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Review article

Effects of folic acid and folic acid plus zinc supplements on the sperm characteristics and pregnancy outcomes of infertile men: A systematic review and meta-analysis

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A R T I C L E I N F O

Keywords:
Folic acid
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A B S T R A C T

Background: Folic acid and zinc supplements have been used to treat male infertility, but their efficacy is still debated.
Objective: To systematically evaluate the effects of folic acid and folic acid plus zinc supplements on sperm characteristics and pregnancy outcomes of infertile men.
Methods: An online systematic search was performed using PubMed, Cochrane Library, and EMBASE databases from inception to August 1, 2022. The goal was to identify randomized controlled trials (RCTs) that used folic acid or folic acid plus zinc to improve sperm characteristics of infertile men. Data were extracted by two investigators who independently screened the literature and assessed for quality according to the criteria. The meta-analysis was performed using RevMan 5.4 software.
Results: A total of 8 RCT studies involving 2168 patients were included. The results showed that compared with the controls, folic acid significantly increased sperm motility (MD, 3.63; 95% CI, −1.22 to 6.05; $P = 0.003$), but did not affect the sperm concentration (MD, 2.53; 95% CI, −1.68 to 6.73; $P = 0.24$) and sperm morphology (MD, −0.02; 95% CI, −0.29 to 0.24; $P = 0.86$) in infertile men. Folic acid plus zinc did not affect sperm concentration (MD, 1.87; 95% CI, −1.39 to 5.13; $P = 0.26$), motility (MD, 1.67; 95% CI, −1.29 to 4.63; $P = 0.27$), and morphology (MD, −0.05; 95% CI, −0.27 to 0.18; $P = 0.69$) in infertile men. Secondary results showed that compared with a placebo, folic acid alone had a higher rate of pregnancy in transferred embryos (35.6% vs. 20.4%, $P = 0.082$), but the difference was not significant. Folic acid plus zinc did not affect pregnancy outcomes.
Conclusions: Based on the meta-analysis, no significant improvements in sperm characteristics with folic acid plus zinc supplements were seen. However, folic acid alone has demonstrated the potential to improve sperm motility and in vitro fertilization-intracytoplasmic sperm injection (IVF–ICSI) outcomes. This indicates that folic acid supplements alone may be a viable treatment option for male infertility.

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1. Introduction

Male infertility is a prevalent issue, affecting around 15% of couples in their reproductive age. In about 50% of cases, male factors are the primary contributor. Genetic causes can play a role, but many cases of male infertility have an unknown etiology. Several factors, such as abnormal sperm DNA methylation [1], abnormal testicular methylenetetrahydrofolate reductase (MTHFR) [2], and damage caused by reactive oxygen species (ROS) [3] may contribute to male infertility.

Folic acid, also known as vitamin B9, is an essential nutrient that is critical in various cellular processes, including DNA synthesis and repair, RNA synthesis, and cell growth and division [4]. It also functions as a potent antioxidant that protects cells from damage caused by free radicals [5]. Scientific evidence suggests that folic acid supplementation may improve male fertility by promoting DNA methylation during spermatogenesis, regulating the expression of MTHFR, and reducing testicular apoptotic gene expression in male mice [6].

Zinc is another critical nutrient for spermatogenesis and is involved in DNA transcription [7]. Both folic acid and zinc have been linked to scavenging ROS. Spermatozoa are highly susceptible to ROS attacks due to their high oxidative phosphorylation activity and low cytoplasmic content, and this can result in decreased sperm quality [8]. Adequate dietary intake of folic acid and zinc is crucial for overall health, and their deficiency can lead to various health problems [9].

The 2012 update of the European Association of Urology Guidelines for Male Infertility proposed that treatments, such as recombinant follicle-stimulating hormone, folic acid plus zinc, or antiestrogens, are potentially beneficial for some patients. However, the scientific evidence supporting this empirical approach was low, and it was unclear which treatment was most effective [10]. Additionally, concerns about high doses of folic acid masking vitamin B12 deficiency and potentially leading to neurological problems exist. Folic acid may also interact with certain medications, such as anti-epileptic drugs, and reduce their effectiveness [11]. Moreover, excess zinc intake could negatively impact prostate health and interfere with the absorption of other minerals like iron and calcium [12,13]. Several published meta-analyses have focused on the genotypic analysis of the methylenetetrahydrofolate reductase (MTHFR) enzyme [14], which is crucial in folate metabolism and male infertility, but fewer meta-analyses of randomized controlled trials (RCTs) have examined the effectiveness of folic acid and zinc supplementation in treating male infertility. As reports on the efficacy of folic acid and folic acid plus zinc supplements in treating male infertility continue to be updated, their efficacy should be clarified.

This study aims to assess the clinical efficacy of folic acid and folic acid plus zinc supplements in treating male infertility through a meta-analysis of randomized controlled trials (RCTs). We hypothesize that folic acid and zinc supplements may enhance male fertility and pregnancy outcomes by reducing reactive oxygen species (ROS), which can damage sperm quality. Further investigation is needed to determine the effectiveness of folic acid and zinc supplements in treating male infertility because it remains a topic.

