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Folic Acid and Zinc Supplementation V Placebo as Infertility Treatment; Randomized Clinical Trial

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Abstract
Dietary supplements for male fertility commonly contain folic acidand zinc depending on limited prior evidence aiming improving semen quality. Never the less, there is nolarge trial has examined the efficacy of this supplements for improving semen qualityand live birth.
OBJECTIVE To determine the effecacy of daily folic acid and zinc supplements on semenquality and live birth.
METHODOLOGYThiswasa multicenter placebo controlled randomized clinical trial. Participants (n = 2370; men aged>18 years and womenaged 18-45 years) seeking infertility treatment were enrolled at 5Saudi major hospitals between January 2016 and December 2019.
INTERVENTIONS Men randomized to receive 5mg of folic acid and 30mg of zinc (n = 1185)or placebo (n = 1185) daily for 6 months.
MAIN OUTCOMES The primary outcomeswere live birth (within 9 months of randomization) and semen quality (sperm concentration, motility, morphology and volume) at 6 months after randomization.
RESULTS Among 2370 men who were randomized 75% attendedthe final 6-month study visit. Live birth outcomes were available for all participants, and 69% ofmen had semen available for analysis at 6 months after randomization. Live birth wasnot significantly different between treatment groups (34% in the folic acid and zincgroup and 35% in the placebo group; risk difference, −0.9%[95%CI, −4.7%to 2.8%]).Semen quality parameters were not significantly different between treatment groups.
CONCLUSIONS Among a general population seeking infertilitytreatment, use of folic acid and zinc supplementation by men, compared withplacebo, did not significantly improve semen quality or live birth rates. Thesefindings do not support the use of folic acid and zinc supplementation by male partners in thetreatment of infertility.

Key words; *Folic acid and Zinc supplementation, Semen Quality, Live births, Clinical Trial*

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I. Introduction

Dietary supplement market is projected toexceed $200 billion in the early 2020s[1]. Worldwide, it is estimated that 32% of adult men useddietary supplements from 1999 to 2017,particularly, among men of couples planning to conceive[2, 3].Many supplements claim benefits for fertility. However, almost no Food and Drug authorities are evaluating these products since it is dietary supplements or food additives, contributing to alargely unregulated industry of products with unprovensafety and efficacy[4]. Furthermore, many supplement aremarketed assexual enhancement[5].

It is noticeable that almost all supplements for male fertility contain folic acidand zinc. Zinc is essential in spermatogenesis as a componentof steroid receptors and metalloenzymes involved in DNAtranscription[6]. Furthermore, zinc's high concentration inseminal fluid, (30 times higher than in blood), suggests a link to semen quality, potentially through its antioxidantfunctions[7, 8]. Sperms are particularly sensitive to oxidativestress[9]. Folate, provides carbons for DNA synthesisand methylation which is essential for spermatogenesis[10].On top of that, Folate Removefree radicals and depends on zinc for proper useand bioavailability [9, 10]. This relation between Zink and Folic acid, demonstrate synergistic properties between them [10-13].

Human trials of folic acid and zinc supplementation haveproduced different results of small number of participants [14].Otherwise,some evidence suggests zinc and folate in combination results in optimal outcomes[11-13]. A meta-analysis concluded that large-scaletrials were needed and it remains unproven whether supplementationcould affect pregnancy rate, which is the most interesting outcome ofinfertile couples[14].Therefore, the aim of this trial was todetermine the efficacy of folic acid and zincsupplementation daily inmenon semen quality and pregnancy rateamong couples seeking infertility treatment.

II. Methods

This is a double blindMulticentre randomized placebocontrolledclinical trial. It was conducted in five hospitals; Prince Meshari General Hospital in Baljurashi, Madinah Maternity and Children Hospital, Makkah Maternity and Children Hospital, Jeddah Maternity and Children Hospital and Jeddah Armed forces Hospital. Trial was conducted for 48 months from 1/1/2016 to 31/12/2019.

Male partners of couples seeking infertility treatment wereenrolled. Participants (men aged ≥18 years and women aged 18-45 years)were ineligible if they were pregnant at enrollment, or ifthe male had obstructive azoospermia or other known infertilitycauses unlikely to benefit from supplementation. Menadvised to abstain from supplements containingfolic acid or zinc, as well as medications thatinteract with folic acid or zinc. Men with knownchronic diseases (e.g., heart disease, diabetes, hypertension,cancer) excluded.

