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Effect of Folic Acid and Zinc Supplementation in Men on Semen Quality and Live Birth Among Couples Undergoing Infertility Treatment
A Randomized Clinical Trial

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IMPORTANCE Dietary supplements marketed for male fertility commonly contain folic acid and zinc based on limited prior evidence for improving semen quality. However, no large-scale trial has examined the efficacy of this therapy for improving semen quality or live birth.

OBJECTIVE To determine the effect of daily folic acid and zinc supplementation on semen quality and live birth.

DESIGN, SETTING, AND PARTICIPANTS The Folic Acid and Zinc Supplementation Trial was a multicenter randomized clinical trial. Couples (n = 2370; men aged ≥18 years and women aged 18-45 years) planning infertility treatment were enrolled at 4 US reproductive endocrinology and infertility care study centers between June 2013 and December 2017. The last 6-month study visit for semen collection occurred during August 2018, with chart abstraction of live birth and pregnancy information completed during April 2019.

INTERVENTIONS Men were block randomized by study center and planned infertility treatment (in vitro fertilization, other treatment at a study site, and other treatment at an outside clinic) to receive either 5 mg of folic acid and 30 mg of elemental zinc (n = 1185) or placebo (n = 1185) daily for 6 months.

MAIN OUTCOMES AND MEASURES The co-primary outcomes were live birth (resulting from pregnancies occurring within 9 months of randomization) and semen quality parameters (sperm concentration, motility, morphology, volume, DNA fragmentation, and total motile sperm count) at 6 months after randomization.

RESULTS Among 2370 men who were randomized (mean age, 33 years), 1773 (75%) attended the final 6-month study visit. Live birth outcomes were available for all couples, and 1629 men (69%) had semen available for analysis at 6 months after randomization. Live birth was not significantly different between treatment groups (404 [34%] in the folic acid and zinc group and 416 [35%] in the placebo group; risk difference, −0.9% [95% CI, −4.7% to 2.8%]). Most of the semen quality parameters (sperm concentration, motility, morphology, volume, and total motile sperm count) were not significantly different between treatment groups at 6 months after randomization. A statistically significant increase in DNA fragmentation was observed with folic acid and zinc supplementation (mean of 29.7% for percentage of DNA fragmentation in the folic acid and zinc group and 27.2% in the placebo group; mean difference, 2.4% [95% CI, 0.5% to 4.4%]). Gastrointestinal symptoms were more common with folic acid and zinc supplementation compared with placebo (abdominal discomfort or pain: 66 [6%] vs 40 [3%], respectively; nausea: 50 [4%] vs 24 [2%]; and vomiting: 32 [3%] vs 17 [1%]).

CONCLUSIONS AND RELEVANCE Among a general population of couples seeking infertility treatment, the use of folic acid and zinc supplementation by male partners, compared with placebo, did not significantly improve semen quality or couples' live birth rates. These findings do not support the use of folic acid and zinc supplementation by male partners in the treatment of infertility.

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Visual Abstract

Supplemental content

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The global dietary supplement market is projected to exceed $200 billion in the early 2020s.[1] In the United States, it was estimated that 45% of adult men used dietary supplements from 1999 to 2012,[2] and supplement use is common in men among couples trying to conceive.[3] Many formulations claim benefits for fertility ranging from sperm count and motility to libido and vitality. However, the US Food and Drug Administration is not permitted to evaluate dietary supplements until after market, contributing to a largely unregulated industry of products with unproven safety and efficacy.[4] Furthermore, supplement tainting with pharmaceutical drugs has occurred, particularly among those marketed for "sexual enhancement."[5]

Most supplements for male fertility contain folic acid and zinc. Zinc is essential in spermatogenesis as a component of steroid receptors and metalloenzymes involved in DNA transcription.[6] Furthermore, zinc's high concentration in seminal fluid (approximately 30 times higher than in blood[7]) suggests a link to semen quality, potentially through its antioxidant functions.[8] Sperm are particularly sensitive to oxidative stress, which is linked to chromatin damage, peroxidation of sperm membranes, impaired motility, and increased apoptosis.[9] Folate, which provides carbons for DNA synthesis and methylation critical to spermatogenesis as well as scavenging of free radicals,[10] also depends on zinc for proper use and bioavailability, demonstrating synergistic properties.[11-13]

Human trials of folic acid and zinc supplementation have been heterogenous and have produced varied results among treatment groups of usually fewer than 30 men[14]; however, some evidence suggests zinc and folate in combination may be optimal.[11-13] A meta-analysis[14] concluded that large-scale trials were needed and it remains unproven whether supplementation could affect live birth, which is the outcome of most interest to couples.

Therefore, the aim of this randomized clinical trial was to determine the effect of quality-controlled folic acid and zinc supplementation daily in men on semen quality and live birth among couples seeking infertility treatment.

Key Points

Question What is the effect of folic acid and zinc supplementation in men on semen quality and live birth among couples planning infertility treatment?

Findings In this randomized clinical trial that included 2370 couples, the use of folic acid and zinc supplementation by male partners, compared with placebo, did not significantly improve couples' live birth rates (34% vs 35%, respectively) or semen quality measured 6 months after randomization.

Meaning These findings do not support the use of folic acid and zinc supplementation by male partners for the treatment of infertility.

Methods

Study Design

The Folic Acid and Zinc Supplementation Trial (FAZST) was a multicenter, double-blind, block-randomized, placebo-controlled clinical trial conducted to assess the effect of folic acid and zinc supplementation in men on semen quality and live birth among couples seeking infertility treatment (**Figure**). The trial protocol (including the statistical analysis plan) appears in Supplement 1 and is described elsewhere.[15] The institutional review boards at all study centers and the data coordinating center approved the trial. Written informed consent was obtained for all participants. A data and safety monitoring board provided external oversight.

Participants

Male partners of couples planning infertility treatment were enrolled at 4 US reproductive endocrinology and infertility care study centers (located in Salt Lake City, Utah; Iowa City, Iowa; Chicago, Illinois; and Minneapolis, Minnesota). Couples (men aged ≥18 years and women aged 18-45 years) were ineligible if they were planning use of donor sperm or a gestational surrogate, were pregnant at enrollment, or if the male had obstructive azoospermia or other known infertility causes unlikely to benefit from supplementation. Men were instructed to abstain from dietary supplements containing folic acid or zinc, as well as medications known to interact with folic acid or zinc. Men with poorly controlled chronic diseases (eg, heart disease, diabetes, hypertension, cancer) were excluded.

Because ovulation induction and intrauterine insemination are commonly provided by general obstetrics and gynecology practitioners, recruitment also included couples planning these treatments in the surrounding community. The trial was designed in this way to be more broadly inclusive of a general infertility care population seeking a range of treatment modalities from the least to the most intensive.[15] Men were excluded initially for anemia (hemoglobin concentration <13 g/dL) using a point-of-care hemoglobin meter to avoid enrolling men with vitamin B_{12} deficiency. After October 30, 2015, men with hemoglobin concentrations less than 13 g/dL were enrolled, with a follow-up serum vitamin B_{12} and methylmalonic acid measurement. Recruitment into the trial was inclusive with respect to race/ethnicity, which was self-reported via provided categories or via an open-text option. Collection of race/ethnicity data is required for research funded by the National Institutes of Health.

