seminal plasma and their correlation with male infertility: A systematic review and meta-analysis. Sci Rep. 2016;6:e.22386.
44. Safarinejad MR, Safarinejad S. The roles of omega-3 and omega-6 fatty acids in idiopathic male infertility. Asian J Androl. 2012;14:514–5.
45. Lafuente R, González-Comadrán M, Solà I, López G, Brassesco M, Carreras R, Checa MA. Coenzyme Q10 and male infertility: A meta-analysis. J Assist Reprod Genet. 2013;30:1147–56.
46. Tvrdá E, Kováčik A, Tušimová E, Massányi P, Lukáč N. Resveratrol offers protection to oxidative stress induced by ferrous ascorbate in bovine spermatozoa. J Environ Sci Heal - Part A Toxic/Hazardous Subst Environ Eng. 2015;50:1440–51.
47. Auger C, Gérain P, Laurent-Bichon F, Portet K, Bornet A, Caporiccio B, Cros G, Teissédre PL, Rouanet JM. Phenolics from commercialized grape extracts prevent early atherosclerotic lesions in hamsters by mechanisms other than antioxidant effect. J Agric Food Chem. 2004;52:5297–302.
48. Majzoub A, Agarwal A. Systematic review of antioxidant types and doses in male infertility: Benefits on semen parameters, advanced sperm function, assisted reproduction and live-birth rate. Arab J Urol. 2018;16:113–24.
49. Agarwal A, Said TM. Carnitines and male infertility. Reprod Biomed Online. 2004;8:376–84.

TABLE CAPTIONS

Table 1. Summary of the RCT studies investigating the effect of nutrition on sperm quality parameters or sexual hormones. The studies are arranged by supplement compounds (A to F) and from the most recent to the oldest study. The studies are classified as: A) Antioxidant supplement studies, B) Folic acid and/or zinc studies, C) Omega-3 fatty acid studies, D) Coenzyme Q10 studies, E) Carnitine studies, F) Dietary supplement studies.

Reference	Location	Age (years)	Population studied	Background diet controlled during the study?	Design	Intervention	Primary outcome	Principal conclusion	ROB1	ROB2	ROB3	ROB4	ROB5	ROB6	ROB7
A) Antioxidant															
(Haghighian *et al.* 2015) (16)	Iran	Intervention (32.98 ± 5.35); placebo (34.12 ± 4.79)	44 patients with iA (23 intervention; 21 placebo)	No	Parallel	12 weeks. Alpha lipoic acid (600 mg) or placebo/day	Sperm parameters (semen volume, sperm count and concentration, sperm motility, vitality and morphology)	Alpha lipoic acid improves sperm count and concentration, and motility (progressive motility). No effect on semen volume, sperm vitality or morphology.	LB	UB	LB	LB	LB	LB	UB
(Hawkes *et al.* 2009) (9)	The USA	18 to 45	42 healthy participants (22 intervention; 20 placebo)	No	Parallel	11 months. Se (300 µg) or placebo (Se < 1.5 µg)/day	Semen parameters (semen volume, sperm count and concentration, sperm motility and morphology) and serum hormones (T, LH, PSA)	Se has no effect on conventional sperm parameters or serum hormones.	UB	UB	LB	LB	LB	UB	UB
Safarinejad *et al.* 2009) (10)	Iran	Intervention Se (31 ± 9); intervention NAC (32 ± 10); intervention NAC + Se (31 ± 8); placebo (31 ± 9)	420 patients with iOAT (314 intervention [105 Se, 105 NAC, 104 NAC + Se]; 106 placebo)	No	Parallel	6 months. Se (200 µg), NAC (600 mg), Se + NAC (200 µg + 600 mg respectively), or placebo/day	Sperm parameters (semen volume, sperm count and concentration, sperm motility and morphology), serum hormones (T, LH, FSH, inhibin B, PRL)	Se, NAC or Se + NAC improve all conventional sperm parameters (semen volume, sperm count and concentration, sperm motility and morphology), except sperm motility in NAC group. Se, NAC or Se + NAC increase T, LH and inhibin B but decrease FSH. No effect on PRL serum hormone.	LB	UB	LB	LB	UB	LB	UB
(Greco *et al.* 2005) (15)	Italy	ND	64 patients with idiopathic infertility (32 intervention) and 32 placebo)	No	Parallel	2 months, Vitamin C (1 g) + vitamin E (1 g), or placebo/day	Semen parameters (sperm count and concentration, sperm motility and morphology), sperm DNA damage	Vitamin C and E improve sperm DNA fragmentation index. No effect on conventional semen parameters.	UB	LB	LB	LB	LB	UB	UB

(Rolf *et al.* 1999) (11)	Germany	Intervention (36.1 ± 5.0); placebo (35.2 ± 4.8)	31 patients with asthenozoospermia or moderate oligoasthenozoospermia (15 intervention; 16 placebo)	No	Parallel	8 weeks. Vitamin C (1,000 mg) + vitamin E (800 mg), or placebo/day	Sperm parameters (semen volume, sperm concentration, sperm motility, sperm vitality and morphology), serum hormones (T, FSH, LH, estradiol)	Vitamin C and E has no effect on conventional semen parameters or hormones.	LB	LB	LB	LB	LB	LB	UB
(Scott *et al.* 1998) (12)	Scotland	Se intervention (32.6 ± 1.1); Se + vitamins intervention (33.9 ± 0.9); placebo (32.9 ± 1.5)	64 patients with asthenozoospermia (46 intervention [16 Se, 30 Se + vitamins]; 18 placebo)	No	Parallel	3 months. Selenium (100 µg), Selenium + vitamin A (1 mg) + vitamin C (10 mg) + vitamin E (15 mg), or placebo/day	Sperm parameters (sperm concentration and motility), conception chance	Selenium improves sperm motility and chance of conception. No effect on sperm concentration.	LB	UB	LB	LB	UB	UB	UB
(Suleiman *et al.* 1996) (14)	Saudi Arabia	Intervention (27-52); placebo (22-45)	87 patients with asthenozoospermia (52 intervention) and 35 placebo)	No	Parallel	6 months. Vitamin E (300 mg) or placebo/day	Sperm parameters (sperm motility) and pregnancy rate	Vitamin E improves sperm motility and pregnancy rate.	UB	UB	LB	LB	UB	UB	UB
(Kessopoulou *et al.* 1995) (13)	England	32 (26 to 49)	30 healthy participants with high levels of ROS	No	Crossover	3 months. 1-month WO, 3 months vitamin E intervention/placebo, 1-month WO, 3 months vitamin E intervention/placebo. vitamin E (600 mg) or placebo/day	Sperm parameters (semen volume, sperm concentration, sperm motility and morphology), zona binding test, ROS	Vitamin E improves the zona binding test. No effect on ROS or conventional sperm parameters.	UB	UB	LB	LB	UB	LB	UB
B) **Folic acid and/or zinc**															
(Raigani et al. 2014) (19)	Iran	ND	83 patients with iOAT (65 intervention [20 folic acid, 24 zinc sulfate, 21 folic acid + zinc sulfate]; 18 placebo)	No	Parallel	16 weeks. Folic acid (5 mg), zinc sulfate (220 mg), folic acid + zinc sulfate (5 mg + 220 mg respectively) or placebo/day	Sperm parameters (sperm concentration, sperm motility, sperm vitality, and morphology), DNA damage and chromatin damage	Zinc sulfate improves sperm chromatin integrity. Folic acid and/or zinc sulfate has no effect on conventional sperm parameters.	UB	UB	LB	LB	UB	LB	UB
(Ebisch *et al.* 2006) (17)	The Netherlands	Subfertile men 35.0 (32.3–37.0); Fertile 34.0 (31.0–38.0)	87 participants (42 intervention [24 fertile, 18 subfertile] and 45 placebo	No	Parallel	6 months. Folic acid (5 mg) + zinc sulfate (66 mg), or placebo/day	Semen parameters (semen volume, sperm concentration, sperm motility and morphology), serum hormones (T, FSH, inhibin B)	Folic acid + zinc sulfate improve sperm concentration. No effect on semen volume, sperm motility or morphology or serum hormones.	LB	UB	LB	LB	UB	LB	UB