2. Methods

We performed a systematic online search using PubMed, Cochrane Library, EMBASE, and ClinicalTrials.gov databases from their inception to August 1, 2020. To ensure methodological rigor, we conducted the review and meta-analysis as per the Cochrane Handbook for Systematic Reviews of Interventions, and we reported our findings following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [15,16]. We registered the review and meta-analysis with PROSPERO under the registration number CRD42021284913.

2.1. Study criteria

2.1.1. Types of research

Randomized controlled trials on folic acid and zinc in infertile men published in English. The time of publication was not restricted.

2.1.2. Study participants

The participants in the studies were adult men with infertility. The diagnostic criteria for sperm characteristics in infertile men were determined by the World Health Organization (WHO) criteria [17]. These criteria, widely recognized in the field, typically involve the evaluation of sperm concentration, motility, and morphology. They are as follows: (1) Semen volume of at least 1.5 mL (mL); (2) Sperm concentration should be at least 15 million per mL. Total sperm count should be at least 39 million; (3) At least 40% of the spermatozoa should be motile (actively moving); and (4) At least 4% of the spermatozoa should have a normal morphology (shape and size). If a man's semen analysis does not meet these criteria, he is diagnosed with male infertility.

2.1.3. Exclusion criteria

(1) Studies that included men with known infertility problems due to varicoceles, genetic diseases, drug abuse, hormonal imbalances, abnormal body mass index (BMI), or other conditions that may contribute to infertility; (2) Studies with incomplete or missing data; and (3) Duplicate published studies (the study with the most comprehensive data was selected).

2.1.4. Interventions

Experimental group: treatment with folic acid and folic acid plus zinc complex; and control group: unlimited.

2.2. Outcome indicators

In this study, participants were included if they had a diagnosis of low fertility or subfertility, defined as having unsuccessfully attempted to conceive for at least one year with at least one abnormal semen parameter according to the WHO 2010 criteria. The abnormal semen parameters were semen volume, sperm concentration, sperm motility, and sperm morphology. Participants were advised to abstain from sexual activity for 3–5 days before collecting semen samples through masturbation in sterile containers. The sperm concentration, morphology, and motility were assessed using the Makler counting chamber method, staining, and flagellar motility proportion, respectively. The primary outcome measures were normal sperm morphology, sperm concentration, and sperm motility. The secondary outcome indicators included beta-human chorionic gonadotropin pregnancy test, clinical intrauterine pregnancy, ectopic pregnancy, multiple pregnancies, early pregnancy loss, cesarean section, preeclampsia or gestational hypertension, gestational diabetes, gestational age, birth weight, or small for gestational age at birth.

2.3. Search strategy

We used a combination of keywords and subject terms in our search, including male infertility, sperm, and folic acid, and involved the combination of keywords with subject terms. To avoid missing potentially relevant studies, the search did not impose any restrictions on publication date or status (see Supplemental Table 1). A detailed description of the search strategy is available at https://www.crd.york.ac.uk/PROSPEROFILES/284913_STRATEGY_20211013.pdf.