Randomization and Masking

Eligible male participants were randomized in a 1:1 ratio to daily folic acid and zinc or placebo by study center and planned infertility treatment stratum (in vitro fertilization [IVF], other treatment onsite, other treatment offsite; additional details appear below). The IVF stratum included couples planning to pursue this type of infertility treatment at the time of enrollment. The 2 other treatment strata (onsite or offsite) included planning ovulation induction procedures, intrauterine insemination procedures, and other forms of fertility optimization.

The computerized randomization algorithm was developed by the trial data coordinating center based on a permuted

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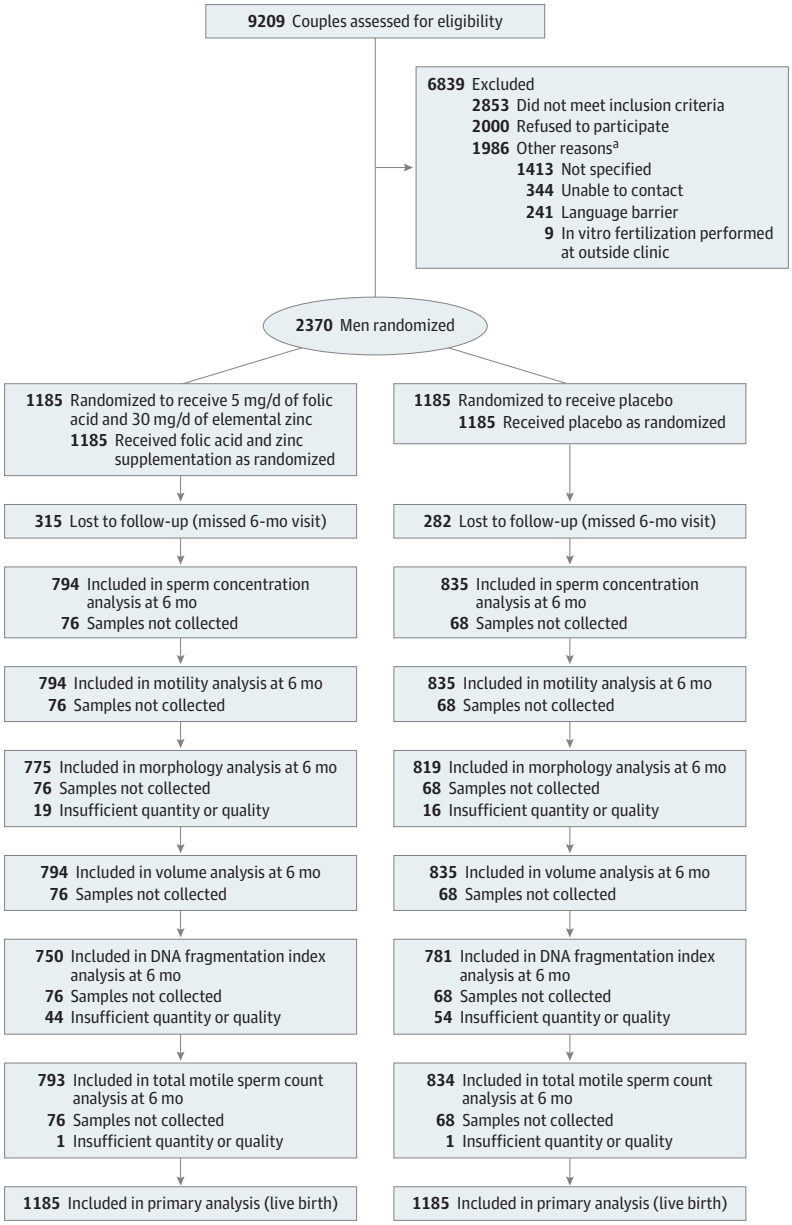
Figure. Patient Recruitment, Randomization, and Follow-up



[a] Categories are not mutually exclusive.

block design with block sizes of 2, 4, or 6 (in random order) within each infertility treatment stratum and study site and was implemented by blinded study coordinators. Participants, trial staff, and investigators were blinded to treatment throughout the trial.

Procedures

Men received daily supplements containing 5 mg of folic acid (dose replicating prior trials[14]) and 30 mg of elemental zinc (lower dose than prior trials and below the upper tolerable limit of 40 mg to improve tolerability and bioavailability[12,14,16]) or placebo for 6 months. The study tablets were manufactured to match in appearance, size, taste, and weight (UPM Pharmaceuticals Inc). Quality control measures for tablets included testing to meet United States Pharmacopeia specifications and quality standards for nutritional and dietary supplements and to prevent issues of potential contamination[5] and general lack of oversight and quality control potentially affecting commercial products.[4,17]

Men were receiving the study intervention for a minimum of 4.5 to 6 weeks before the ovulatory phase of the first infertility treatment cycle. Although the spermatogenic cycle is approximately 74 days, this timing ensures a minimum time of receiving the intervention that covers the stages of spermatocytogenesis (mitotic and meiotic phases) as well meeting the practical needs of patients to initiate infertility treatment promptly.

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Male participants completed in-person study visits, which included semen and other biospecimen collection, at baseline and at 2, 4, and 6 months after randomization. Adverse event and adherence questionnaires were administered at each visit to assess relevant symptoms and frequency of skipped doses. Female participants were followed up for 9 months after randomization, and up to 9 additional months for pregnancy outcomes, with brief monthly questionnaires assessing infertility treatment, pregnancy status, and pregnancy outcomes. Women also were asked to report directly to research staff any positive home urine pregnancy tests or positive serum β-human chorionic gonadotropin tests. Pregnancy outcomes were ascertained through medical record review, including obstetric records of the prenatal care clinician and hospital records for incidental visits and delivery.

Primary Outcomes

The co-primary outcomes were live birth (determined by medical record abstraction) and semen quality parameters (assessed by quantification of sperm concentration, motility [including percentage of progressive motile sperm and percentage of nonprogressive motile sperm], morphology [percentage of normal[18] forms], volume, DNA fragmentation index [which measures the integrity of sperm DNA as the percentage of sperm in the ejaculate containing excess single- and double-strand DNA breaks], and total motile sperm count [calculated as volume × sperm concentration × motility]).

Each laboratory underwent standardized training and interlaboratory quality control testing to comply with World Health Organization criteria (fifth edition).[18] In addition, the DNA fragmentation index was determined at a central laboratory using a Comet assay, which is a single-cell gel electrophoresis approach for measuring DNA breaks.[19,20] Interpretation of the overall trial findings was determined by using live birth as the key outcome of infertility treatment. Possible divergent results in semen quality parameters were to be interpreted as a function of the number of parameters that differed, and the magnitude and direction of the differences.

Secondary Outcomes

Prespecified secondary outcomes included β-human chorionic gonadotropin–detected pregnancy (serum β-human chorionic gonadotropin level >5 mIU/mL), clinical intrauterine pregnancy (visualized gestational sac in the uterus using ultrasonography), ectopic pregnancy, pregnancy with multiple fetuses, early pregnancy losses (including serum β-human chorionic gonadotropin level >5 mIU/mL followed by a decline), and clinically recognized pregnancy losses (clinical pregnancy followed by a pregnancy loss at <20 weeks' gestation).