			[23 fertile, 22 subfertile])												
(Wong *et al.* 2002) (18)	The Netherlands	Subfertile men 34.3 ± 3.9; fertile men 34.2 ± 4.2	193 healthy participants (99 fertile, 94 subfertile)	No	Parallel	6 months. Folic acid (5 mg), zinc sulfate (66 mg), folic acid + zinc sulfate (5 mg + 66 mg respectively), or placebo/day	Sperm parameters (semen volume, sperm count and concentration, motility and morphology)	Folic acid + zinc sulfate improves sperm concentration and morphology; folic acid improves sperm morphology in subfertile patients. Zinc improves sperm morphology in fertile patients. No effect on semen volume, sperm count or motility.	LB	LB	LB	LB	LB	LB	UB
(Omu *et al.* 1998) (20)	Kuwait	Intervention (37.8 ± 7.9); control (38.1 ± 8.2)	97 patients with asthenozoopspermia (49 intervention; 48 control)	No	Parallel	3 months. Zinc (500 mg)/day, or no therapy	Sperm parameters (semen volume, sperm concentration, sperm motility and morphology), HOS, serum hormones (T, FSH, LH, PRL), pregnancy, abortion, delivered or on-going pregnancies	Zinc improves sperm concentration, sperm motility, sperm integrity membrane (HOS), fertilizing capacity, conception and pregnancy.	UB	UB	UB	UB	LB	LB	UB
C) Omega-3 fatty acid															
(Martínez-Soto *et al.* 2016) (21)	Spain	Intervention (35 ± 0.8); placebo (35.6 ± 1)	57 patients attending an infertility clinic (32 intervention; 25 placebo)	No	Parallel	10 weeks. Omega-3 DHA enriched oil (990 mg DHA + 135 mg EPA) or placebo (1,500 mg sunflower oil)/day	Sperm parameters (semen volume, sperm count and concentration, sperm motility and morphology), sperm DNA fragmentation	DHA reduces the percentage of spermatozoa with DNA damage. No effect on any conventional sperm parameter.	UB	UB	LB	LB	UB	LB	UB
(Safarinejad 2011) (22)	Iran	Intervention (32 ± 9); placebo (32 ± 10)	211 patients with iOAT (106 intervention; 105 placebo)	No	Parallel	32 weeks. Omega-3 group (1.12 g EPA + 0.72 g DHA) or placebo/day	Sperm parameters (semen volume, sperm count and concentration, sperm motility and morphology), serum hormones (T, LH, FSH, estradiol, PRL)	EPA and DHA improve sperm count and concentration, sperm motility and morphology. No effect on semen volume or serum hormones.	UB	UB	LB	LB	UB	LB	UB
D) Coenzyme Q10															
(Nadjarzadeh *et al.* 2014) (23)	Iran	Intervention (34.17 ± 4.52); placebo (34.67 ± 6.69)	47 patients with iOAT (23 intervention; 24 placebo)	Yes	Parallel	12 weeks. CoQ10 (200 mg), or placebo (containing lactose)/day	Sperm parameters (sperm concentration, sperm motility, and morphology)	CoQ10 has no effect on conventional sperm parameters.	UB	UB	LB	LB	UB	LB	UB
(Safarinejad *et al.* 2012) (24)	Iran	Intervention (31); placebo (32)	191 patients with iOAT (96 intervention; 95 placebo)	No	Parallel	6 months. Ubiquinol, or reduced CoQ10, (200 mg) or placebo/day	Sperm parameters (semen volume, sperm count and concentration, sperm motility and morphology), and serum hormones (T, LH, FSH, inhibin B, PRL)	Ubiquinol improves sperm count and concentration and sperm motility, increases inhibin B and reduces LH and FSH. No effect on semen volume, sperm morphology, T or PRL.	LB	LB	LB	LB	UB	LB	UB