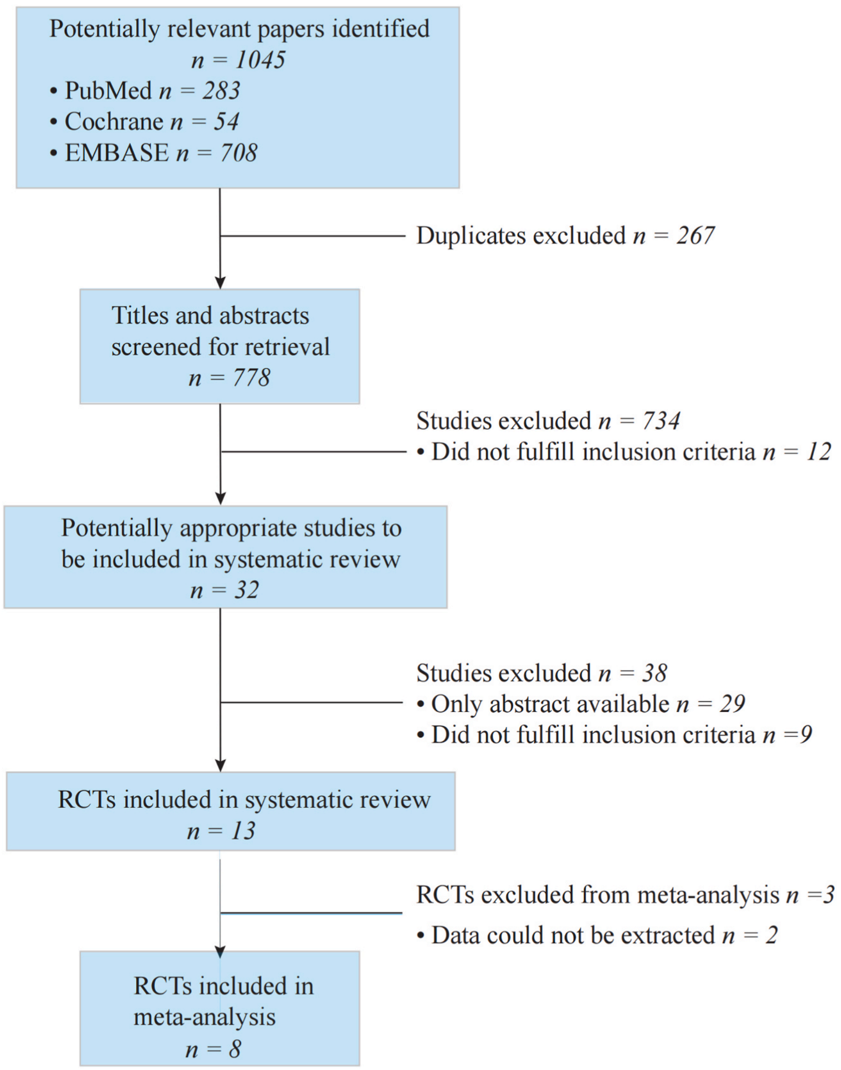

Fig. 1. Flowchart of included studies.

Table 1
Characteristics of included studies.

Study	Country	Cause of male infertility	Time (weeks)	Days of sexual abstinence (days)	Age (years)	Type of intervention			Control group		Outcome
						intervention	Dosage	Sample size	intervention	Sample size	
Wong, 2002 [18]	South Africa	Suboptimal semen quality	26	3to 5	UNKNOWN	Folic acid	5 mg/ day	22	placebos	24	Volume, Sperm concentration, Motility, morphology
						Folic acid and zinc sulfate	5 mg/ day 66 mg/day	24	placebos	24	
Ebisch, 2003 [19]	Netherlands	Suboptimal semen quality	26	3to 5	UNKNOWN	Folic acid and zinc sulfate	5 mg/ day 66 mg/day	19	placebos	21	sperm concentration
Ebisch, 2006 [20]	Netherlands	Suboptimal semen quality	26	3 or higher	UNKNOWN	Folic acid and zinc sulfate	5 mg/ day 66 mg/day	18	placebos	22	Serum Folate and zinc measurements
da Silva, 2013 [21]	Brazil		13	5 to 7	35.3 ± 7.7	Folic acid	5 mg/ day	23	placebos	26	Perm concentration, Motility, morphology
.Raigani, 2014 [22]	Iran	Oligoasthenoteratozoospermia	16	2 to 3	UNKNOWN	Folic acid	5 mg/ day	20	placebos	18	Sperm concentration, Motility, morphology
						Folic acid and zinc sulfate	5 mg/ day 220 mg/day	21	placebos	18	
Boonyarangkul, A.2015 [23]	Thailand	Oligoteratoasthenozoospermia (OAT)	Evaluated three and six months after treatment.	2 to 5	35.93 ± 1.35	Folic acid	5 mg/ day	15	placebos	15	Sperm concentration, Motility, morphology
D'argent, 2021 [24]	France	Suboptimal semen quality	13	3 to 5	36.5 ± 6.2	Folic acid	15 mg/ day	83	placebos	79	Sperm concentration, Motility, morphology. Additional secondary endpoints were: the number of oocytes retrieved, number of embryos obtained, number of top quality embryos, and number of transferred and frozen embryos.
Schisterman, 2020 [25]	USA	Suboptimal semen quality	For a minimum of 4.5–6 weeks	UNKNOWN	32.5 ± 5.7	Folic acid and zinc	5 mg/ day 30 mg/day	794	placebos	835	Volume, Sperm Concentration, Motility, Morphology Primary Outcomes 1 Live Birth (Determined by Medical record abstraction

2.4. Evaluation of literature quality and data extraction

Two researchers independently screened the retrieved literature, and then compared the screening results. If there were any differences, a third researcher was asked to assist in deciding whether to include the literature. The extracted data included the first author's name, publication time, intervention measures, control measures, outcome indicators, etc. The methodological quality of the included studies was evaluated using the risk of bias assessment tool in the Cochrane Systematic Reviewers Manual 5.4 (RevMan 5.4).

2.5. Risk of bias assessment

The risk of bias assessment on included studies was done using the tools recommended by the Cochrane Handbook for RCTs as follows: (1) Whether the randomization method was correct; (2) Whether the allocation was concealed; (3) Whether the subjects and investigators were blinded; (4) The completeness of the data results; (5) Whether there was selective reporting of the study results; and (6) Other sources of bias. The risk of bias was assessed by two evaluators individually, and any disagreements were discussed and resolved.

