REVIEW

The Effect of Folate and Folate Plus Zinc Supplementation on Endocrine Parameters and Sperm Characteristics in Sub-Fertile Men: A Systematic Review and Meta-Analysis

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Purpose: To evaluate the effect of folate and folate plus zinc supplementation on endocrine parameters and sperm characteristics in sub fertile men.

Materials and Methods: We conducted a systematic review and meta-analysis. Electronic databases of Medline, Scopus , Google scholar and Persian databases (SID, Iran medex, Magiran, Medlib, Iran doc) were searched from 1966 to December 2016 using a set of relevant keywords including "folate or folic acid AND (infertility, infertile, sterility)".All available randomized controlled trials (RCTs), conducted on a sample of sub fertile men with semen analyses, who took oral folic acid or folate plus zinc, were included. Data collected included endocrine parameters and sperm characteristics. Statistical analyses were done by Comprehensive Meta-analysis Version 2.

Results: In total, seven studies were included. Six studies had sufficient data for meta-analysis. "Sperm concentration was statistically higher in men supplemented with folate than with placebo ($P < .001$)". However, folate supplementation alone did not seem to be more effective than the placebo on the morphology ($P = .056$) and motility of the sperms ($P = .652$). Folate plus zinc supplementation did not show any statistically different effect on serum testosterone ($P = .86$), inhibin B ($P = .84$), FSH ($P = .054$), and sperm motility ($P = .169$) as compared to the placebo. Yet, folate plus zinc showed statistically higher effect on the sperm concentration ($P < .001$), morphology ($P < .001$), and serum folate level ($P < .001$) as compared to placebo.

Conclusion: Folate plus zinc supplementation has a positive effect on sperm characteristics in sub fertile men. However, these results should be interpreted with caution due to the important heterogeneity of the studies included in this meta-analysis. Further trials are still needed to confirm the current findings.

Keywords: folate; folic acid; zinc sulfate; male infertility; sub fertility; sperm; endocrine.

INTRODUCTION

Sub fertility is defined as the failure to get pregnant after one year of regular, unprotected intercourse[1-2]. Approximately 15% of couples are sub fertile, a male factor being involved in half of the cases. It is estimated that, in the general population, one in twenty men is sub fertile[1-4]. Coping with a permanent childlessness, because of sub fertility, can be very difficult for couples[5,6], who would, therefore, resort to assisted reproduction techniques (ART). Yet, these techniques do not treat the cause of sub fertility. A large number of andrologic disorders remain unexplained, a condition named idiopathic male infertility which is mainly related to changes in lifestyle, environmental exposure and eating habits.[2,7-9] Some researchers observed an enhancement in semen parameters with the use of antioxidants, suggesting that such substances minimize the toxic effects of oxidative stress in spermatozoa. One of these antioxidant agents is folate, a vitamin from the B group involved in a large number of biochemical processes, particularly in the DNA synthesis (10). Indeed, DNA synthesis is important for the development of spermatozoa. Several enzymes involved in DNA synthesis are zinc or vitamin B dependents[11]. Folate, is implicated in the oxidative pathway. Derangements in the oxidative pathway play a role in the pathogenesis of sub fertility. It has been reported that folic acid, the synthetic form of the folate, effectively scavenges oxidizing free radicals, and, as such, can be regarded as an antioxidant[12]. Despite its water-soluble character, folic acid inhibits lipid peroxidation (LPO). Therefore, folic acid can protect bio-constituents such as cellular membranes or DNA from free radical damage[13]. Zinc is also an important micronutrient that serves as a cofactor for more than 80 metalloenzymes involved in DNA transcription and protein synthesis[14]. Moreover, zinc finger proteins are implicated in the genetic expression of steroid hormone receptors[15], and also have antioxidant[16] and antiapop-

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Table 1. Characteristics of randomized controlled trials included in the systematic review.