(Nadjarzadeh *et al.* 2011) (25)	Iran	Intervention (34.17 ± 4.52); placebo (34.67 ± 6.69)	47 patients with iOAT (23 intervention; 24 placebo)	Yes	Parallel	12 weeks. CoQ10 (200 mg), or placebo (containing lactose)/day	Sperm parameters (semen volume, pH, sperm count and concentration, sperm motility and morphology)	CoQ10 has no effect on conventional sperm parameters.	UB	UB	LB	LB	UB	LB	UB
(Balercia *et al.* 2009) (26)	Italy	32 (27-39)	55 patients with iA (28 intervention; 27 placebo)	No	Parallel	6 months. CoQ10 (200 mg) or placebo/day	Semen parameters (sperm concentration, sperm motility and morphology)	CoQ10 improves sperm motility. No effect on sperm concentration or morphology.	UB	UB	LB	LB	LB	LB	UB
(Safarinejad 2009) (27)	Iran	Intervention (28 ± 9); placebo (28 ± 10)	194 patients with iOAT (98 intervention; 96 placebo)	No	Parallel	6 months. CoQ10 (300 mg) or placebo/day	Sperm parameters (semen volume, sperm count and concentration, sperm motility and morphology), acrosome-reacted spermatozoa, serum hormones (T, LH, FSH, inhibin B, PRL)	CoQ10 improves sperm count and concentration, sperm motility and morphology. Se reduces FSH and LH, and increases inhibin B and acrosome-reacted spermatozoa.	LB	UB	LB	LB	LB	LB	UB
E) Carnitine															
(Balercia *et al.* 2005) (28)	Italy	24 to 38	59 patients with iA (44 intervention [15 LC, 15 LAC, 14 LC + LAC]; 15 placebo)	No	Parallel	6 months. LC (3 g), LAC (3 g), LC + LAC (2 g+1 g respectively), or placebo (malic acid, sodium benzoate, sodium saccharinate dihydrate, anhydrous sodium citrate, pineapple flavoring, and demineralized water)/day	Sperm parameters (semen volume, sperm concentration, sperm motility and morphology)	LC, LAC or LC + LAC improve sperm motility. LAC improves sperm concentration and LC sperm morphology. No effect on semen volume.	UB	UB	LB	LB	LB	LB	UB
(Lenzi *et al.* 2004) (29)	Italy	20 to 40	56 patients with iOAT (30 intervention; 26 placebo)	No	Parallel	6 months. LC (2 g) + LAC (1 g), or placebo/day	Sperm parameters (semen volume, sperm concentration, sperm motility and morphology)	LC + LAC improve sperm motility. No effect on semen volume, sperm concentration or morphology.	UB	UB	LB	LB	LB	LB	UB
(Lenzi *et al.* 2003) (30)	Italy	20 to 40	86 infertile patients	No	Crossover	2 months WO, 2 months LC intervention/placebo, 2 months WO and 2 months LC intervention/placebo. LC (2 g), or placebo/day	Sperm parameters (semen volume, sperm concentration, sperm motility and morphology)	LC improves sperm concentration and motility. No effect on semen volume or sperm morphology.	UB	UB	LB	LB	UB	LB	UB
F) Dietary supplements															

(Maretti *et al.* 2017) (31)	Italy	Intervention (37; 32–42); placebo (36; 30–43)	41 patients with iOAT (20 intervention; 21 placebo)	No	Parallel	6 months. Flortec (*Lactobacillus paracasei* B21060 $5x10^9$ CFUs + arabinogalctan 1,243 mg + oligo-fructosaccharides 700 mg + L-glutamine 500 mg) or placebo (alimentary starch)/day	Sperm parameters (semen volume, pH, sperm count and concentration, sperm motility and morphology), serum hormones (T, LH, FSH, E2, PRL)	Flortec improves semen volume, sperm count, sperm concentration, progressive motility, and morphology, also improves FSH, LH, and T levels. No effect on PRL and E2.	LB	UB	LB	LB	LB	LB	UB
(Calogero *et al.* 2015) (32)	Italy	Intervention (28 ± 9); placebo (28 ± 10)	194 patients with idiopathic infertility (98 intervention; 96 placebo)	No	Parallel	3 months. Inofolic (4 g MI + 400 µg folic acid) or placebo (400 µg folic acid alone)/day	Sperm parameters (semen volume, sperm count and concentration, sperm motility and morphology), acrosome-reacted spermatozoa, serum hormones (T, LH, FSH, inhibin B, PRL)	Mi increases sperm count and concentration, motility (progressive motility) and T levels, and decreases acrosome-reacted spermatozoa, LH and FSH levels. No effect on semen volume, PRL or inhibin B.	UB	UB	LB	LB	LB	UB	UB
(Tremellen *et al.* 2007) (33)	Australia	Intervention (37.1 ± 5.1); placebo (35.5 ± 4.3)	60 patients with idiopathic infertility attending an infertility clinic (n=ND)	No	Parallel	3 months. Menevit (Lycopene 6 mg, vitamin E 400 IU, vitamin C 100 mg, zinc 25 mg, selenium 26 µg, folate 0.5 mg, garlic 1 g, palm oil) or placebo (palm oil)/day	Sperm parameters (sperm concentration, sperm motility, sperm vitality and morphology), SDF and fecundability parameters (cleavage stage embryo quality, oocyte fertilization rate and pregnancy rates)	Menevit improves pregnancy rates during IVF-ICSI treatment. No effect on conventional sperm parameters.	LB	UB	LB	LB	LB	LB	UB
(Kolahdooz *et al.* 2014) (34)	Iran	Intervention (31.5 ± 1.1); placebo (32.1 ± 0.8)	68 patients with idiopathic infertility (34 intervention; 34 placebo)	No	Parallel	2 months. *Nigella sativa* seed oil (5 mg of *N.sativa* oil) or placebo (liquid paraffin)/day	Sperm parameters (semen volume, pH, sperm concentration, sperm motility and morphology)	*N.sativa* improves semen volume and pH, sperm concentration, sperm motility, sperm morphology, and semen round cells in the ejaculate (reduction of this type of cells).	UB	LB	LB	LB	UB	LB	UB
(Robbins *et al.* 2012) (35)	The USA	Intervention (25.6 ± 4.0); control (24.8 ± 3.7)	107 healthy participants (55 intervention; 52 control)	Yes	Parallel	12 weeks. Walnuts (75 g) or no tree nuts consumption (control) in the context of a westernized diet	Sperm parameters (sperm concentration, sperm motility, sperm vitality and morphology), and sperm aneuploidy (X, Y, 18)	Walnuts improve sperm motility, sperm vitality and morphology. No effect on semen volume, sperm concentration or aneuploidy.	LB	UB	LB	LB	LB	LB	UB
(Safarinejad *et al.* 2011) (36)	Iran	Intervention (28.4 ± 5.2); placebo (28.8 ± 5.6)	230 patients with iOAT (114 intervention; 116 placebo)	No	Parallel	6 months. Saffron (60 mg *Crocus sativa*) or placebo/day	Sperm parameters (semen volume, sperm count and concentration, sperm motility and morphology), serum hormones (T, LH, FSH, PRL, TSH)	Saffron has no effect on conventional sperm parameters or serum hormones.	UB	UB	LB	LB	UB	LB	UB