2.6. Statistical analysis

Meta-analysis was performed using RevMan 5.4 statistical software, and the primary outcome indicators were continuous variables with a mean difference (MD) and 95% confidence interval (CI) as effect sizes. Secondary outcome indicators were not fully described due to the limited number of included studies. Data for which median and maximal or minimal values were reported in the included studies were transformed according to a predetermined formula and then combined for analysis. The statistical analysis of the relationship between the two groups was conducted using the Chi-square test. The criteria for significant heterogeneity between included studies were ($P < 0.1$, $I^2 > 50$). A meta-analysis was conducted with heterogeneity testing using a random effects model. Subgroup analyses were performed for different interventions. Sensitivity analysis was conducted by sequentially excluding individual studies, re-running the meta-analysis, and assessing the difference between the excluded results and the original combined results. Because fewer than 10 studies were included in this paper, publication bias testing was not performed.

3. Results

3.1. Search results

We obtained 1054 articles, and after removing duplicates, 778 articles remained, then 746 articles were excluded by reading the abstract and title. Finally, 8 RCTs with a total of 2165 patients were included by reading the full text [18–25]. The literature screening process and results are shown in Fig. 1.

3.2. General characteristics of the included studies

The sample size of infertile men ranged from 15 to 794, and 1063 participants were randomly assigned to the treatment group and 1105 participants to the placebo group. Four of the articles reported mean patient ages ranging from 32.5 to 36.5 years, a maximum follow-up of 26 weeks, and patient abstinence of 2–7 days. Three of the eight articles reported folic acid as a supplement [21,23,24], three reported mixtures of folic acid and zinc as a supplement [19,20,25], and two reported both folic acid and folic acid plus zinc as a

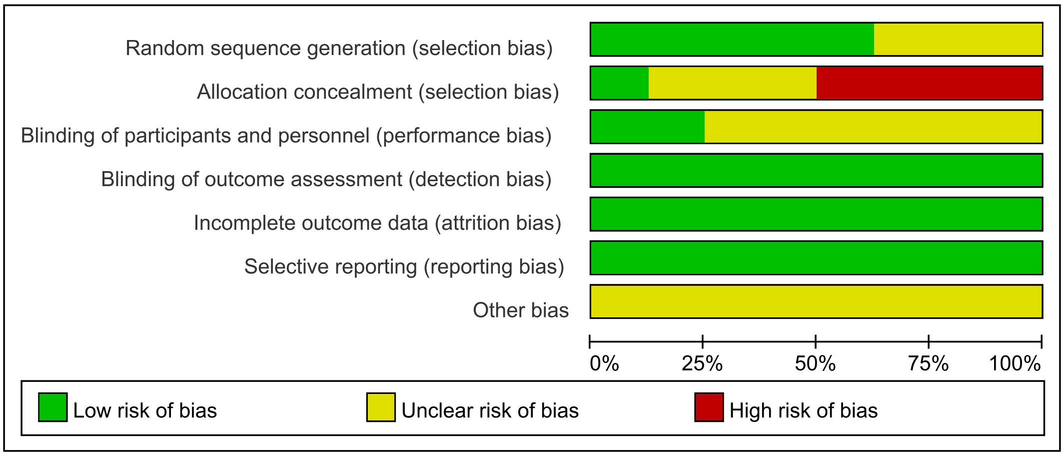

Fig. 2. Risk of bias graph: authors' guide of each risk of bias item presented as percentages across all included studies.

supplement [18,22]. The doses used ranged from 5 to 15 mg/day for folic acid and 30–220 mg/day for zinc. Only two articles reported on secondary outcome indicators [24,25]. The characteristics of the included studies are listed in Table 1.

3.3. Risk of bias assessment

Of the included studies in this analysis, five studies used computers for the random assignment [18–20,24,25], and three studies mentioned randomization without providing details [21–23]. Three studies used a randomization list for allocation concealment [18–20], one study used envelopes for allocation concealment [21], and four articles did not mention allocation concealment schemes [22–25]. Two studies blinded participants, trial staff, and investigators [18,25], and six study articles did not clearly describe blinding methods [18–25]. None of the included studies exhibited selective reporting bias, and the data integrity was good (Fig. 2).

3.4. Meta-analysis results

3.4.1. Sperm concentration

A total of seven RCTs [12,13,16–19] with 1063 cases were included in this analysis. The random effects model analysis showed that folic acid and folic acid plus zinc supplements had no statistically significant effect on sperm concentration in infertile men (MD, 1.87; 95% CI, −1.39 to 5.13; $P = 0.26$). Six RCTs [18,19,22–24] showed that folic acid did not have a statistically significant effect on sperm concentration in infertile men (MD, 2.53; 95% CI, −1.68 to 6.73; $P = 0.24$). Five RCTs [18–20,22,25] showed that folic acid and zinc supplements did not have a statistically significant effect on sperm concentration in infertile men (MD, 0.95; 95% CI, −4.54 to 6.45; $P = 0.73$) (Fig. 3).