Men received daily supplements containing 5 mg offolic acid and 30 mg of elementalzinc or placebo for 6 months.Men were receiving the study intervention for a minimumof 12 to 16 weeks before the ovulatory phase of thefirst infertility treatment cycle. This timing ensures a minimumtime of receiving the intervention that covers thestages of spermatocytogenesis as well meeting the practical needs of patients to initiateinfertility treatment promptly.

The studytablets were available in Ministry of Health (MOH) hospitals and armed forces hospitals. Placebo was supplied by different pharmaceutical companies to match study tablets in appearance,size, taste, and weight.

Eligible male participants were randomized in a 1:1 ratio todaily folic acid and zinc or placebo. Randomization was done using last digit in the participant file number, if its odd he will receive folic acid and zinc supplement and if it is even number he will receive placebo. Blinding was conducted through keeping Participantsand investigatorsblinded to treatmentthroughout the trial.

Male participants completed in-person study visits,which included semen collection, atbaseline and at 2, 4, and 6 months after randomization.Adverse event and adherence questionnaires discussedat each visit to assess symptoms and adherence to treatment. Female participants were followedup for 9 months after randomization, with brief monthly questionnairesassessing infertility treatment, pregnancy status,and pregnancy outcomes. Women asked to reportdirectly to research staff any positive serum β-human chorionic gonadotropintests.

Primary outcomes were live birth and semen quality (assessed by quantification of sperm concentration, Motility, morphology and volume). Secondary outcomes included clinical intrauterinepregnancy (visualized gestational sac in the uterususing ultrasonography), ectopic pregnancy, pregnancywith multiple fetuses, early pregnancy losses (includingserum β-human chorionic gonadotropin level >5mIU/mLfollowed by a decline) and clinically recognized pregnancylosses (clinical pregnancy followed by a pregnancy loss at<20 weeks' gestation). Added to that, cesarean delivery, preeclampsia or gestational hypertension, gestational diabetes, birth weight and small for gestational age at birth.

Analyzed data arerepresented either as simple statistics (number,percentage or mean ± standard deviation). The statisticalanalyses involved two-sample testand chi-squared test,as appropriate. P value of <0.05 was consideredsignificant.The ethics committee of MOH and Armed Forces Health services approved this study.

III. Results

2370menrandomized 1185 to the folic acid andzinc supplements group and 1185 to the placebo group. The baseline characteristics of the maleand female participants were similar between the groups(Table 1).

	Folic Acid and Zinc (n = 1185) No (%)	Placebo (n = 1185) No (%)
Male Partner		
Age, mean (SD), y	32.5 (5.7)	32.7 (6.0)
Body mass index, mean (SD)	30.1 (6.7)	29.6 (6.7)
Race/ethnicity		
Saudi	1108/1182 (94)	1110/1178 (94)
Non-Saudi	74/1182 (6)	68/1178 (6)
Education level		
High school degree or less	198/1173 (17)	173/1168 (15)
Bachelor's degree	763/1173 (65)	775/1168 (66)
Master's degree or higher	212/1173 (18)	220/1168 (19)
Employment status		
Unemployed	149/1096 (14)	148/1095 (14)
employed	947/1096 (86)	947/1095 (86)
Male factor infertility diagnosis		
Yes	160/758 (21)	165/759 (22)
No	598/758 (79)	594/759 (78)

Female Partner		
Age, mean (SD), y	30.6 (5.0)	30.8 (5.2)
Body mass index, mean (SD)	28.9 (8.3)	28.1 (8.1)
Race/ethnicity		
Saudi	1129/1180 (96)	1128/1178 (96)
Non-Saudi	51/1180 (4)	50/1178 (4)
Education level		
High school degree or less	130/1170 (11)	145/1167 (12)
Bachelor's degree	822/1170 (70)	814/1167 (70)
Master's degree or higher	218/1170 (19)	208/1167 (18)
Female factor infertility diagnosis		
Yes	281/758 (37)	265/757 (35)
No	477/758 (63)	492/757 (65)

Table 1: Participants baseline characteristics

Participant adherence was high overall.Most participants did not report missing more than 5 doses during the interval between each follow-up visit.

For the primary outcome of live birth, 820 participants(35%) attained a live birth, which did not significantly differby intervention group overall (404 [34%] in the folic acid andzinc group vs 416 [35%] in the placebo group; risk difference,−0.9% [95% CI, −4.7% to 2.8%]) (Table 2).