693 Abbreviations are ordered alphabetically. CoQ10: Coenzyme Q10, DHA: Docosahexaenoic acid, E2: Estradiol, EPA: Eicosapentaenoic acid, FSH:
694 Follicle-stimulating hormone, HOS: Hypo-osmotic swelling, iA: Idiopathic asthenozoospermia, ICSI: Intra-citoplasmatic sperm injection, iOA:
695 Idiopathic oligoasthenozoospermia, iOAT: Idiopathic oligoasthenoteratozoospermia, IVF: *In-vitro* fertilization, LAC: L-acetyl carnitine, LB: Low risk
696 of bias, LC: L-carnitine, LH: Luteinizing hormone, MI: Myoinositol, NAC: N-acetyl cysteine, PRL: Prolactin, PSA: Prostatic specific antigen, RCT:
697 Randomized clinical trial, ROB: Risk of bias, ROB1: random sequence generation; ROB2: allocation concealment; ROB3: blinding of participants
698 and personnel; ROB4: blinding of outcome assessment; ROB5: incomplete outcome data; ROB6: selective reporting; ROB7: other bias; ROS:
699 Reactive oxygen species, SDF: Sperm DNA fragmentation, Se: Selenium, T: Testosterone, TSH: Thyroid-stimulating hormone, UB: Unclear risk
700 of bias, WO: Washout period.

FIGURE CAPTIONS

Figure 1. Mean differences (MD) and 95% confidence intervals (CI) for the effects of selenium supplements on sperm concentration, sperm total motility and sperm morphology.

Notes: Forest plots of the studies using generic-inverse variance and a fixed-effects estimate method. The points for each study indicate the MD, the size of the boxes indicates the weight of the study and the horizontal lines indicate the 95% CI for each study. The bold data represent the total number of participants for all studies and the diamond represents the pooled MD.

Figure 2. Mean differences (MD) and 95% confidence intervals (CI) for the effects of zinc supplements on sperm concentration, sperm total motility and sperm morphology.

Notes: Forest plots of the studies using generic-inverse variance and a fixed-effects estimate method. The points for each study indicate the MD, the size of the boxes indicates the weight of the study and the horizontal lines indicate the 95% CI for each study. The bold data represent the total number of participants for all studies and the diamond represents the pooled MD.

Figure 3. Mean differences (MD) and 95% confidence intervals (CI) for the effects of omega-3 fatty acid supplements on total sperm count, sperm concentration, sperm total motility and sperm morphology.

Notes: Forest plots of the studies using generic-inverse variance and a fixed-effects estimate method. The points for each study indicate the MD, the size of the boxes indicates the weight of the study and the horizontal lines indicate the 95% CI for each study. The bold data represent the total number participants for all studies and the diamond represents the pooled MD.

Figure 4. Mean differences (MD) and 95% confidence intervals (CI) for the effects of coenzyme-Q10 supplements on total sperm count, sperm concentration, sperm progressive motility, sperm total motility and sperm morphology.

Notes: Forest plots of the studies using generic-inverse variance and a fixed-effects estimate method. The points for each study indicate the MD, the size of the boxes indicates the weight of the study and the horizontal lines indicate the 95% CI for each study. The bold data represent the total number of participants for all studies and the diamond represents the pooled MD. The two articles by Nadjarzadeh *et al.* (2011 and 2014) are computed as one study.

Figure 5. Mean differences (MD) and 95% confidence intervals (CI) for the effects of carnitine (LC, LAC or LC+LAC) supplements on sperm concentration, sperm progressive motility, sperm total motility and sperm morphology.

Notes: Forest plots of the studies using generic-inverse variance and a fixed-effects estimate method. The points for each study indicate the MD, the size of the boxes indicates the weight of the study and the horizontal lines indicate the 95% CI for each study. The bold data represent the total number of participants for all studies and the diamond represents the pooled MD. Abbreviations: LAC, L-acetyl carnitine; LC, L-carnitine.

Figure

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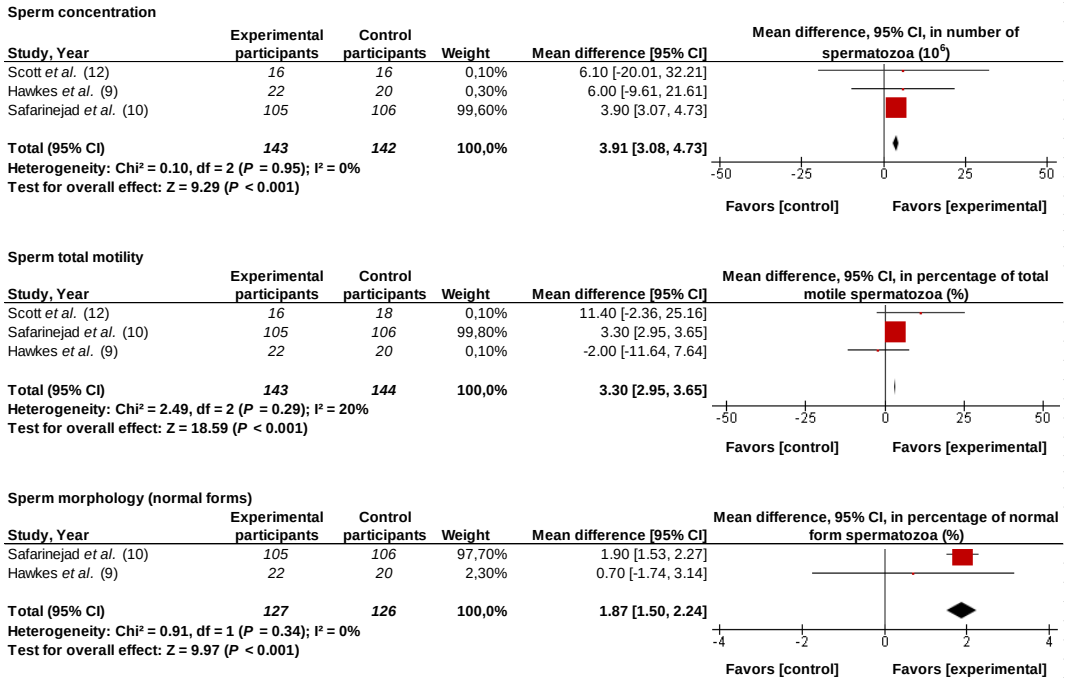