Author, Country, Year	Duration ; Wk	Mean age, Y	Dosage zinc\folicacid	design	Cause of male infertility	Outcome	drop out%	Type of interventions	Treatment		Control	randomization technique	Treatment Blinding	Baseline Major comparability relevant findings
Azizollahi, Iran, 2013	26	29.07±6.8	66 and 5 mg	A prospective randomized controlled study	varicocele	semen parameters1) number Normal 2) sperm morphology 3)Sperm motility 4)progressive sperm	30% (112\160)	Zinc sulfate (ZS), Folic acid (FA), Zinc sulfate/Folic acid (ZF), and placebo (PL).	26FA 29ZF	25PL 32ZS	NO randomaly allocated	YES	YES	Folic acid increased the sperm number FA vs. Placebo ($p < 0.05$) co-administration of zinc and folic acid improvementin sperm number, motility and sperm progression ($p < 0.05$)
Silva, Brazil, 2013	26	35.3 ±7.7	5mg folic acid	randomized, double-blind and placebo-controlled clinical trial	oligozoospermy, astenozoospermy, teratozoospermy oligo-astheno-teratozoospermy	1) sperm concentration 2) sperm vitality 3) motility 4) morphology	30% (70/49)	folic, placebo	23FA	26PL	NO	YES	YES	Folic acid does not improve semen
Raigani, Iran, 2013	16	unclear	220/5mg	intervention randomized, double-blind clinical trial	oligoasthenoter-atozoos permic (OAT)	1)Sperm concentration, 2)motility 3)morphology 4)sperm viability 5)sperm mitochondrial Function 6)sperm chromatin status using toluidine blue, 7)aniline blue, 8)acridine orange and chromomycin A3 staining; and 9)semen and blood folate, zinc, B12, 10)total antioxidant capacity (TAC) 11)malondialdehyde (MDA) concentrations	0%	folic /placebo, folic /zinc , zinc /placebo, placebo/ placebo.	21ZF 20FA	18PL 24ZS	NO randomly allocated	YES	YES	did not improve sperm functional parameters(p=0/05)
Ebisch, Nederland, 2003	26	unclear	66 and 5 mg	Double-blind, placebo-controlled intervention study	unclear	1)Sperm concentration, 2)motility 3)morphology Prevalence of C677T MTHFR polymorphism	0%	Zinc sulfate (ZS), Folic acid (FA), Zinc sulfate/Folic acid (ZF), and placebo (PL	19ZF 24F	23Z 21PL	Yes	Double-blind	YES	sperm count was found only in the combined folic acid and zinc sulfate intervention group increases in sperm concentration in wild-types for the C677T MTHFR (P.005
Wong, Netherlands, 2002	26	34. 2 ± 4.2	66 and 5 mg	Double-blind, placebo-controlled interventional study	unclear	1)Sperm concentration, 2)motility 3)morphology 4)blood folate, zinc	fertile 8% infertile 9%	folic /placebo, folic /zinc zinc /placebo or placebo/ placebo	24ZF/PL 22FA/pL 23ZS/pL 25PL/pL	26ZF/PL 24FA/pL 25ZS/pL 24PL/pL	Yes	YES	YES	sperm count increases after combined zinc sulfate and folic acid in both sub fertile and fertile men.
Ebisch,, Netherlands, 2005,	26		66 and 5 mg	double-blind, placebo-controlled intervention study		1)Sperm concentration, 2)motility 3)morphology 4) serum folate, zinc 5)follicle-stimulating hormone (FSH), 6)testosterone 7) inhibin B		placebo, folic acid/zinc	18ZF 22PL	24F/Z 23PL	No	YES	No	increased sperm concentration in sub ertile males Other semen and endocrine parameters were not affected by intervention
Nematollahi, Iran, 2012	26	29.07±6.8	66 and 5 mg	A prospective randomized controlled study	varicocele	serum hormonal level 1) inhibin b , 2) FSH , 3)testosterone, 4) nitric oxide, 5)superoxide dismutase, 6)total antioxidant capacity		Zinc sulfate (ZS), Folic acid (FA), Zinc sulfate/Folic acid (ZF), and placebo (PL).	26FA 29ZF	25PL 32ZS	NO randomly allocated	YES	YES	zinc sulfate and folic acid co-administration, rise in inhibin B and seminal plasma activity ($P \leq 0.05$). improvement in SOD activity in zinc sulfate and folic acid co-

totic properties[17]. Furthermore, it has been shown that zinc is important in testicular development, spermatogenesis and sperm motility, as reviewed by Wong et al. (2000)[18]. Recently, some studies have demonstrated a 74% increase in normal sperm count and a 3% increase in abnormal morphology in sub-fertile men after

Table 2.Subgroup analyses of the effects of folic acid/ zinc and only folic acid on endocrine parameters and sperm characteristics in sub fertile men .Cochrane Q value, p-value, effect size and I-squared.