Specific prespecified pregnancy outcomes included cesarean delivery, preeclampsia, gestational diabetes, gestational age at delivery, preterm birth (delivery before 37 weeks' gestation), birth weight, small for gestational age (as a marker of growth restriction; defined as <10th percentile of birth weight[21]), severe postpartum maternal morbidity (including postpartum hemorrhage, anemia requiring transfusion, sepsis, seizure, HELLP [hemolysis, elevated level of liver enzymes, low platelet count] syndrome, and preeclampsia with pulmonary edema), major neonatal complications (including structural malformations, chromosomal anomalies, bronchopulmonary dysplasia, necrotizing enterocolitis, severe intraventricular hemorrhage, periventricular leukomalacia, and retinopathy of prematurity), stillbirth, and neonatal death.

In the IVF stratum, embryonic development parameters were considered, including fertilization rates and method, number of cells and embryo morphology on day 3 and day 5, number and proportion of good quality embryos on day 5, number and quality of embryos transferred, number of embryos cryopreserved, and sperm penetration assay results. When available, information regarding the chromosomal complement of embryos was assessed. Reproductive hormones and certain other biomarkers were also prespecified as secondary outcomes but are not reported herein.

Participant adverse events were closely monitored and were reported using standardized forms and standard guidance for immediacy of reporting to the study sponsor and the data and safety monitoring board. An internal adverse events committee routinely evaluated adverse events. The data and safety monitoring board reviewed adverse events at least annually.

Statistical Analysis

A sample size of 2310 couples (rounded to 2400), divided equally between the folic acid and zinc supplementation group and the placebo group, was targeted to provide 90% power at a 2-sided α level of .05 to detect a risk difference of 7% in live birth (implying a risk ratio of 1.10), with continuity correction and allowing for a dropout rate of 15%. A cumulative live birth rate of 63% for the placebo group assumed multiple cycles of assisted conception within 9 months of follow-up and varying success rates by different treatment modalities.[22,23] A risk difference of 7% is clinically meaningful and is in line with other large trials assessing pharmacological agents among couples undergoing infertility treatment.[24]

In all analyses, participants were kept in the treatment group to which they were randomized. Live birth was analyzed among all randomized couples, permitting the prespecified strict intent-to-treat analysis. Although the same principles were applied, a pure intent-to-treat analysis of semen quality was not possible due to incomplete participant visit attendance and semen collection. Analyses were performed using a complete case approach overall and by infertility treatment stratum. Risk differences and risk ratios were estimated for live birth and for the secondary outcomes with binary end points using generalized linear models, adjusting for infertility treatment stratum and study site to improve precision,[25,26] and the first occurrence of each outcome was counted per couple.

Semen quality parameters were compared at 6 months after randomization using analysis of covariance and accounting for the same factors. A permutation test based on

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the sum of scores from the *t* tests across semen quality parameters (sperm concentration, motility, morphology, and sperm DNA fragmentation) was performed. To assess the robustness of the findings for semen quality to distributional assumptions, nonparametric testing was applied. Unadjusted risk differences were estimated using the standard *z* score (the normal approximation to the binomial distribution) for live birth and binary secondary outcomes. Unadjusted risk ratios were assessed using the Mantel-Haenszel test.

Even though the unadjusted analyses were prespecified, the adjustment for randomization block and study site was appropriate and, as expected, the adjusted and unadjusted findings were similar; therefore, only the adjusted results are presented. A fixed-effects approach was used for adjustment given the model convergence issues that arise when using mixed-effects models. Analyses of embryonic parameters among couples in the IVF stratum used generalized linear models and estimating equation methods to account for multiple IVF cycles per couple and multiple embryos per cycle; however, this approach was not prespecified in the statistical analysis plan.

An interim analysis (detailed elsewhere[15]) was conducted by the data coordinating center under the direction of the data and safety monitoring board after 50% of participants completed the 6-month visit to determine whether the study should be stopped for strong evidence of harm to semen quality. Briefly, the sequential approach of Lan and DeMets[27] was used with Bonferroni adjustment to distribute the 1-sided type I error rate among 3 continuous semen quality parameters (sperm concentration, morphology, and motility). To account for the α spent from the interim tests, the confidence intervals reported herein for sperm concentration, morphology, and motility represent 95.1% confidence intervals. Neither the live birth rate nor any of the secondary outcomes was evaluated in the interim analysis and thus no α was spent.

Several post hoc sensitivity analyses were conducted beginning with semen quality, and applying inverse probability weighting to account for men who missed the 6-month visit to ameliorate potential bias from dropout associated with adverse effects of the intervention, the female partner becoming pregnant prior to study completion, and other baseline characteristics. In addition, we evaluated semen quality parameters by setting samples that had inadequate sperm concentration for analysis to missing in an effort to align with studies that exclude men with azoospermia or a low sperm count. We also examined the sensitivity of findings with preterm birth to the gestational age cut point of 37 weeks, repeating the analysis using cut points of 36 and 38 weeks.

We additionally stratified the analysis of live births by time receiving the intervention (ie, the time until the ovulatory phase of the first treatment cycle or 2 weeks' gestation among pregnancies occurring [1] before 74 days after randomization or [2] more than 74 days after randomization) to explore whether the findings varied among those with vs those without a full spermatogenic cycle during use of the intervention prior to fertilization. To emulate a trial enrolling only men with known male factor or semen quality impairments, we examined outcomes restricted to men with baseline indicators of male factor infertility or poor semen quality according to World Health Organization criteria (fifth edition).[18,28]

All analyses were performed using SAS version 9.4 (SAS Institute Inc). Tests were 2-sided with a level of significance of .05. Because of the potential for type I error due to multiple comparisons for the co-primary and secondary end points, the findings should be interpreted as exploratory.

Results

Trial recruitment occurred between June 2013 and December 2017 and 2370 men were randomized (1185 to the folic acid and zinc group and 1185 to the placebo group; Figure). The last 6-month study visit for semen collection occurred during August 2018 and chart abstraction of clinical data was completed during April 2019. Results of the interim analysis did not reach the prespecified bound (Supplement 1 and detailed elsewhere[15]) for harm on semen parameters; therefore, the trial continued unchanged. The baseline characteristics of the male and female participants were balanced between the groups (**Table 1**).

Participant adherence was high overall and across time. Most participants reported missing no more than 5 daily doses during the interval between each follow-up visit (from baseline to 2 months, 87% adherence; 2-4 months, 86%; and 4-6 months, 83%). Similarly, high adherence for the folic acid and zinc group was seen from baseline to 2 months, 86%; from 2 to 4 months, 86%; and from 4 to 6 months, 82% vs 88%, 86%, and 84%, respectively, for the placebo group. A participant who reported missing more than 10 daily doses was uncommon (only occurred among 4% of participants from baseline to 2 months; 5% of participants from 2-4 months; and 7% of participants from 4-6 months).

For the primary outcome of live birth, 820 participants (35%) attained a live birth, which did not significantly differ by intervention group overall (404 [34%] in the folic acid and zinc group vs 416 [35%] in the placebo group; risk difference, −0.9% [95% CI, −4.7% to 2.8%]) and within infertility treatment stratum (**Table 2**). Live birth was assessed for all 2370 participants; however, semen quality parameters were missing for 31% of men because they were lost to follow-up (n = 597 [25%]) or lacked samples at the 6-month study visit (n = 144 [6%]). Some additional missingness occurred because of insufficient sample quantity or quality for morphology (n = 35 [1%]), DNA fragmentation index (n = 98 [4%]), and total motile sperm count (n = 2 [<1%]) (Figure).