Figure

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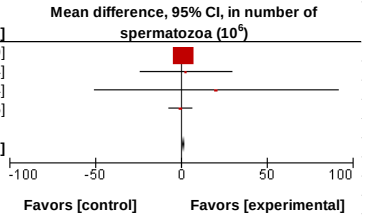
Sperm concentration

Study, Year	Experimental participants	Control participants	Weight	Mean difference [95% CI]
Omu *et al.* (20)	*49*	*48*	98,50%	1.50 [0.71, 2.29]
Wong *et al.* (19) a	*23*	*25*	0,10%	3.00 [-23.84, 29.84]
Wong *et al.* (19) b	*23*	*25*	0,00%	20.50 [-50.44, 91.44]
Raigani *et al.* (17)	*24*	*18*	1,40%	-0.20 [-6.86, 6.46]
Total (95% CI)	***119***	***116***	**100,0%**	**1.48 [0.69, 2.27]**

Heterogeneity: Chi² = 0.54, df = 3 (*P* = 0.91); I² = 0%
Test for overall effect: Z = 3.69 (*P* < 0.001)

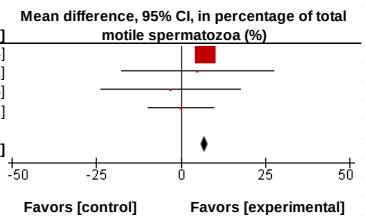


Footnotes
a. Subfertile
b. Fertile

Sperm total motility

Study, Year	Experimental participants	Control participants	Weight	Mean difference [95% CI]
Omu *et al.* (20)	*49*	*48*	98,50%	7.13 [6.13, 8.14]
Wong *et al.* (19) a	*23*	*25*	0,20%	5.00 [-17.42, 27.42]
Wong *et al.* (19) b	*23*	*25*	0,20%	-3.00 [-23.65, 17.65]
Raigani *et al.* (17)	*24*	*18*	1,10%	0.00 [-9.61, 9.61]
Total (95% CI)	***119***	***116***	**100,0%**	**7.03 [6.03, 8.03]**

Heterogeneity: Chi² = 3.03, df = 3 (*P* = 0.39); I² = 1%
Test for overall effect: Z = 13.78 (*P* < 0.001)

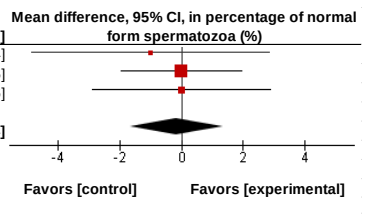


Footnotes
a. Subfertile
b. Fertile

Sperm morphology (normal forms)

Study, Year	Experimental participants	Control participants	Weight	Mean difference [95% CI]
Wong *et al.* (19) a	*23*	*25*	15,00%	-1.00 [-4.84, 2.84]
Wong *et al.* (19) b	*23*	*25*	58,00%	0.00 [-1.95, 1.95]
Raigani *et al.* (17)	*24*	*18*	27,00%	0.00 [-2.86, 2.86]
Total (95% CI)	***70***	***68***	**100,0%**	**-0.15 [-1.64, 1.34]**

Heterogeneity: Chi² = 0.22, df = 2 (*P* = 0.90); I² = 0%
Test for overall effect: Z = 0.20 (*P* = 0.84)



Footnotes
a. Subfertile
b. Fertile

Figure

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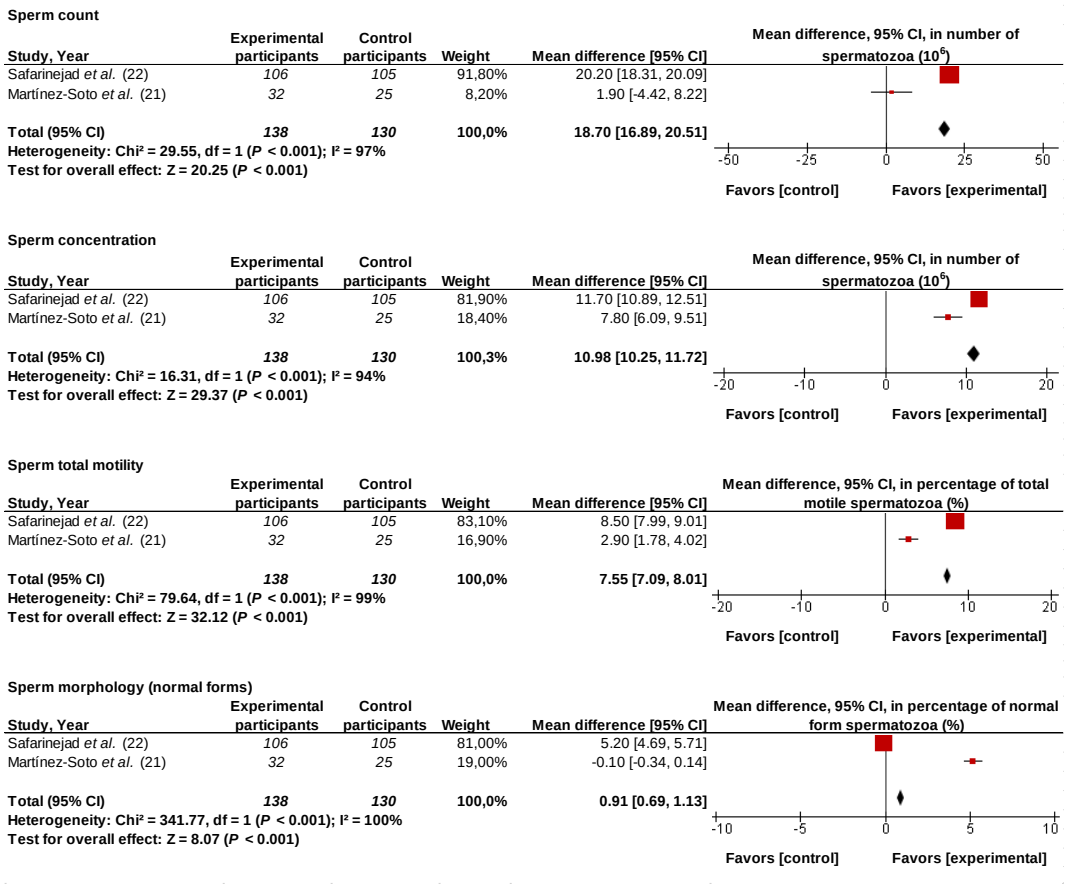