intervention	Variable	Reference	Number of RCTs	Random effect model SMD (95% CI)	p-value	Q- value	I-squared	p-value for heterogeneity
folic acid and Zinc	Sperm concentration	(19, 21, 24, 33)	4	1.829 (0.969 to 2.690)	0.000	17.535	82.892	0.001
	Motility	(19, 21, 33)	3	0.595(-0.253 to 1.442)	0.169	11.817	83.075	0.003
	Morphology	(19, 21, 33)	3	1.095(0.737 to 1.452)	0.000	0.261	0.000	0.878
	FSH	(20, 24)	2	-0.430(-0.867 to0.007)	0.054	1.122	10.867	0.290
	Testosterone	(20, 24)	2	0.151(-0.600 to1.902)	0.866	16.589	93.972	0.000
	Inhibin B	(20, 24)	2	0.114(1.005 to 1.233)	0.842	7.153	86.019	0.007
	serum folate level	(21, 24)	2	3.693(2.964 to 4.421)	0.000	0.785	0.000	0.376
Folic acid	Sperm concentration	(19, 21, 22, 33)	4	2.075(0.939 to 3.211)	0.000	28.942	89.635	0.000
	Motility	(19, 21, 22, 33)	4	-0.089(-0.473 to 0.296)	0.652	5.148	47.721	0.161
	Morphology	(19, 21, 22, 33)`	4	-0.286(-0.579 to0.008)	0.056	2.695	0.000	0.441

supplementation of folic acid in combination with zinc sulphate[19].

Only limited knowledge is available on the impact of folic acid and zinc on sub fertility. There has been a growing interest in folic acid and zinc among sub-fertile men according to scientific literature[14]. Some studies have demonstrated the importance of the effects of folic acid and zinc on spermatogenesis[19-21]. The effect of folic acid has been comprehensively assessed in several Randomized Control Trial (RCT) studies and showed a range from no beneficial effect[22] to significant effects [19-21,23,24]. Yet, the essential mechanisms involved in the useful effects of synthetic folic acid and zinc on spermatogenesis remain unclear. Folic acid and zinc may affect endocrine parameters, for example, by stimulating the function of the Sertoli cells. These cells provide the necessary microenvironment for normal germ cell production. Sertoli cells are the main producers of inhibin B in the human body. The serum inhibin B concentration is positively associated with sperm concentration, testicular volume and the state of the spermatogenetic epithelium[25,26]. Moreover, inhibin B appears to be a marker of advanced stages of spermatogenesis[27,28] Thus, the inhibin B concentration reflects the quality of the Sertoli cell function and spermatogenesis and, as such, can be used as a sensitive marker of spermatogenesis in humans.

A systematic review by Forges and Barbarino[29] showed a possible role of folates in male sub fertility. However, there is a dearth of systematic reviews on the effects of micronutrient supplementation on sperm parameters, the current systematic review and meta-analysis was conducted to assess the effect of folic acid and folate plus zinc on endocrine parameters including inhibin B, FSH and testosterone as well as sperm characteristics such as sperm concentration, morphology and motility in sub fertile men.

MATERIALS AND METHODS

Data sources and search strategy

MEDLINE (1966 to December 2016) and Scopus (1990 to December 2016), were searched for published randomized controlled trials (RCTs). Search keywords were "folat or folic acid AND (infertility, infertile, sterility)". Persian databases (SID, Iran medex, Magiran, Medlib, Iran doc) and Google Scholar were also searched using equivalent keywords until December 2016. 47 pages and 476 results were considered in google scholar. In addition, reference section of relevant trials, systematic reviews and meta-analysis were manually checked to identify further trials missed by electronic search. Authors were contacted for additional missing data. Publication bias was assessed by funnel plots and Egger's test. The selection process of RCTs included in the systematic review is described in **Figure 1**.

Inclusion criteria

Trials were included in the systematic review if they met the following criteria:

(1) Conducted on a sample of sub fertile men with semen or endocrine analyses.

(2) Designed as a randomized controlled trial (RCT).

(3) Compared oral folic acid supplementation as the mono-preparations or in combination with zinc sulfate in the intervention group regardless of the control group type.

Outcome measures

Primary outcome measures included:

Semen parameters: 1) Sperm concentration, 2) Sperm motility, and 3) Sperm abnormal morphology

Endocrine parameters:1) Follicle stimulating hormone (FSH), 2) Inhibin B, and 3) Testosterone

Secondary outcome measure included blood serum level of folate.

Data extraction

For each study, we extracted the following data according to a pre-defined checklist: first author, study design, study duration and quality of trials. The latter was assessed by two reviewers using Oxford Center for Evidence Based Medicine checklist for therapeutic studies. Data were independently assessed by two authors and disparities were resolved by discussion with a third researcher.