3.4.2. Sperm motility

A total of six RCTs [18,21–25] with 1002 cases were included in the analysis. The random effects model analysis showed that folic acid and folic acid plus zinc supplements had no statistically significant effect on sperm motility in infertile men (MD, 1.72; 95% CI, 0.31–3.12; $P = 0.27$). Statistically significant results were observed in five RCTs that investigated the effect of folic acid supplements on sperm motility in infertile men (MD, 4.00; 95% CI, −1.99 to 6.00; $P < 0.003$) [18,21–24]. Whereas, three RCTs [18,22,25] that studied the effect of folic acid and zinc supplements on sperm motility showed no statistically significant effect (MD, −0.49; 95% CI, −2.46 to 1.49; $P = 0.63$) (Fig. 4).

3.4.3. Sperm morphology

A total of five RCTs [18,21–23,25] with 1124 cases were included. The random effects model analysis showed that folic acid alone or in combination with zinc had no statistically significant effect on sperm morphology in infertile men (MD, −0.05; 95% CI, −0.27 to 0.18; $P = 0.69$). Four RCTs [18,21–23] showed that folic acid alone had no statistically significant effect on sperm morphology in infertile men (MD, −0.02; 95% CI, −0.29 to 0.24; $P = 0.86$). Additionally, three RCTs [18,22,25] that investigated the effect of folic acid and zinc supplements on sperm morphology in infertile men did not find a statistically significant effect (MD, −0.11; 95% CI,

	Folic acid			Placebo				Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI
3.1.1 Effects of folic acid on sperm concentration								
Boonyarangkul 2015	-8.2	5.95	15	-16.44	16.56	15	13.4%	8.24 [-0.66, 17.14]
D'argent 2021	-1.2	25.7	83	-1.2	11.3	79	28.9%	0.00 [-6.06, 6.06]
da Silva 2013	0.17	24.13	23	-0.81	18.25	26	7.3%	0.98 [-11.12, 13.08]
Ebisch 2003	8.39	164.79	24	-15.11	202.05	21	0.1%	23.50 [-85.19, 132.19]
Raigani 2014	3.5	13.68	20	0.4	17.88	18	10.2%	3.10 [-7.11, 13.31]
Wong 2002	4	135.52	22	1.5	118.45	25	0.2%	2.50 [-70.73, 75.73]
Subtotal (95% CI)			**187**			**184**	**60.1%**	**2.53 [-1.68, 6.73]**
Heterogeneity: Tau² = 0.00; Chi² = 2.47, df = 5 (P = 0.78); I² = 0%								
Test for overall effect: Z = 1.18 (P = 0.24)								
3.1.2 Effect of folic acid plus zinc on sperm concentration								
Ebisch 2003	22.27	162.39	19	-15.11	202.05	21	0.1%	37.38 [-75.75, 150.51]
Ebisch 2006	5	16.09	18	-2.8	14.21	22	11.8%	7.80 [-1.71, 17.31]
Raigani 2014	1.5	9.19	21	0.4	17.88	18	12.7%	1.10 [-8.05, 10.25]
Schisterman 2020	-3.4	84.9	794	1.5	87.72	835	15.1%	-4.90 [-13.28, 3.48]
Wong 2002	4.5	190.05	24	1.5	118.45	25	0.1%	3.00 [-86.09, 92.09]
Subtotal (95% CI)			**876**			**921**	**39.9%**	**0.95 [-4.54, 6.45]**
Heterogeneity: Tau² = 2.74; Chi² = 4.26, df = 4 (P = 0.37); I² = 6%								
Test for overall effect: Z = 0.34 (P = 0.73)								
Total (95% CI)			**1063**			**1105**	**100.0%**	**1.87 [-1.39, 5.13]**
Heterogeneity: Tau² = 0.00; Chi² = 6.96, df = 10 (P = 0.73); I² = 0%								
Test for overall effect: Z = 1.12 (P = 0.26)								
Test for subgroup differences: Chi² = 0.20, df = 1 (P = 0.66), I² = 0%								

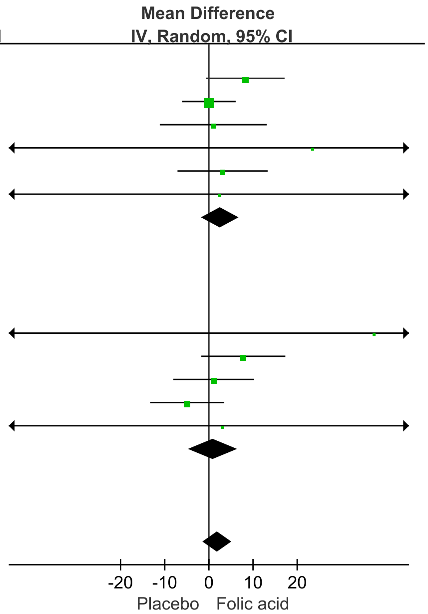

Fig. 3. Effects of folic acid and folic acid plus zinc supplements on sperm concentration in infertile men.