For the semen quality parameters, sperm concentration, motility, morphology, volume, and total motile sperm count were not significantly different after 6 months (**Table 3**). A statistically significant increase in DNA fragmentation index was observed with folic acid and zinc supplementation overall (mean of 29.7% for percentage of DNA fragmentation vs 27.2% for the placebo group; mean difference, 2.4% [95% CI, 0.5% to 4.4%]). The permutation test for

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Table 1. Characteristics of Participants

	No./Total No. (%)[a]	
	Folic Acid and Zinc (n = 1185)	Placebo (n = 1185)
Male Partner		
Age, mean (SD), y	32.5 (5.7)	32.7 (6.0)
Body mass index, mean (SD)[b]	30.1 (6.7)	29.6 (6.7)
Systolic blood pressure, mean (SD), mm Hg	126.8 (12.8)	126.5 (13.9)
Diastolic blood pressure, mean (SD), mm Hg	78.4 (10.8)	78.0 (10.9)
Race/ethnicity		
Non-Hispanic white	974/1182 (82)	962/1178 (82)
Non-Hispanic black	21/1182 (2)	34/1178 (3)
Asian	43/1182 (4)	46/1178 (4)
Hispanic or Latino	70/1182 (6)	68/1178 (6)
Other[c]	74/1182 (6)	68/1178 (6)
Education level		
High school degree or less	198/1173 (17)	173/1168 (15)
Some college	428/1173 (36)	386/1168 (33)
Bachelor's degree	335/1173 (29)	389/1168 (33)
Master's degree or higher	212/1173 (18)	220/1168 (19)
Employment status		
Unemployed	149/1096 (14)	148/1095 (14)
Employed part-time	58/1096 (5)	53/1095 (5)
Employed full-time	802/1096 (73)	798/1095 (73)
Full-time student	87/1096 (8)	96/1095 (9)
Taking multivitamin within past 3 mo		
Yes	298/751 (40)	284/752 (38)
No	453/751 (60)	468/752 (62)
Male factor infertility diagnosis		
No	598/758 (79)	594/759 (78)
Yes[d]	160/758 (21)	165/759 (21)
Low sperm count	87/157 (55)	93/161 (58)
Low sperm motility	80/157 (51)	71/161 (44)
Abnormal morphology	70/157 (45)	76/161 (47)
Other	10/157 (6)	9/161 (6)
Poor DNA fragmentation[e]	1/157 (<1)	1/161 (<1)
Testicular failure	2/157 (1)	0/161
Past reproductive conditions, No. (%)		
Varicocele	96 (8)	82 (7)
Inguinal hernia repair	35 (3)	34 (3)
Vasectomy reversal	20 (2)	18 (2)
Hydrocele	16 (1)	13 (1)
Injury to testicles	14 (1)	15 (1)
Baseline semen quality		
No. of participants	1141	1153
Sperm concentration, mean (SD), million/mL	88.2 (84.6)	87.5 (90.2)
Motility, mean (SD), % motile[f]	53.7 (20.9)	53.7 (19.7)
Morphology, mean (SD), % normal[g]	5.7 (4.8)	5.9 (4.4)
Total motile sperm count, mean (SD), million[h]	196 (223)	192 (236)
Female Partner		
Age, mean (SD), y	30.6 (5.0)	30.8 (5.2)
Body mass index, mean (SD)[b]	28.9 (8.3)	28.1 (8.1)
Systolic blood pressure, mean (SD), mm Hg	114.7 (13.9)	115.2 (14.2)
Diastolic blood pressure, mean (SD), mm Hg	73.6 (10.9)	73.1 (10.4)
Race/ethnicity		
Non-Hispanic white	994/1180 (84)	962/1178 (82)
Non-Hispanic black	22/1180 (2)	21/1178 (2)
Asian	61/1180 (5)	70/1178 (6)
Hispanic or Latino	52/1180 (4)	75/1178 (6)
Other[c]	51/1180 (4)	50/1178 (4)

(continued)

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Table 1. Characteristics of Participants (continued)

	No./Total No. (%)[a]	
	Folic Acid and Zinc (n = 1185)	Placebo (n = 1185)
Education level		
High school degree or less	130/1170 (11)	145/1167 (12)
Some college	404/1170 (35)	383/1167 (33)
Bachelor's degree	418/1170 (36)	431/1167 (37)
Master's degree or higher	218/1170 (19)	208/1167 (18)
Employment status		
Unemployed	171/1087 (16)	177/1085 (16)
Employed part-time	184/1087 (17)	185/1085 (17)
Employed full-time	696/1087 (64)	694/1085 (64)
Full-time student	36/1087 (3)	29/1085 (3)
Female factor infertility diagnosis		
No	477/758 (63)	492/757 (65)
Yes[d]	281/758 (37)	265/757 (35)
Polycystic ovary syndrome	131/258 (51)	117/247 (47)
Anovulation	49/258 (19)	46/247 (19)
Endometriosis	58/258 (22)	34/247 (14)
Other	39/258 (15)	38/247 (15)
Diminished ovarian reserve	18/258 (7)	22/247 (9)
Blocked fallopian tubes	21/258 (8)	15/247 (6)
Uterine abnormalities	13/258 (5)	10/247 (4)
Hypothalamic amenorrhea	0/258	4/247 (2)
Hyperprolactinemia	3/258 (1)	0/247
Household/Couple		
Marital status		
Married or living with partner	1180/1184 (99)	1179/1183 (99)
Single or other	4/1184 (<1)	4/1183 (<1)
Annual household income		
<$40 000	176/1111 (16)	157/1123 (14)
$40 000-$74 999	422/1111 (38)	456/1123 (41)
$75 000-$99 999	261/1111 (23)	232/1123 (21)
≥$100 000	252/1111 (23)	278/1123 (25)
Male health insurance		
Yes	1108/1173 (94)	1117/1171 (95)
No	65/1173 (6)	54/1171 (5)
Male infertility insurance		
Yes	297/1106 (27)	266/1116 (24)
No	493/1106 (45)	495/1116 (44)
Do not know	316/1106 (29)	355/1116 (32)
Female health insurance		
Yes	1134/1174 (97)	1144/1173 (98)
No	40/1174 (3)	29/1173 (2)
Female infertility insurance		
Yes	363/1133 (32)	319/1144 (28)
No	500/1133 (44)	514/1144 (45)
Do not know	270/1133 (24)	311/1144 (27)
Time trying to conceive		
No. of participants	1102	1105
Median (interquartile range), mo	19 (12-36)	18 (12-36)

[a] Unless otherwise indicated. Column percentages may not sum to 100% due to rounding.

[b] Calculated as weight in kilograms divided by height in meters squared.

[c] Included American Indian/Alaskan Native, Native Hawaiian/Pacific Islander, mixed race, and nonspecified other.

[d] Infertility at baseline was based on self-report of past diagnoses; therefore, standardized clinical definitions of these diagnoses may not apply. Participants could list more than 1 infertility diagnosis.

[e] Defined as a measure of sperm DNA integrity based on excess DNA strand breaks. There are no clinically recognized cut points for abnormal DNA fragmentation index; however, greater than 35% fragmentation is generally seen as an elevated level.

[f] Abnormal semen parameter is 40% or less total motile sperm.

[g] Abnormal semen parameter is less than 4% normal forms.