Figure

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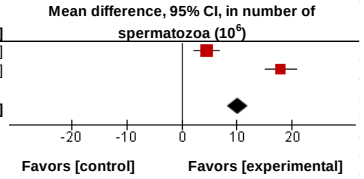
Sperm count

Study, Year	Experimental participants	Control participants	Weight	Mean difference [95% CI]
Safarinejad (27)	*98*	*96*	57,80%	4.50 [2.11, 6.89]
Safarinejad *et al.* (24) a	*96*	*95*	42,20%	17.90 [15.10, 20.70]
Total (95% CI)	***194***	***191***	**100,0%**	**10.15 [8.34, 11.97]**

Heterogeneity: Chi² = 50.98, df = 1 (*P* < 0.001); I² = 98%
Test for overall effect: Z = 10.96 (*P* < 0.001)

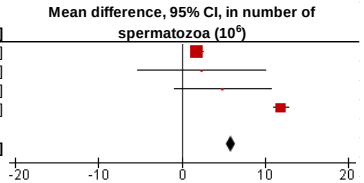


Footnotes
a. End-of-treatment derived values

Sperm concentration

Study, Year	Experimental participants	Control participants	Weight	Mean difference [95% CI]
Safarinejad (27)	*98*	*96*	57,80%	1.80 [1.04, 2.56]
Balercia *et al.* (26)	*28*	*27*	0,60%	2.37 [-5.27, 10.01]
Nadjarzadeh *et al.* (23,25)	*23*	*24*	1,00%	4.90 [-0.89, 10.69]
Safarinejad *et al.* (24) a	*96*	*95*	40,60%	11.90 [11.00, 12.80]
Total (95% CI)	***245***	***242***	**100,0%**	**5.93 [5.36, 6.51]**

Heterogeneity: Chi² = 283.54, df = 3 (*P* < 0.001); I² = 99%
Test for overall effect: Z = 20.22 (*P* < 0.001)

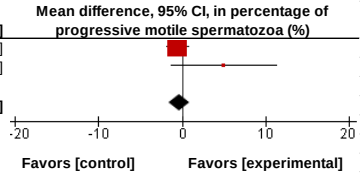


Footnotes
a. End-of-treatment derived values

Sperm progressive motility

Study, Year	Experimental participants	Control participants	Weight	Mean difference [95% CI]
Balercia *et al.* (26)	*28*	*27*	95,90%	-0.58 [-1.88, 0.72]
Nadjarzadeh *et al.* (23,25)	*23*	*24*	4,10%	4.95 [-1.37, 11.27]
Total (95% CI)	***51***	***51***	**100,0%**	**-0.35 [-1.63, 0.92]**

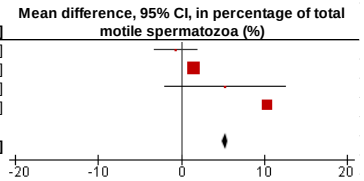
Heterogeneity: Chi² = 2.82, df = 1 (*P* = 0.09); I² = 65%
Test for overall effect: Z = 0.59 (*P* = 0.62)



Sperm total motility

Study, Year	Experimental participants	Control participants	Weight	Mean difference [95% CI]
Safarinejad (27)	*98*	*96*	1,60%	1.50 [1.07, 1.93]
Balercia *et al.* (26)	*28*	*27*	55,20%	-0.70 [-3.22, 1.82]
Nadjarzadeh *et al.* (23,25)	*23*	*24*	0,20%	5.24 [-2.06, 12.54]
Safarinejad *et al.* (24) a	*96*	*95*	43,00%	10.40 [9.91, 10.89]
Total (95% CI)	***245***	***242***	**100,0%**	**5.30 [4.98, 5.62]**

Heterogeneity: Chi² = 749.27, df = 3 (*P* < 0.001); I² = 100%
Test for overall effect: Z = 32.65 (*P* < 0.001)

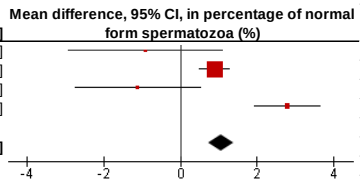


Footnotes
a. End-of-treatment derived values

Sperm morphology (normal forms)

Study, Year	Experimental participants	Control participants	Weight	Mean difference [95% CI]
Safarinejad, 2009	*98*	*96*	2,90%	0.90 [0.51, 1.29]
Balercia *et al.* (26)	*28*	*27*	76,80%	-0.91 [-2.92, 1.10]
Nadjarzadeh *et al.* (23,25)	*23*	*24*	4,30%	-1.12 [-2.76, 0.52]
Safarinejad *et al.* (24) a	*96*	*95*	16,00%	2.80 [1.95, 3.65]
Total (95% CI)	***245***	***242***	**100,0%**	**1.06 [0.72, 1.41]**

Heterogeneity: Chi² = 27.10, df = 3 (*P* < 0.001); I² = 89%
Test for overall effect: Z = 6.12 (*P* < 0.001)



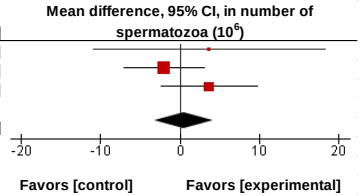
Footnotes
a. End-of-treatment derived values

Figure

Sperm concentration

Study, Year	Experimental participants	Control participants	Weight	Mean difference [95% CI]
Lenzi *et al.* (30) a	*86*	*86*	6,50%	3.70 [-10.96, 18.36]
Lenzi *et al.* (29) b	*30*	*26*	55,50%	-2.03 [-7.06, 3.00]
Balercia *et al.* (28) c	*44*	*45*	38,00%	3.67 [-2.41, 9.75]
Total (95% CI)	***160***	***157***	**100,0%**	**0.51 [-3.24, 4.26]**

Heterogeneity: Chi² = 2.20, df = 2 (*P* = 0.33); I² = 9%
Test for overall effect: Z = 0.27 (*P* = 0.79)

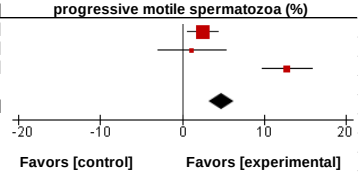


Footnotes
a. LC; imputated SE; crossover design
b. LC+LAC
c. Pooled intervention arms