Quality assessment of the included studies

The quality of the included studies was evaluated by Oxford Center for Evidence Based Medicine checklist for RCTs.[30](**Table 1**)

Risk of bias assessment

Two authors independently assessed the risk of bias for each study using the criteria outlined in the Cochrane Handbook for Systematic Reviews of Interventions. [31] The Cochrane risk of bias tool consists in six domains: Random sequence generation; allocation concealment;

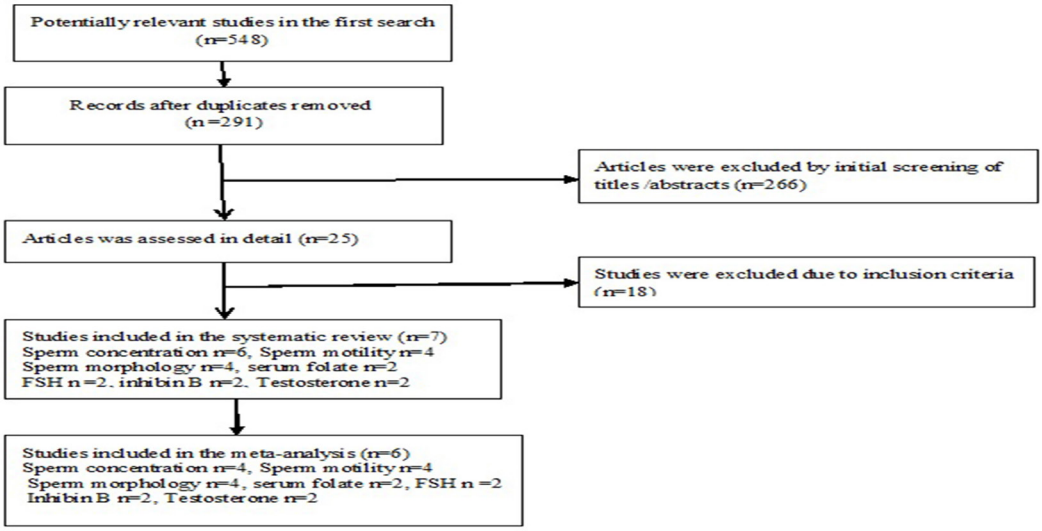

Figure 1. PRISMA Flow chart for systematic review and meta-analysis.

blinding of participants and personnel, blinding of outcome assessment; incomplete outcome data; selective outcome reporting; and other source of bias. Risk of bias for each domain was assessed as low, unclear or high. Disagreements were resolved by discussion, and, if necessary, further information was sought from the primary author.

Description of risk of bias

The risk of bias for all studies is summarized in **Figure 2**. **Figure 3** illustrates the risk of bias for each study. Random sequence generation and allocation concealment were assessed as adequate in two studies.[19, 32] Ebisch et al.(2003) considered the issue of allocation concealment using computer -generated random numbers. Wong et al.(2002) reported generating an adequate allocation concealment through using blinded medications kept in hospital's pharmacy. Based on the randomization list, capsules were coded by the hospital pharmacy and distributed to the research assistant who would prescribe them to the patients. Subfertile men received capsules coded from 1 to 105 and fertile men capsules coded from 501 to 605. Each participant was given the randomized code in order of intake and receive one bottle containing folic acid (5 mg) or placebo capsules and a second bottle containing zinc sulfate (66 mg) or placebo capsules. Allocation concealment was not reported in the studies done by Azizollahi et al. (2014) , Nematollahi (2012), Silva (2013), Raigani (2013) and Ebischet al. (2005).

Blinding of participants was reported in all studies. Wong et al. (2002) conducted a double-blind, randomized, controlled trial in fertile and sub fertile men to determine whether administration of folic acid, with or without the addition of zinc sulfate, increased semen quality. The studies of Silva (2013), Nematollahi (2012) and Raigani (2013) were also double-blind. Blinding of the outcome assessment, for semen analysis and sperm function, was carried out individually and blindly by two laboratory experts in the study of Raigani (2013). The other six included studies had an unclear risk of detection bias, as the blinding of outcome assessment

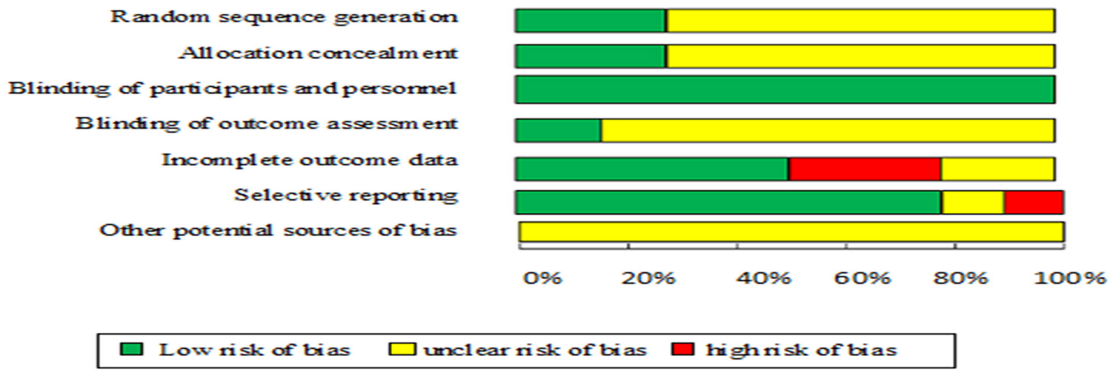

Figure 2. Risk of bias graph: Systematic review. Authors' judgments of each risk of bias item presented as percentages across all included studies.