[h] Abnormal semen parameter is less than 20 million sperm per ejaculate.

semen quality parameters (sperm concentration, motility, morphology, and DNA fragmentation index) indicated a statistically significant difference with lower overall semen quality in the folic acid and zinc group (t_{sum4} = −6.06; P = .03). This result was largely driven by the DNA fragmentation index because an exploratory permutation test of the semen quality parameters of sperm concentration, motility, and morphology showed no significant difference (t_{sum3} = −3.76; P = .10). In the other treatment onsite stratum, poorer morphology was observed.

Results of the co-primary outcomes were also assessed in several post hoc sensitivity analyses. For live birth, similar results were found when stratifying men by first fertilization attempt before 74 days after randomization (280 [51%] in the folic acid and zinc group vs 289 [52%] in the placebo group; risk difference, −1.0% [95% CI, −6.8% to

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Table 2. Primary Outcome of Live Birth

	No./Total No. (%)		Adjusted Risk Difference (95% CI), %	Adjusted Risk Ratio (95% CI)
	Folic Acid and Zinc	Placebo		
Primary Outcome: Live Birth[a]				
Overall	404/1185 (34)	416/1185 (35)	−0.9 (−4.7 to 2.8)[b]	0.98 (0.88 to 1.09)[b]
Infertility treatment stratum[c]				
In vitro fertilization	97/185 (52)	91/188 (48)	3.8 (−6.1 to 13.8)[d]	1.08 (0.88 to 1.31)[d]
Other treatment onsite[e]	264/831 (32)	277/827 (33)	−1.7 (−6.2 to 2.8)[d]	0.95 (0.82 to 1.09)[d]
Other treatment offsite[e]	43/169 (25)	48/170 (28)	−2.7 (−12.0 to 6.7)[d]	0.90 (0.63 to 1.26)[d]

[a] Female participants were followed up for a minimum of 9 months after randomization. If a woman became pregnant at any time during those 9 months, the pregnancy was followed up until its completion (ie, pregnancy loss or live birth).

[b] Adjusted for infertility treatment stratum and study site.

[c] Indicates the planned infertility treatment at the time of randomization.

[d] Adjusted for study site.

[e] Included ovulation induction, intrauterine insemination, and natural fertility optimization methods obtained at any of the reproductive endocrinology and infertility specialist study centers (onsite) or with a community provider (offsite).

4.8%]) vs by first fertilization attempt more than 74 days after randomization (124 [20%] in the folic acid and zinc group vs 127 [20%] in the placebo group; risk difference, −0.7% [95% CI, −5.1% to 3.7%]).

Regarding the semen quality parameters, the results were similar when accounting for those lost to follow-up using weighted sensitivity analyses (far right column in Table 3 [adjusted weighted mean difference]) and when using nonparametric testing (eTable 1 in Supplement 2). Furthermore, setting samples with inadequate sperm concentration as missing produced null findings for the DNA fragmentation index and similar results for the other semen quality parameters (eTable 2 in Supplement 2). The findings also were similar when restricted to men with known male factor infertility or poor semen quality at baseline (eTables 3 and 4 in Supplement 2).

There was no statistically significant effect of supplementation on most of the prespecified secondary outcomes, including β-human chorionic gonadotropin–detected pregnancy, clinical intrauterine pregnancy, ectopic pregnancy, pregnancy with multiple fetuses, early pregnancy loss, cesarean delivery, preeclampsia or gestational hypertension, gestational diabetes, gestational age, birth weight, or small for gestational age at birth (**Table 4**). A statistically significant increase in preterm delivery was observed with folic acid and zinc supplementation overall (67 [6%] vs 45 [4%] in the placebo group; risk difference, 1.9% [95% CI, 0.2% to 3.6%]) (Table 4). Early embryonic development parameters in the IVF stratum were not significantly different by treatment group (eTable 5 in Supplement 2). A post hoc sensitivity analysis for preterm birth indicated no significant effects using cut points at 36 weeks' gestation (41 [3.5%] in the folic acid and zinc group vs 33 [2.8%] in the placebo group; risk difference, 0.68% [95% CI, −0.70% to 2.08%]) or at 38 weeks' gestation (103 [8.7%] in the folic acid and zinc group vs 93 [7.8%] in the placebo group; risk difference, 0.87% [95% CI, −1.33% to 3.07%]).

Folic acid and zinc supplementation in male partners did not notably affect stillbirth, neonatal morbidity, neonatal mortality, or severe postpartum maternal morbidity. There were 29 structural malformations reported (26 among births and 3 among pregnancy losses). Twenty-one malformations were categorized as major defects (6 with known genetic cause), 6 were minor, and 2 could not be classified. Adverse events were more frequent in the folic acid and zinc group (32% vs 27% in the placebo group), reflective of more frequent gastrointestinal symptoms and erythema (**Table 5**). The trial recorded a total of 12 serious adverse events (7 in the folic acid and zinc group and 5 in the placebo group) and none were judged to be related to the intervention.

Discussion

In this randomized clinical trial, supplementation with 5 mg of folic acid and 30 mg of zinc in men did not improve semen quality parameters or increase couples' live birth rates among patients seeking infertility treatment using IVF or other treatment modalities. Furthermore, this lack of efficacy was accompanied by some increased mild gastrointestinal adverse effects. This trial's findings do not support the use of folic acid and zinc supplementation in male partners to improve semen quality and couples' infertility treatment outcomes.

This report addresses the long-standing need for a rigorous large-scale trial to examine the effects of folic acid and zinc supplementation on semen quality. Although these findings disagree with the conclusion from a recent meta-analysis[14] that a supplement combination with folate and zinc improved semen quality, primarily in sperm concentration, the authors of the meta-analysis had urged caution given the heterogeneity of the included studies. It is possible prior findings indicated a potential benefit of supplementation due to exclusion of men with azoospermia.

A recent smaller trial,[29] which was not included in the meta-analysis,[14] examining a commercial formula of multiple antioxidant nutrients (but with lower doses of folic acid and zinc than used herein) reported no benefit of supplementation on semen quality, which is consistent with the current trial. Specific subgroups remain to be examined in this trial population; for example, a methylenetetrahydrofolate reductase gene polymorphism was shown to

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Table 3. Primary Outcome of Semen Quality Parameters After 6 Months