Sperm progressive motility

Study, Year	Experimental participants	Control participants	Weight	Mean difference [95% CI]
Lenzi *et al.* (30) a	*86*	*86*	63,80%	2.50 [0.64, 4.36]
Lenzi *et al.* (29) b	*30*	*26*	12,40%	1.18 [-3.02, 5.38]
Balercia *et al.* (28) c	*44*	*45*	23,80%	12.85 [9.81, 15.88]
Total (95% CI)	***160***	***157***	**100,0%**	**4.80 [3.32, 6.28]**

Heterogeneity: Chi² = 35.77, df = 2 (*P* < 0.001); I² = 94%
Test for overall effect: Z = 6.35 (*P* < 0.001)

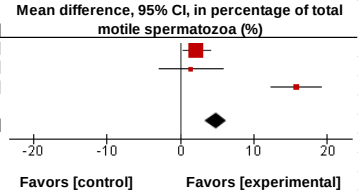


Footnotes
a. LC; imputated SE; crossover design
b. LC+LAC
c. Pooled intervention arms

Sperm total motility

Study, Year	Experimental participants	Control participants	Weight	Mean difference [95% CI]
Lenzi *et al.* (30) a	*86*	*86*	67,60%	2.20 [0.35, 4.05]
Lenzi *et al.* (29) b	*30*	*26*	12,50%	1.47 [-2.84, 5.78]
Balercia *et al.* (28) c	*44*	*45*	19,90%	15.82 [12.41, 19.23]
Total (95% CI)	***160***	***157***	**100,0%**	**4.82 [3.30, 6.34]**

Heterogeneity: Chi² = 49.94, df = 2 (*P* < 0.001); I² = 96%
Test for overall effect: Z = 6.21 (*P* < 0.001)

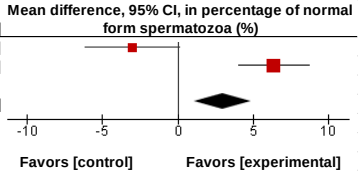


Footnotes
a. LC; imputated SE; crossover design
b. LC+LAC
c. Pooled intervention arms

Sperm morphology (normal forms)

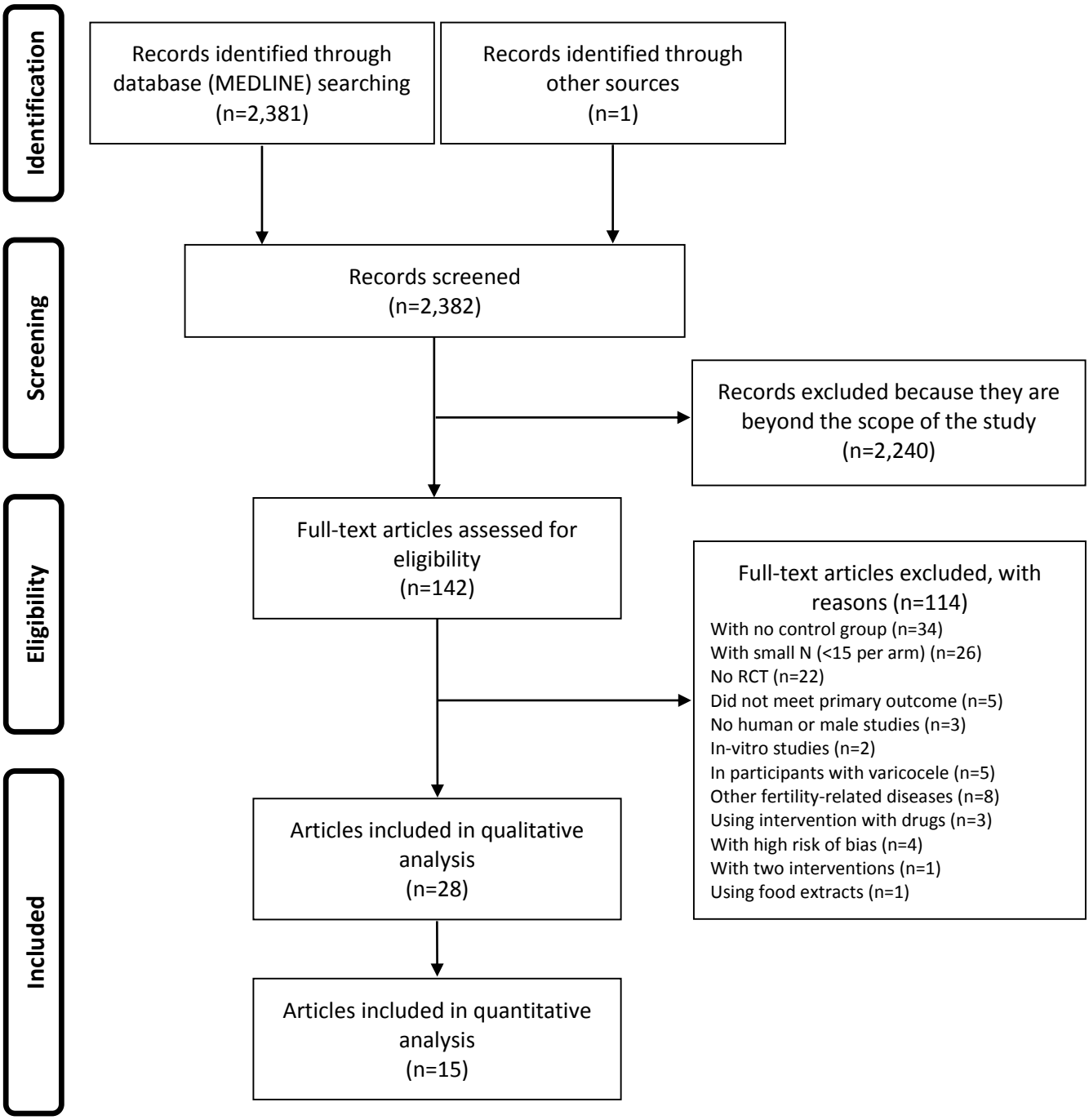
Study, Year	Experimental participants	Control participants	Weight	Mean difference [95% CI]
Lenzi *et al.* (29) a	*30*	*26*	36,10%	-3.03 [-6.17, 0.11]
Balercia *et al.* (28) b	*44*	*45*	63,90%	6.37 [4.02, 8.73]
Total (95% CI)	***74***	***71***	**100,0%**	**2.98 [1.10, 4.87]**

Heterogeneity: Chi² = 22.06, df = 1 (*P* < 0.001); I² = 95%
Test for overall effect: Z = 3.10 (*P* = 0.002)