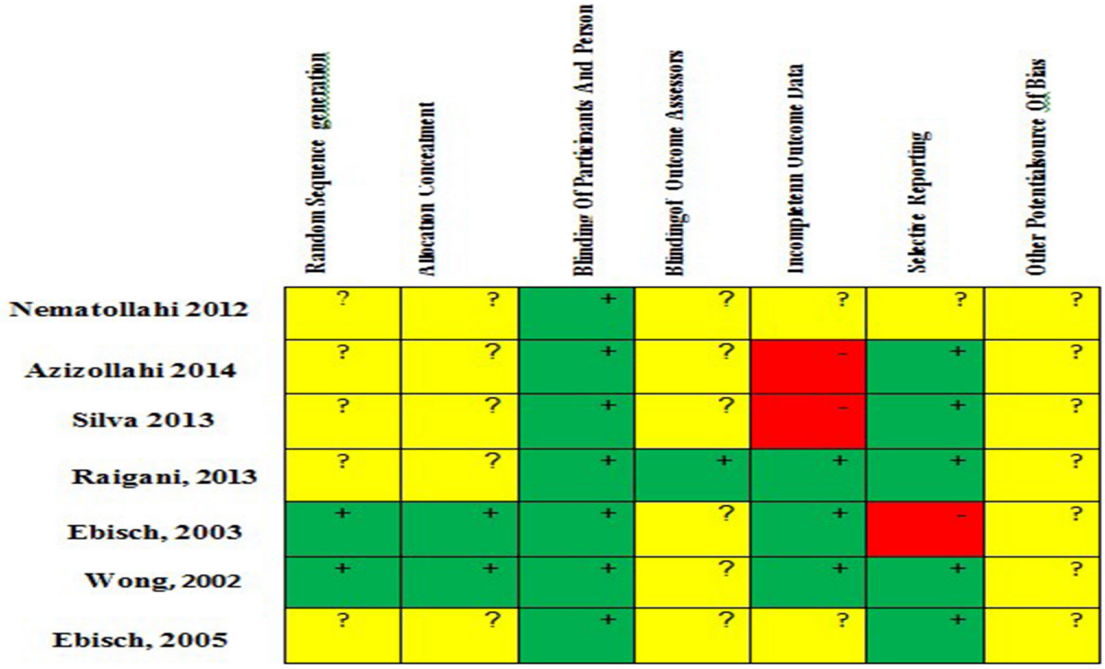

Figure 3. Risk of bias summary: Systematic review. Authors' judgments of each risk of bias item for each included study.

to group allocation was not mentioned. Raigani et al. (2013), nd Ebisch et al .(2003) experienced no participant drop out. Azizollahi et al. (2014) and Silva et al. (2013) reported a 30% rate of drop out and considered having a high risk of attrition bias. Wong (2002) reported 8% of drop out. Nematollahi (2012) and Ebisch (2005) did not report any drop out. Azizollahi et al. (2014), Ebischet al. (2005) , Silva et al. (2013) and Wong et al. (2002) adequately reported their outcomes; however, the two other studies were considered to have a high risk of reporting bias. Nematollahi et al.(2012) reported that data were statistically analyzed but significant p-value was not found. Ebischet al. (2003) reported the results in figures where the outcome data for the mean and standard deviations of semen parameters were not reported. Some authors gave little information on the eventual bias. such as which supplementary food and which type of diet the control group received.

Statistical analyses

We estimated the difference between means in two ways: difference in means (MD) and standardized dif-