Semen Quality Parameters	Mean (SD)		Adjusted Mean Difference (95% CI)[a]	Adjusted Weighted Mean Difference (95% CI)[a]
	Folic Acid and Zinc	Placebo		
Overall				
No. of participants	794	835		
Sperm concentration, million/mL[b]	84.8 (85.2)	89.0 (85.0)	−4.3 (−12.5 to 3.9)	−5.2 (−13.6 to 3.1)
Motility, %[c]	52.7 (21.2)	53.2 (20.1)	−0.5 (−2.5 to 1.5)	−0.6 (−2.7 to 1.4)
Morphology, % normal[d]	5.7 (4.2)	6.0 (4.8)	−0.4 (−0.8 to 0.1)	−0.4 (−0.9 to 0)
Volume, mL	3.5 (1.7)	3.5 (1.8)	0 (−0.2 to 0.2)	0 (−0.1 to 0.2)
DNA fragmentation index, %[e]	29.7 (20.5)	27.2 (17.8)	2.4 (0.5 to 4.4)	2.3 (0.3 to 4.3)
Total motile sperm count, million[f]	183 (226)	182 (212)	1.4 (−19.7 to 22.5)	0.3 (−20.9 to 21.4)
In Vitro Fertilization[g]				
No. of participants	124	135		
Sperm concentration, million/mL[b]	81.8 (96.5)	76.1 (78.6)	6.3 (−14.6 to 27.1)[h]	7.4 (−13.2 to 28.1)[h]
Motility, %[c]	51.7 (21.9)	51.7 (20.0)	−0.3 (−5.4 to 4.9)[h]	−0.1 (−5.4 to 5.1)[h]
Morphology, % normal[d]	5.2 (4.3)	5.4 (4.7)	−0.3 (−1.4 to 0.9)[h]	−0.4 (−1.6 to 0.7)[h]
Volume, mL	3.5 (1.5)	3.5 (1.7)	−0.1 (−0.5 to 0.3)[h]	0 (−0.4 to 0.4)[h]
DNA fragmentation index, %[e]	27.1 (19.6)	26.8 (19.6)	0 (−5.0 to 5.0)[h]	0.1 (−4.8 to 5.1)[h]
Total motile sperm count, million[f]	165 (221)	152 (188)	11.4 (−37.0 to 59.9)[h]	12.9 (−35.2 to 61.0)[h]
Other Infertility Treatment Onsite[g,i]				
No. of participants	566	590		
Sperm concentration, million/mL[b]	85.0 (83.1)	92.2 (84.8)	−7.1 (−16.8 to 2.6)[h]	−8.8 (−18.8 to 1.2)[h]
Motility, %[c]	52.5 (21.1)	53.9 (19.5)	−1.3 (−3.6 to 1.1)[h]	−1.5 (−3.9 to 0.8)[h]
Morphology, % normal[d]	5.6 (4.0)	6.2 (4.9)	−0.6 (−1.2 to −0.1)[h]	−0.7 (−1.2 to −0.2)[h]
Volume, mL	3.5 (1.7)	3.5 (1.8)	0 (−0.2 to 0.2)[h]	0 (−0.2 to 0.2)[h]
DNA fragmentation index, %[e]	30.0 (20.3)	27.0 (16.8)	3.0 (0.8 to 5.2)[h]	2.7 (0.4 to 5.0)[h]
Total motile sperm count, million[f]	186 (226)	188 (207)	−1.8 (−26.6 to 23.0)[h]	−3.8 (−28.9 to 21.3)[h]
Other Infertility Treatment Offsite[g,i]				
No. of participants	104	110		
Sperm concentration, million/mL[b]	87.2 (83.0)	87.7 (92.5)	−0.4 (−24.6 to 23.8)[h]	−0.6 (−24.5 to 23.4)[h]
Motility, %[c]	55.0 (20.9)	51.5 (22.8)	3.2 (−2.8 to 9.3)[h]	3.7 (−2.5 to 9.8)[h]
Morphology, % normal[d]	6.7 (4.9)	5.6 (4.3)	1.1 (−0.2 to 2.4)[h]	1.1 (−0.2 to 2.4)[h]
Volume, mL	3.5 (1.7)	3.4 (1.7)	0 (−0.4 to 0.5)[h]	0 (−0.4 to 0.5)[h]
DNA fragmentation index, %[e]	30.7 (22.7)	28.5 (20.5)	1.6 (−4.5 to 7.8)[h]	2.2 (−3.9 to 8.3)[h]
Total motile sperm count, million[f]	192 (233)	184 (262)	7.8 (−60.3 to 75.9)[h]	8.2 (−59.8 to 76.1)[h]

[a] The CIs for the semen quality parameters of sperm concentration, motility, and morphology represent 95.1% CIs to properly account for the α spent in the interim analysis. The mean differences for the values in the overall section were adjusted for infertility treatment stratum and study site.

[b] If sperm concentration was rare or too few to count, no sperm in sample, or retrograde, then motility and morphology were set to zero and DNA fragmentation index was set to [(100 − *max*)/$\sqrt{2}$ + *max*], where the *max* measured value for DNA fragmentation index was 98.4%.

[c] Includes the percentage of progressive motile and the percentage of nonprogressive motile. Abnormal semen parameter is 40% or less total motile sperm.

[d] The World Health Organization (fifth edition) criteria[18] definition for normal morphology is 4% or greater normal forms. Abnormal semen parameter is less than 4% normal forms.

[e] Defined as a measure of sperm DNA integrity based on excess DNA strand breaks. There are no clinically recognized cut points for abnormal DNA fragmentation index; however, greater than 35% fragmentation is generally seen as an elevated level.

[f] Calculated as volume × sperm concentration × motility. Abnormal semen parameter is less than 20 million sperm per ejaculate.

[g] Indicates the planned infertility treatment at the time of randomization.

[h] Adjusted for study site.

[i] Included ovulation induction, intrauterine insemination, and natural fertility optimization methods obtained at any of the reproductive endocrinology and infertility specialist study centers (onsite) or with a community provider (offsite).

modify the effect of folic acid and zinc supplementation on sperm count in 1 small trial.[30]

In addition to examining the effects of folic acid and zinc supplementation on clinical measures of semen quality, the present trial examined their effects on DNA fragmentation index, a measure of sperm DNA damage from oxidative stress previously associated with infertility[31-35] and potentially ameliorated by folic acid and zinc.[7-9] Although prior data on folic acid and zinc and DNA fragmentation index are limited, the current findings suggest increased sperm DNA damage associated with supplementation. However, the present trial results agree with a prior small trial of a general antioxidant supplement in men with a prior elevated DNA fragmentation index (37 in the antioxidant group and 40 in the placebo group)

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Table 4. Couple-Based Secondary Outcomes