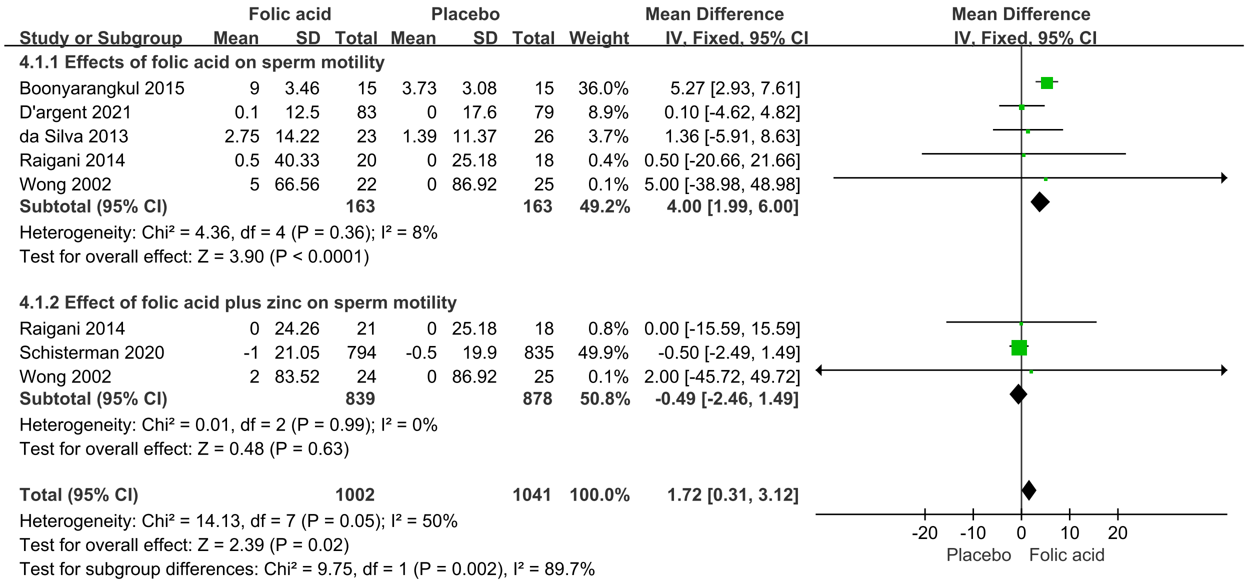

Fig. 4. Effects of folic acid and folic acid plus zinc supplements on sperm motility in infertile men.

−0.55 to 0.33; $P = 0.63$) (Fig. 5).

3.4.4. Secondary (pregnancy) outcomes

Two RCTs reported on the effects of folic acid and zinc supplements on pregnancy outcomes. One study found that folic acid and zinc supplements had no statistically significant effect on outcomes, including the detection of β-human chorionic gonadotropin hormone, clinical intrauterine pregnancy, ectopic pregnancy, multiple pregnancies, early pregnancy loss, cesarean delivery, pre-eclampsia or gestational hypertension, gestational diabetes, gestational age, birth weight, orpremature infant. However, this same study observed a significant increase in preterm births with overall folic acid and zinc supplements (67 [6%] vs. 45 [4%] in the placebo group; risk difference, 1.9% [95% Cl, 0.2%–3.6%) risk difference, 1.9% [95% CI, 0.2%–3.6%]). Furthermore, the study also reported that folic acid and zinc supplements did not have a significant impact on stillbirth, neonatal morbidity, neonatal mortality, or severe postpartum maternal morbidity. However, the study reported 29 cases of structural malformations, with 26 at birth and 3 occurring as pregnancy losses. Of these malformations, 21 were considered major defects, 6 had a known genetic cause, and 2 were unclassifiable. In terms of adverse events, the folic acid and zinc group had a higher incidence of adverse events compared to the placebo group (32% vs. 27% in the placebo group). They were primarily gastrointestinal symptoms and erythema. Furthermore, the study recorded a total