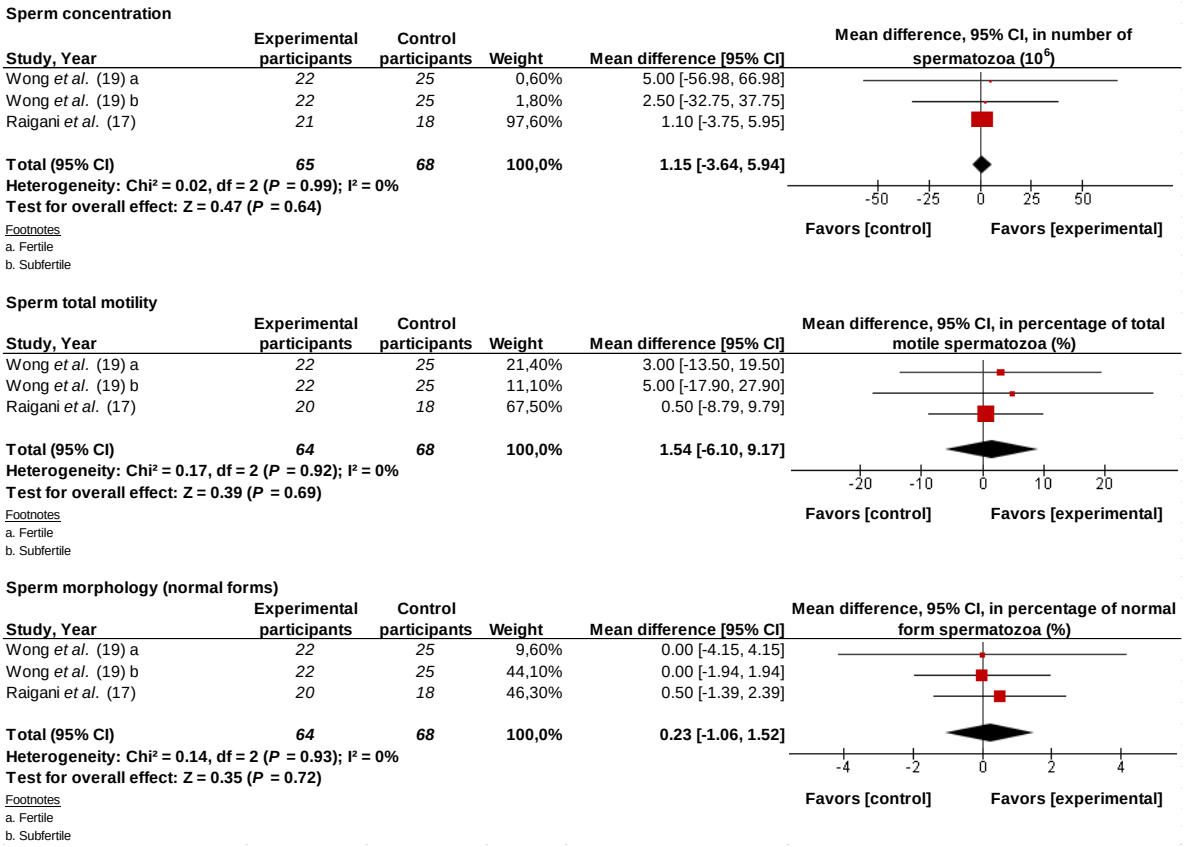
Footnotes
a. LC+LAC
b. Pooled intervention arms

Supplementary data



Supplemental Figure 1. Flow chart of the literature search and selection process.

Supplementary data



Supplemental Figure 2. Mean differences (MD) and 95% confidence intervals (CI) for the effects of folic acid supplements on sperm concentration, sperm total motility and sperm morphology.

Notes: Forest plots of the studies using generic-inverse variance and a fixed-effects estimate method. The points for each study indicate the MD, the size of the boxes indicates the weight of the study and the horizontal lines indicate the 95% CI for each study. The bold data represent the total number of participants for all studies and the diamond represents the pooled MD.

Supplementary data

Supplemental Appendix. Complete search strategy for the literature published between the earliest available online indexing year and October 2017 in the MEDLINE-Pubmed database.

Search terms:

((((((((((((((((((fertility[MeSH Terms]) OR infertility[MeSH Terms]) OR male infertility[MeSH Terms]) OR male fertility[MeSH Terms]) OR fertility) OR infertility) OR male infertility) OR male fertility) OR sperm dysfunction) OR sperm dysfunctions) OR sperm dna damage) OR varicocele[MeSH Terms]) OR asthenozoospermia[MeSH Terms]) OR oligozoospermia[MeSH Terms]) OR oligoasthenozoospermia) OR oligoasthenoteratozoospermia) OR teratozoospermia)) AND ((((((((((((((((((((((((((((((((diet[MeSH Terms]) OR nutrients[MeSH Terms]) OR food[MeSH Terms]) OR food supplement) OR dietary supplement) OR probiotic) OR nuts[MeSH Terms]) OR vitamin c[MeSH Terms]) OR vitamin e[MeSH Terms]) OR zinc[MeSH Terms]) OR antioxidants[MeSH Terms]) OR cereals[MeSH Terms]) OR meat[MeSH Terms]) OR vegetable[MeSH Terms]) OR fruit[MeSH Terms]) OR fishes[MeSH Terms]) OR legumes[MeSH Terms]) OR milk[MeSH Terms]) OR yogurt[MeSH Terms]) OR cheese[MeSH Terms]) OR seeds[MeSH Terms]) OR eggs[MeSH Terms]) OR dairy product[MeSH Terms])) OR micronutrient[MeSH Terms]) OR micronutrients[MeSH Terms]) OR vitamins[MeSH Terms]) OR alcohol consumption[MeSH Terms]) OR zinc) OR l-carnitine) OR n-acetylcysteine) OR glutathione) OR coenzyme q10) OR selenium[MeSH Terms]) OR fatty acids[MeSH Terms]) OR sugar)))

Inclusion filters:

Case Reports, Classical Article, Clinical Conference, Clinical Study, Clinical Trial, Clinical Trial, Phase I, Clinical Trial, Phase II, Clinical Trial, Phase III, Clinical Trial, Phase IV, Congresses, Controlled Clinical Trial, Dataset, English Abstract, Evaluation Studies, Introductory Journal Article, Journal Article, Letter, Meta-Analysis, Multicenter Study, Pragmatic Clinical Trial, Randomized Controlled Trial, Systematic Reviews, Abstract, Humans, English, Male.