	Folic Acid and Zinc, No. (%)[a]	Placebo, No. (%)[a]	Adjusted RD (95% CI), %[b]	Adjusted RR (95% CI)[b]
Overall				
No. of participants	1185	1185		
Pregnancy and pregnancy loss				
hCG-detected pregnancy	479 (40)	490 (41)	−0.9 (−4.7 to 3.0)	1.00 (0.91 to 1.09)
Clinical intrauterine pregnancy	449 (38)	462 (39)	−1.0 (−4.9 to 2.8)	0.98 (0.89 to 1.08)
Any indication of pregnancy	519 (44)	535 (45)	−1.3 (−5.2 to 2.7)	0.98 (0.90 to 1.07)
Ectopic pregnancy	6 (<1)	5 (<1)	0.1 (−0.5 to 0.6)	1.21 (0.37 to 3.97)
Early pregnancy loss (prior to 20 wk)	137 (12)	150 (13)	−1.1 (−3.7 to 1.5)	0.93 (0.75 to 1.15)
Pregnancy with multiple fetuses	42 (4)	42 (4)	0 (−1.5 to 1.5)	1.00 (0.66 to 1.52)
Pregnancy or delivery complications				
Preeclampsia or gestational hypertension	47 (4)	51 (4)	−0.3 (−1.9 to 1.3)	0.92 (0.63 to 1.36)
Gestational diabetes	26 (2)	34 (3)	−0.7 (−1.9 to 0.6)	0.77 (0.47 to 1.27)
Cesarean delivery	143 (12)	129 (11)	1.2 (−1.4 to 3.8)	1.12 (0.89 to 1.39)
Preterm delivery	67 (6)	45 (4)	1.9 (0.2 to 3.6)	1.49 (1.04 to 2.16)
Singleton	37 (3)	29 (2)	0.7 (−0.6 to 2.0)	1.28 (0.80 to 2.07)
Small for gestational age	62 (5)	59 (5)	0.3 (−1.5 to 2.0)	1.05 (0.75 to 1.49)
Gestational age, mean (SD), wk	38.6 (2.5)	38.8 (2.2)	−0.2 (−0.5 to 0.1)[c]	
Birth weight, mean (SD), g	3062 (731)	3133 (654)	−64.7 (−156.0 to 26.4)[c]	
Stillbirth (loss at or after 20 wk)	1 (<1)	4 (<1)	−0.2 (−0.6 to 0.1)	0.25 (0.03 to 2.24)
Neonatal mortality	3 (<1)	2 (<1)	0.1 (−0.3 to 0.5)	1.50 (0.25 to 8.95)
Major neonatal complications[d]	2 (<1)	1 (<1)	0.1 (−0.2 to 0.4)	1.99 (0.18 to 21.9)
Structural malformations[e]	15 (1)	14 (1)	0.1 (−0.8 to 1.0)	1.08 (0.53 to 2.21)
Chromosomal anomalies[e]	4 (<1)	5 (<1)	−0.1 (−0.6 to 0.4)	0.80 (0.22 to 2.97)
Severe maternal morbidity	15 (1)	10 (<1)	0.4 (−0.4 to 1.3)	1.50 (0.68 to 3.32)
In Vitro Fertilization[f]				
No. of participants	185	188		
Pregnancy and pregnancy loss				
hCG-detected pregnancy	112 (61)	111 (59)	1.3 (−8.3 to 10.8)	1.02 (0.90 to 1.16)
Clinical intrauterine pregnancy	107 (58)	107 (57)	0.7 (−9.0 to 10.5)	1.01 (0.86 to 1.19)
Any indication of pregnancy	117 (63)	116 (62)	1.3 (−8.2 to 10.8)	1.04 (0.90 to 1.20)
Ectopic pregnancy	1 (<1)	0		
Early pregnancy loss (prior to 20 wk)	31 (17)	28 (15)	1.8 (−5.5 to 9.1)	1.13 (0.71 to 1.80)
Pregnancy with multiple fetuses	18 (10)	16 (9)	1.1 (−4.7 to 6.9)	1.13 (0.60 to 2.12)
Pregnancy or delivery complications				
Preeclampsia or gestational hypertension	12 (6)	12 (6)	0.1 (−4.8 to 5.1)	1.01 (0.47 to 2.18)
Gestational diabetes	7 (4)	8 (4)	−0.6 (−4.5 to 3.4)	0.90 (0.33 to 2.42)
Cesarean delivery	41 (22)	31 (16)	5.6 (−2.4 to 13.5)	1.35 (0.89 to 2.04)
Preterm delivery	22 (12)	16 (9)	3.5 (−2.6 to 9.7)	1.43 (0.78 to 2.63)
Singleton	6 (3)	10 (5)	−1.9 (−6.0 to 2.1)	0.65 (0.24 to 1.73)
Small for gestational age	15 (8)	15 (8)	0 (−5.5 to 5.5)	1.00 (0.51 to 1.99)
Gestational age, mean (SD), wk	38.0 (3.0)	38.3 (2.3)	−0.3 (−1.1 to 0.4)[c]	
Birth weight, mean (SD), g	2896 (850)	3068 (631)	−193 (−393 to 7.4)[c]	
Stillbirth (loss at or after 20 wk)	0	2 (1)		
Neonatal mortality	0	1 (<1)		
Major neonatal complications[d]	0	0		
Structural malformations[e]	3 (2)	7 (4)	−2.1 (−5.3 to 1.1)	0.44 (0.12 to 1.66)
Chromosomal anomalies[e]	0	1 (<1)		
Severe maternal morbidity	4 (2)	4 (2)	0 (−3.0 to 2.9)	1.00 (0.25 to 3.94)

(continued)

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Table 4. Couple-Based Secondary Outcomes (continued)

	Folic Acid and Zinc, No. (%)[a]	Placebo, No. (%)[a]	Adjusted RD (95% CI), %[b]	Adjusted RR (95% CI)[b]
Other Infertility Treatment Onsite[f,g]				
No. of participants	831	827		
Pregnancy and pregnancy loss				
hCG-detected pregnancy	328 (39)	325 (39)	0.2 (−4.5 to 4.9)	1.00 (0.89 to 1.13)
Clinical intrauterine pregnancy	303 (36)	308 (37)	−0.8 (−5.4 to 3.9)	0.98 (0.86 to 1.11)
Any indication of pregnancy	348 (42)	355 (43)	−1.1 (−5.8 to 3.7)	0.97 (0.87 to 1.09)
Ectopic pregnancy	4 (<1)	4 (<1)		
Early pregnancy loss (prior to 20 wk)	94 (11)	102 (12)	−1.0 (−4.1 to 2.1)	0.92 (0.71 to 1.20)
Pregnancy with multiple fetuses	20 (2)	24 (3)	−0.5 (−2.0 to 1.0)	0.83 (0.46 to 1.49)
Pregnancy or delivery complications				
Preeclampsia or gestational hypertension	30 (4)	37 (4)	−0.9 (−2.8 to 1.0)	0.81 (0.51 to 1.30)
Gestational diabetes	17 (2)	24 (3)	−0.9 (−2.4 to 0.6)	0.70 (0.38 to 1.30)
Cesarean delivery	88 (11)	85 (10)	0.3 (−2.6 to 3.3)	1.03 (0.78 to 1.37)
Preterm delivery	42 (5)	26 (3)	1.9 (0 to 3.8)	1.61 (1.00 to 2.60)
Singleton	29 (3)	17 (2)	1.4 (−0.1 to 3.0)	1.70 (0.94 to 3.07)
Small for gestational age	37 (4)	40 (5)	−0.4 (−2.4 to 1.6)	0.92 (0.60 to 1.43)
Gestational age, mean (SD), wk	38.7 (2.3)	38.8 (2.2)	−0.1 (−0.5 to 0.3)[c]	
Birth weight, mean (SD), g	3125 (682)	3143 (657)	−17.3 (−128.0 to 93.1)[c]	
Stillbirth (loss at or after 20 wk)	1 (<1)	1 (<1)		
Neonatal mortality	3 (<1)	1 (<1)		
Major neonatal complications[d]	2 (<1)	1 (<1)		
Structural malformations[e]	12 (1)	6 (<1)	0.7 (−0.3 to 1.7)	2.00 (0.76 to 5.28)
Chromosomal anomalies[e]	3 (<1)	4 (<1)		
Severe maternal morbidity	11 (1)	6 (<1)	0.6 (−0.4 to 1.6)	1.83 (0.68 to 4.91)
Other Infertility Treatment Offsite[f,g]				
No. of participants	169	170		
Pregnancy and pregnancy loss				
hCG-detected pregnancy	39 (23)	54 (32)	−8.3 (−17.7 to 1.1)	0.74 (0.52 to 1.05)
Clinical intrauterine pregnancy	39 (23)	47 (28)	−4.3 (−13.5 to 4.9)	0.84 (0.58 to 1.21)
Any indication of pregnancy	54 (32)	64 (38)	−5.3 (−15.4 to 4.7)	0.83 (0.62 to 1.11)
Ectopic pregnancy	1 (<1)	1 (<1)		
Early pregnancy loss (prior to 20 wk)	12 (7)	20 (12)	−4.5 (−10.7 to 1.7)	0.61 (0.31 to 1.21)
Pregnancy with multiple fetuses	4 (2)	2 (1)	1.2 (−1.6 to 4.0)	1.97 (0.37 to 10.60)
Pregnancy or delivery complications				
Preeclampsia or gestational hypertension	5 (3)	2 (1)	1.8 (−1.2 to 4.8)	2.50 (0.50 to 12.6)
Gestational diabetes	2 (1)	2 (1)	0 (−2.3 to 2.3)	1.00 (0.14 to 6.99)
Cesarean delivery	14 (8)	13 (8)	0.7 (−5.1 to 6.4)	1.07 (0.52 to 2.20)
Preterm delivery	3 (2)	3 (2)	0 (−2.8 to 2.8)	1.01 (0.21 to 4.94)
Singleton	2 (1)	2 (1)	0 (−2.3 to 2.3)	1.03 (0.15 to 7.17)
Small for gestational age	10 (6)	4 (2)	3.6 (−0.7 to 7.8)	2.54 (0.81 to 7.92)
Gestational age, mean (SD), wk	38.6 (1.6)	39.2 (2.0)	−0.4 (−1.2 to 0.3)[c]	
Birth weight, mean (SD), g	3092 (653)	3205 (686)	−96.4 (−373.0 to 180.0)[c]	
Stillbirth (loss at or after 20 wk)	0	1 (<1)		
Neonatal mortality	0	0		
Major neonatal complications[d]	0	0		
Structural malformations[e]	0	1 (<1)		
Chromosomal anomalies[e]	1 (<1)	0		
Severe maternal morbidity	0	0		