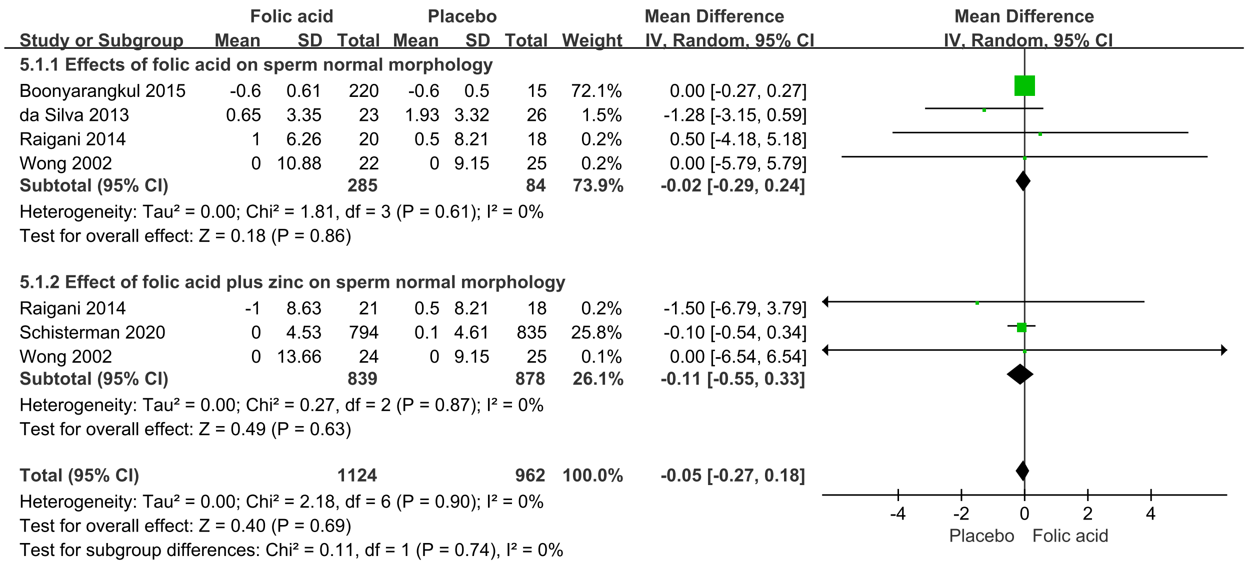

Fig. 5. Effects of folic acid and folic acid plus zinc supplements on sperm normal morphology in infertile men.

of 12 serious adverse events, with 7 occurring in the folic acid and zinc group and 5 in the placebo group. However, none of the serious adverse events were related to the intervention [25].

According to another study, the total number of pregnancies (spontaneous and IVF-ICSI) for the intention-to-treat population (N = 162) was 26 in the folic acid group and 15 in the placebo group (31.33% vs. 18.99%, $P = 0.071$) [24]. Between randomized grouping and IVF-ICSI attempts, five spontaneous pregnancies were observed in each group. After IVF-ICSI, the number of biochemical pregnancies (serum hCG >100 IU/mL) was 26 and 11 in the folic acid and placebo groups, respectively ($P = 0.02$). The folic acid group had higher biochemical pregnancy rates than the placebo group for extracted oocytes (38.8% vs. 16.9%, $P = 0.005$). Moreover, the biochemical pregnancy rate by embryo transfer was significantly higher in the placebo group (44.1% and 22.4%, respectively, $P = 0.018$). Although clinical pregnancy rates by embryo transfer tended to be higher in the folic acid group, the difference did not reach statistical significance (35.6% vs. 20.4%, $P = 0.082$). The early pregnancy loss rate was comparable in both groups (2.0% vs. 8.5%, $P = 0.146$).

4. Discussion

Male infertility is a complex condition that affects numerous couples worldwide, and deficiencies in micronutrients such as folic acid and zinc may contribute to it. There is research on the effects of folic acid and folic acid plus zinc supplements on sperm characteristics and pregnancy outcomes of infertile men. To investigate this further, we conducted a systematic review and meta-analysis of the available evidence. Our analysis indicated that folic acid plus zinc supplements did not have a significant effect on sperm characteristics in infertile men. However, folic acid alone may improve sperm motility in infertile men and lead to improved outcomes in IVF-ICSI.

Our study supports previous findings that folic acid supplements have a positive impact on sperm quality. Wong et al. observed that zinc sulfate and folic acid increased the total number of normal sperm in infertile men, regardless of their fertility level [18]. Similarly, Ebisch et al. found that folic acid and zinc sulfate significantly increased sperm concentration in infertile men with a specific genetic polymorphism [19]. Ebisch et al. also reported a significant increase in sperm concentration in men with low fertility who received folic acid and zinc sulfate supplements [20]. Raigani et al. further demonstrated that folic acid and zinc sulfate supplements increased sperm concentration in infertile men who received combined treatment or folic acid treatment only [22]. However, only one study by Boonyarangkul et al. showed that folic acid alone improved sperm viability after three months of treatment, which was consistent with our study. This is potentially due to reduced sperm DNA fragmentation and diminished sperm damage [23].

Our research adds to the existing body of evidence demonstrating that folic acid supplements can have a positive impact on sperm motility in infertile men. This may be due to the role of folic acid in reducing oxidative stress and improving antioxidant activity [26]. Oxidative stress has been shown to impair sperm motility by damaging the cell and mitochondrial membrane of sperm cells, leading to decreased energy production and disrupted ion channels, which are necessary for sperm motility [27–29]. Folate has been shown to scavenge free radicals and prevent oxidative damage to proteins and DNA [30]. Additionally, animal studies suggest that folate supplements can increase antioxidant enzyme activity, potentially by acting as a co-factor for enzymes involved in antioxidant production such as glutathione. Glutathione is an effective antioxidant that can neutralize ROS and prevent oxidative stress, which may contribute to the improvement of sperm vitality in patients [31].