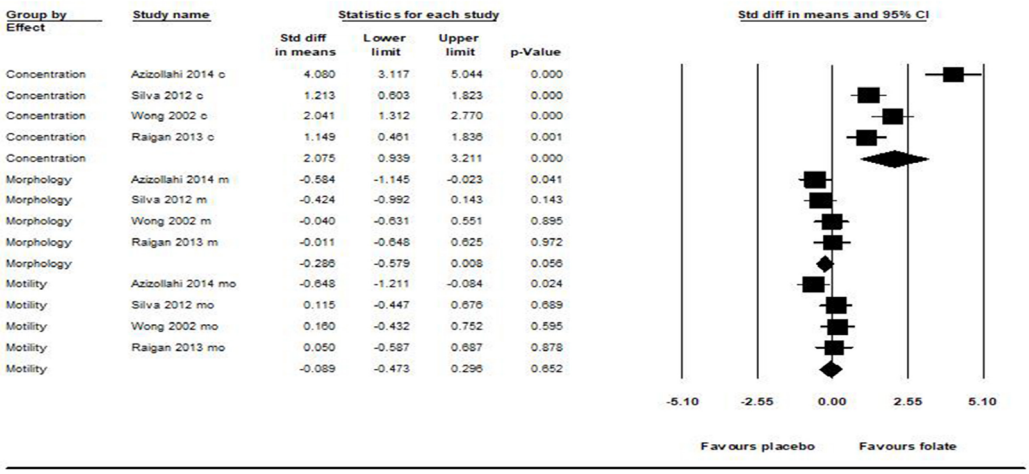

Figure 4. Effects of folic acid only supplementation on sperm characteristics , the horizontal lines denote the 95% CI, ■ point estimate.

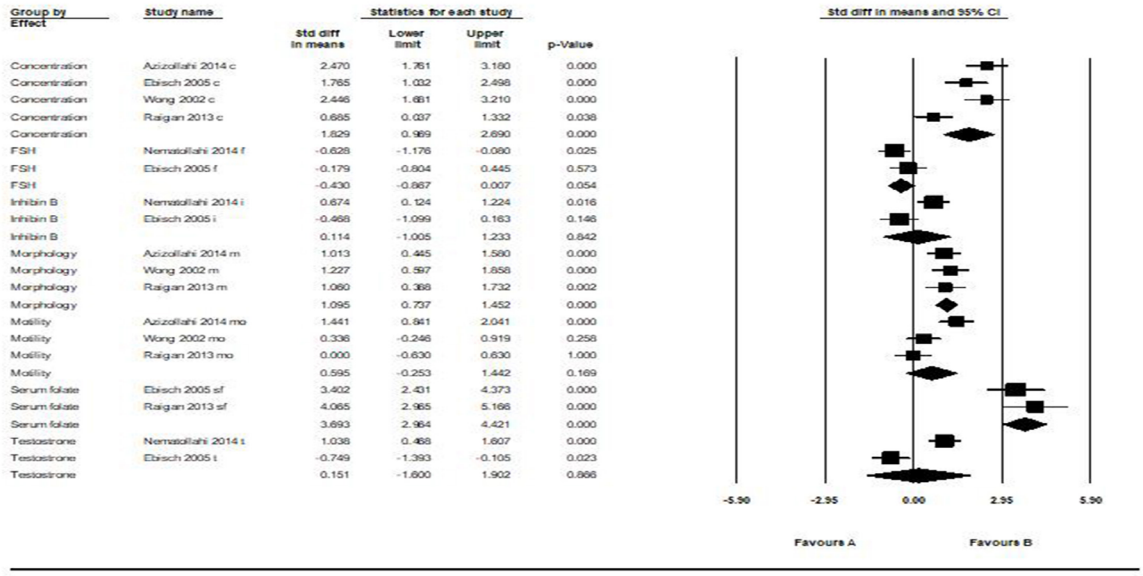

Figure 5. Effects of folic acid/ zinc supplementation on sperm characteristics and endocrine parameters, the horizontal lines denote the 95% CI, ■ point estimate.

ference in means (SMD). The latter was used when studies included in the meta-analysis measured the same outcome by different measurement units. Changes in mean (Sperm concentration, motility, abnormal morphology, serum folate, FSH, Inhibin B, testosterone) at baseline and endpoint were assessed.

The main effect size used in this meta-analysis was standardized difference in means of changes in variables in the folate and placebo groups. We used the Cochrane recommendations for effect size calculations[24]. We interpreted the results using random effects model (Der-simonian and Laird method) because of the large heterogeneity among the trials included in this meta-analysis. For heterogeneity evaluation, Cochrane Q test ($P < .05$ as statistically significant) and I-squared index were used. The latter was used to assess how much of the variance across studies was likely to be real and was not due to sampling errors. All statistical analyses were done by Comprehensive Meta-analysis Version 2 (Biostat, Englewood, NJ, USA).

RESULTS

Out of 548 relevant publication trials, seven RCTs [19-22, 24,32,33] met the inclusion criteria.

Of these seven studies, six RCTs reported sperm concentration parameter, four RCTs reported sperm motility and sperm morphology and two RCTs reported serum folate, FSH, inhibin B and Testosterone. Six trails had sufficient data for meta-analysis, of which four RCTs[19,21,22,33] had reported sperm concentration, sperm motility, and sperm morphology and two RCTs [20,24] had reported serum folate, FSH, inhibin B and testosterone (**Figure 1**). The summarized characteristics of the included studies are shown in **Table 1**.