Abbreviations: hCG, human chorionic gonadotropin; RD, risk difference; RR, risk ratio.

[a] Unless otherwise indicated.

[b] For analyses with an insufficient number of events, the RDs and RRs were not calculated. The values in the overall section were adjusted for infertility treatment stratum and study site.

[c] Data are expressed as an adjusted mean difference.

[d] Bronchopulmonary dysplasia, necrotizing enterocolitis, severe intraventricular hemorrhage, periventricular leukomalacia, and retinopathy of prematurity.

[e] Among live births and pregnancy losses.

[f] Planned at the time of randomization. The RDs and RRs were adjusted for study site.

[g] Included ovulation induction, intrauterine insemination, and natural fertility optimization methods obtained at any of the reproductive endocrinology and infertility specialist study centers (onsite) or with a community provider (offsite).

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Table 5. Frequency of the Most Commonly Reported Adverse Events in Men

	No. (%)			
	Folic Acid and Zinc (n = 1185)	Placebo (n = 1185)	Adjusted Risk Difference (95% CI), %[a]	Adjusted Risk Ratio (95% CI)[a]
Serious adverse events	7 (<1)	5 (<1)	0.2 (−0.4 to 0.7)	1.40 (0.45 to 4.41)
Gastrointestinal symptoms				
Abdominal discomfort or pain	66 (6)	40 (3)	2.2 (0.6 to 3.8)	1.62 (1.12 to 2.34)
Nausea	50 (4)	24 (2)	2.2 (0.9 to 3.6)	2.06 (1.29 to 3.29)
Vomiting	32 (3)	17 (1)	1.3 (0.2 to 2.4)	1.88 (1.06 to 3.34)
General disorders and administration site conditions				
Pyrexia	66 (6)	62 (5)	0.4 (−1.4 to 2.2)	1.08 (0.78 to 1.50)
Infections and infestations				
Influenza	21 (2)	11 (<1)	0.8 (0 to 1.8)	1.91 (0.93 to 3.94)
Respiratory, thoracic, and mediastinal disorders				
Oropharyngeal pain	57 (5)	60 (5)	−0.2 (−1.9 to 1.4)	0.94 (0.68 to 1.32)
Nasopharyngitis	32 (3)	40 (3)	−0.8 (−2.2 to 0.6)	0.77 (0.49 to 1.21)
Skin and subcutaneous tissue disorders				
Erythema	23 (2)	8 (<1)	1.3 (0.4 to 2.2)	2.88 (1.29 to 6.41)
Pruritus	20 (2)	17 (1)	0.3 (−0.7 to 1.2)	1.21 (0.66 to 2.24)
Rash	21 (2)	12 (1)	0.8 (−0.1 to 1.7)	1.81 (0.91 to 3.60)

[a] Adjusted for infertility treatment stratum and study site.

when azoospermic or low sperm concentration samples were treated as missing (common in other studies), indicating null findings for DNA fragmentation index or clinical semen parameters.[36] Further research is needed to understand the clinical importance of small differences in DNA fragmentation index.

The frequency of fetal and maternal complications was similar between groups (though the trial was not powered for these outcomes), except an unexpected increase in preterm birth in the folic acid and zinc group. Despite a statistically significant risk difference of 1.9% in preterm birth, mean gestational ages and birth weights were not significantly different. A sensitivity analysis indicated no significant effects using cut points at 36 or 38 weeks for preterm birth. Verification of this result is needed, which could be mediated by paternal influences on placental function,[37] but may be a chance finding. However, there were more frequent adverse events in men randomized to folic acid and zinc supplementation compared with placebo, indicating these doses of folic acid and zinc may be poorly tolerated by some men. Previous studies of zinc have reported higher rates of gastrointestinal adverse effects.[16,38]

Limitations

This study has several limitations. First, the present findings are generalizable to a general infertility clinic population and not subfertile men specifically; most patients were white and non-Hispanic, with high socioeconomic status, thus limiting generalizability.

Second, because this was a pragmatic trial of couples planning infertility treatment, couples may have conceived via sperm produced prior to initiating the intervention. However, the median time receiving the intervention prior to the first date of attempted fertilization (eg, intrauterine insemination date or equivalent) was 85 days, which is even later than the theoretically ideal target of 74 days. Late-stage exposure of sperm to the intervention would not affect any hypothesized effects of supplementation on protection of sperm quality during maturation and storage. In addition, sensitivity analyses stratifying men by first fertilization attempt before 74 days after randomization vs more than 74 days after randomization produced similar results.

Third, due to couples pursuing fewer cycles of infertility treatment than anticipated, the cumulative live birth rate observed for the placebo group was substantially lower than assumed in the sample size calculations. However, this lower rate had little effect on the power to detect a meaningful risk difference of 7%, which was outside the observed 95% CI for the difference in live birth rates in this trial (95% CI, −4.7% to 2.8%).

Fourth, although live birth was assessed passively for all couples, 6-month semen quality was missing for 31% of men. The reweighted sensitivity analysis suggests that the semen analysis findings were not affected by this limitation.

Fifth, because of the potential for type I error due to multiple comparisons for the co-primary and secondary end points, statistically significant findings should be interpreted as exploratory.

Conclusions

Among a general population of couples seeking infertility treatment, the use of folic acid and zinc supplementation by male partners, compared with placebo, did not significantly improve semen quality or couples' live birth rates. These findings do not support the use of folic acid and zinc supplementation by male partners in the treatment of infertility.

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Correction: This article was corrected on March 24, 2020, to fix 2 transposed rows in Table 1.

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Author Contributions: Dr Schisterman had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
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