Research has demonstrated that folic acid plays a significant role in DNA synthesis by participating in the synthesis of the nucleotide precursor molecule 5,10-methylene tetrahydrofolate [32]. This molecule provides a one-carbon unit for the synthesis of purine and pyrimidine nucleotides, which are used to construct new DNA chains [33]. Furthermore, folic acid is crucial in producing S-adenosyl methionine (SAM), which provides a methyl group for DNA methylation [34]. Thus, folic acid is important for sperm DNA synthesis and methylation, which impacts sperm characteristics and fertilization ability in patients.

Zinc, an essential nutrient, serves as a cofactor for over 80 metalloenzymes that are involved in synthesizing large molecules such as DNA and tRNA [35]. Studies have shown that zinc is essential for testicular development and that seminal plasma zinc concentration affects various sperm characteristics [36]. Additionally, folate-related genes have been linked to male infertility [37], and folate deficiency may lead to oxidative stress, disrupted methylation reactions [32], insufficient protein synthesis, and inadequate sperm production [38]. Zinc's effectiveness through 5α-reductase is also critical for testosterone synthesis in Leydig cells [31].

Excess ROS can cause harmful effects on sperm membranes due to the rich content of polyunsaturated fatty acids. This leads to reduced sperm motility, structural DNA damage, and cell apoptosis [27]. Folic acid and zinc act as antioxidants, thus mitigating the excess production of ROS and its harmful effects [26,39]. However, our study found no significant beneficial effects from the combined treatment. This could be due to inadequate dosage or durations of treatment. Higher doses or longer durations of treatment may be needed for a positive outcome. The combined treatment may not be effective for all types of infertility. Additionally, the mechanism of action of folic acid and zinc remains unclear. They may even have neutralizing or antagonistic effects on each other. Therefore, further investigation is required.

Several studies have investigated the effects of folic acid and zinc supplements on pregnancy outcomes in infertile men. One study involving couples seeking infertility treatment did not find significant improvements in semen quality or live birth rates for male partners who took folic acid and zinc supplements compared with those who took a placebo [26]. However, another randomized controlled study found that high-dose folic acid supplements in men with infertility who required IVF-ICSI led to improved outcomes. Although these results are promising, further research is needed to determine the optimal dosage and duration of supplementation and underlying mechanisms [25]. The evidence regarding the efficacy of antioxidants in treating infertility in men is currently uncertain. Therefore, further large, well-designed randomized placebo-controlled trials that study infertile men and report on pregnancies and live births are needed to clarify the exact role of antioxidants.

Additionally, we have noted the potential benefits of folic acid and zinc in the cryopreservation of semen. Research has found that supplementing the cryopreservation medium with these nutrients can improve the quality and survival of sperm post-thaw [40]. A study reported that zinc supplements improved the quality of cryopreserved bovine sperm by minimizing oxidative stress and preserving sperm membrane integrity [41]. Similarly, human studies have shown that including folic acid and zinc in the cryopreservation medium can improve the viability, motility, and DNA quality of sperm [42]. These findings suggest that folic acid and zinc may have a protective effect on reproductive cells during cryopreservation, which could potentially enhance the success rates of semen-freezing procedures. However, further research is needed to determine the optimal dosage and duration of supplementation and the underlying mechanisms of action.

It is noteworthy that there are additional factors that may also affect male fertility, such as BMI, drug abuse, hormone usage, and biochemical variables. Also, studies have not fully addressed genetic diseases that can impact male fertility. Therefore, further research is needed to gain a better understanding of the potential impact of these factors on fertility and to develop effective interventions for infertile men.

Our study has several limitations. The effectiveness of folic acid and zinc supplements may have been influenced by variations in dosage, duration, and formulations used in different studies included in the meta-analysis. Furthermore, differences in determining sperm characteristics according to the WHO guidelines may have affected the results.

The meta-analysis did not find significant improvements in sperm characteristics with folic acid plus zinc supplements, but folic acid alone showed potential in improving sperm motility and IVF-ICSI outcomes. This suggests that folic acid supplements alone may be a viable treatment option for male infertility. Further research is needed to determine the optimal dosage and duration of folic acid supplementation and the underlying mechanisms that improve sperm motility. Clinicians should consider recommending folic acid supplementation to infertile men undergoing IVF-ICSI, but should also consider addressing other factors that may be contributing to their infertility.

5. Conclusion

Based on this meta-analysis, there was no significant improvement in sperm characteristics when folic acid plus zinc supplements were used. However, folic acid alone has demonstrated the potential to improve sperm motility and IVF-ICSI outcomes. This indicates that folic acid supplementation alone may be a viable treatment option for male infertility. Clinicians recommending folic acid supplements to infertile men undergoing IVF-ICSI should also consider addressing other factors that may contribute to their infertility.

Author contribution statement

All authors listed have significantly contributed to the development and the writing of this article.

Data availability statement

Available in repository. PROSPERO 2021: CRD42021284913.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e18224.

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