Figure 4 shows the forest plots of the meta-analysis of folic acid only supplementation studies. As shown in the figure, the effect of folate on the concentration was statistically higher than the placebo (2.07; [95% CI: 0.939 to 3.21], $P < .001$; heterogeneity I2 = 89 %; $P < .001$). From the four trials included in our meta-analysis [19,21,22,33], the RCT by Ebisch et al.[32] was not included in the quantitative analysis due to incomplete reporting. This study included one hundred and thirteen fertile and 77 sub fertile males. The interventions consisted in 26 weeks of a daily dose of folic acid (5 mg) or placebo. Before and after the intervention, one standardized semen sample was obtained from every participant for semen analysis according to the World Health Organization (WHO) guidelines.[30,34] The results of the study showed no statistically significant increase in sperm concentration in men who received folate supplementation alone as compared to the placebo. Moreover, folate supplementation alone did not seem to be more effective than placebo on the morphology (-0.286; [95% CI: -0.579 to 0.008], $P = .056$; heterogeneity I2 = 0 %: $P = .44$) and motility of the sperms (-0.089 [95% CI: -0.473 to 0.0.296], $P = .652$; heterogeneity;I2 = 41 %: $P = .161$).

Figure 5 shows the forest plots of the meta-analysis of the folate plus zinc supplementation studies. Folate plus Zinc supplementation did not show any statistically different effect on serum testosterone (0.151 ; [95% CI: -1.6 to 1.9], $P = .86$; heterogeneity I2 = 93 %; $P < .001$), inhibine B(0.114 ; [95% CI: -1.0 to 0.32], $P = .84$; heterogeneity I2 = 86 %; $P = .007$), FSH(-0.430 ; [95% CI: -0.8 to 0.007], $P = .054$; heterogeneity I2 = 10 %; $P = .29$), and sperm motility (0.595 ; [95% CI: -0.25 to 1.44], $P = .169$; heterogeneity I2 =83 %; $P = .003$) as compared to the placebo [20,21,24,33]. On the other hand, folate plus zinc showed a statistically higher effect on sperm concentration (1.829 ; [95% CI: 0.969 to 2.690], $P < .001$; heterogeneity I2 = 82 %; $P < .001$), morphology (1.095 ; [95% CI: 0.737 to 1.452], $P < .001$; heterogeneity I2 = 0 %; $P = .87$), and serum folate level (3.693 ; [95% CI: 2.964 to 4.421], $P < .001$; heterogeneity I2 = 0 %; $P = .376$) as compared to placebo. For sperm concentration, out of the four trials included in this meta- analysis [19,21,24,33] , the trial by Ebisch et al.32 was not included in the quantitative analysis due to incomplete reporting. This study included one hundred and thirteen fertile and 77 sub fertile males.

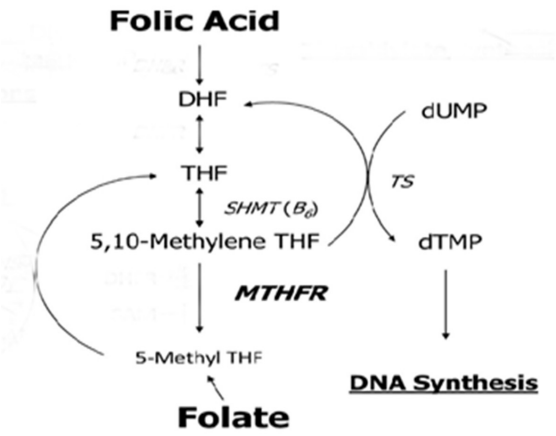

Figure 6. Folic acid metabolism. This schematic shows the process by which folate/folic acid is used for DNA synthesis. The MTHFR 677C/T variant reduces enzyme activity (37) . DHF, dihydrofolate ; dTMP, thymidylate; dUMP, deoxyuridine monophosphate; MTHFR, methylenetetrahydrofolate reductase; THF, tetrahydrofolate ;TS, thymidylate synthase.

The interventions consisted in 26 weeks of a daily dose of zinc sulfate and folic acid (combined), or placebo. The study showed a statistically significant increase in sperm concentration in patients supplemented with folate plus zinc as compared to the placebo (P=.005). Table 2 shows the Cochrane Q values and I-squared indices of the meta-analysis.

DISCUSSION

Folate had a positive effect on sperm concentration in sub fertile men. The meta-analysis also showed that folate plus zinc supplementation had a positive effect on sperm concentration, morphology and serum folate level in sub fertile men. Folate is important for the synthesis of DNA, transfer RNA and the amino acids cysteine and methionine. It has also been reported that folate effectively cleans oxidizing free radicals, and, as such, can be regarded as an antioxidant.[11] Therefore, folic acid can protect bio-constituents such as cellular membranes or DNA from free radical damage[11]. When folic acid is administered, it has to be converted into the biologically active form 5-methyltetrahydrofolate to use its functions. This conversion is done by MTHFR, one of the important enzymes in folate metabolism. MTHFR converts 5, 10-methylenetetrahydrofolate into 5-methyl tetrahydrofolate, which afterwards provides a methyl group to cobalamin, to form methylcobalamin and tetrahydrofolate. Methylcobalamin serves as a cofactor in the conversion of 5-methyl tetrahydrofolate to tetrahydrofolate, in which homocysteine is remethylated to form the essential amino acid methionine in human (**Figure 6**). In the MTHFR gene, a common polymorphism, resulting from a cytosine to thymine substitution (C677T), may be present.[14] The incidence of heterozygosity or homozygosity for this variation is around 40 and 10% in Caucasians[35], and this amount varies between populations. This polymorphism is associated with reduced activity of MTHFR, resulting in a remaining enzyme activity of 65% for heterozygous carriers and of only 30% for homozygous carriers.[36, 37] People with the homozygous genotype have significantly higher plasma homocysteine concentrations compared to heterozygotes, especially when folate concentrations are low[38]. The C677T polymorphism in the MTHFR gene is accompanied by an altered folate metabolism and an impaired homocysteine remethylation, resulting in an increased folate need. The effect of supplementation with folic acid on semen parameters is, therefore, likely to be dependent on the MTHFR genotype. The impact of MTHFR gene on infertility risk has been studied in various ethnic groups.[32,39-49] Most commonly studied MTHFR, c.677C.T, has been established as a risk factor for male infertility in some populations.[44,46-48,50-52]. Stern et al. found that the 677T-homozygous genotype was associated with lower DNA methylation capacity compared with the 677C-homozygous genotype.[53] Bezold et al. reported a higher frequency of the homozygous 677T MTHFR genotype in infertile men and suggested that products of MTHFR may have a role in the pathogenesis of male infertility. They furthermore stated that homozygous men, in particular, may benefit from folic acid supplementation[52]. Mfady et al. showed an association between MTHFR 677TT genotype and male infertility.[54]

Animal in vivo and in vitro studies have stated that zinc deficiency reduces the absorption and metabolism of dietary folate (55, 56) because of its function as a cofactor for the folate-metabolizing. If zinc itself is not a cofactor of the MTHFR enzyme, it is, however, a cofactor for methionine synthesis and for betaine homocysteine methyltransferase. Although folic acid and zinc both have antioxidant properties; this is still another mechanism whereby these micronutrients can affect apoptosis, as it seems that oxidative stress could influence apoptosis. The inhibitory effects of zinc on apoptosis have been assumed to contain two mechanisms: early in the apoptotic pathways, zinc may inhibit caspases (proteases involved in programmed cell death), whereas later in the apoptotic chain of events, zinc may stop calcium- and magnesium-dependent endonucleases, which cause apoptotic DNA fragmentation.[17,57] Our meta-analysis showed that some sperm characteristics including sperm concentration and morphology significantly improved after folic acid plus zinc sulfate intervention. Animal in vivo and in vitro studies have shown that zinc deficiency decreases the absorption and metabolism of dietary folate,[55,58] because of its function as a cofactor for the folate-metabolizing enzymes dihydrofolate reductase and *y*-glutamyl hydrolase. To the best of our knowledge, this is the first systematic review and meta-analysis to assess the effect of folate and folate plus zinc supplementation on endocrine parameters and sperm characteristics. Although we reported the results using random effects model, a large heterogeneity was present among trials. It can be attributed to folate bioavailability, variability between individuals, amount of administered folic acid and zinc, infertility status, and variability of folate and zinc received by other food-sources. The assessed quality of almost all included studies in this systematic review was also suboptimal and can therefore decrease the reliability of our results. Future trials should base their design on the CONSORT guideline in order to increase the quality. Folate plus zinc supplementation had a positive effect

of sperm concentration, morphology, and serum folate level but did not show any statistically different effect on sperm motility in sub fertile men. Our data, however, showed the absence of an effect of folic acid and zinc sulfate on endocrine parameters. However, the interpretation of results of the current study is limited due to large heterogeneity among included studies. Further trials are still needed to confirm the current findings.

CONCLUSIONS

Folate plus zinc supplementation had a positive effect on sperm characteristics in sub fertile men. However, the interpretation of results of the current study is limited due to the large heterogeneity among included studies. Further trials are still needed to confirm the current findings.

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CONFLICT OF INTEREST

The authors report no conflict of interest.

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