	Folic Acid and Zinc (n = 1185) No (%)	Placebo (n = 1185) No (%)	Risk Difference (95% CI)
Live Birth	404/1185 (34)	416/1185 (35)	−0.9 (−4.7 to 2.8)

Table 2: Primary outcome of live birth

For the semen quality parameters, sperm concentration, motility, morphology and volumewere not significantly different after 6 months (Table 3).

Semen Quality Parameters	Folic Acid and Zinc Mean (SD)	Placebo Mean (SD)	Risk Difference (95% CI)
No. of participants	794	835	
Sperm concentration, million/mL	84.8 (85.2)	89.0 (85.0)	−4.3 (−12.5 to 3.9)
Motility, %	52.7 (21.2)	53.2 (20.1)	−0.5 (−2.5 to 1.5)
Morphology, % normal	5.7 (4.2)	6.0 (4.8)	−0.4 (−0.8 to 0.1)
Volume, mL	3.5 (1.5)	3.5 (1.7)	−0.1 (−0.5 to 0.3)

Table 3: Primary outcome of semen quality

There was no statistically significant effect of supplementationon 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, cesareandelivery, preeclampsia or gestational hypertension, gestationaldiabetes, gestational age, birth weight, or small forgestational age at birth (Table 4).Significantincrease in preterm delivery observed with folic acid andzinc supplementation overall (67 [6%] vs 45 [4%] in the placebogroup; risk difference, 1.9% [95% CI, 0.2% to 3.6%]).

	Folic Acid and Zinc No (%)	Placebo No (%)	Risk Difference (95% CI)
No. of participants	1185	1185	
hCG-detected pregnancy	479 (40)	490 (41)	−0.9 (−4.7 to 3.0)
Clinical intrauterine pregnancy	449 (38)	462 (39)	−1.0 (−4.9 to 2.8)
Ectopic pregnancy	6 (<1)	5 (<1)	0.1 (−0.5 to 0.6)
Early pregnancy loss (prior to 20 wk)	137 (12)	150 (13)	−1.1 (−3.7 to 1.5)
Pregnancy with multiple fetuses	42 (4)	42 (4)	0 (−1.5 to 1.5)
Preeclampsia or gestational hypertension	47 (4)	51 (4)	−0.3 (−1.9 to 1.3)
Gestational diabetes	26 (2)	34 (3)	−0.7 (−1.9 to 0.6)
Cesarean delivery	143 (12)	129 (11)	1.2 (−1.4 to 3.8)
Preterm delivery	67 (6)	45 (4)	1.9 (0.2 to 3.6)
Small for gestational age	62 (5)	59 (5)	0.3 (−1.5 to 2.0)

Table 4: Secondary outcomes

IV. Discussion

In this randomized clinical trial, supplementation with 5 mgof folic acid and 30 mg of zinc in men did not improvesemen quality parameters or increase couples live birthrates among patients seeking infertility treatment. Furthermore, some increased mild gastrointestinal adverse effects accompanied this lack of efficacy.

This trialdiscusses the need for large-scale trial to examine the effects of folic acidand zinc supplementation on semen quality. Althoughthese findings disagree with the conclusion from a meta-analysis that a supplement combination with folateand zinc improved semen quality,the authors of the meta-analysis had urged cautiongiven the heterogeneity of the included studies[14].

The frequency of maternal complications wassimilar between groups, except an unexpected increase in pretermbirth in the folic acid and zinc group. Verification of this result is needed, which may be a chance finding[15]. However, there were lowadverse events in men randomized to folic acid andzinc supplementation compared with placebo, indicatingthese doses of folic acid and zinc are well tolerated bymen. Previous studies of zinc have reported higherrates of gastrointestinal adverse effects[16, 17].

This study has several limitations. First, the present findingsare generalizable to a general infertility clinic population andnot subfertile men specifically; most patients were whiteand Saudi men, with high socioeconomic status, thus limitinggeneralizability.

Second, due to couples pursuing fewer cycles of infertilitytreatment than anticipated, the cumulative live birth rateobserved for the placebo group was lower thanassumed in the sample size calculations. On the other hand, thislower rate had little effect on the power to detect a meaningfulrisk difference.

V. Conclusions

Among a general population of couples seeking infertility treatment,the use of folic acid and zinc supplementation by malepartners, compared with placebo, did not significantly improvesemen quality or couples live birth rates. These findingsdo not support the use of folic acid and zinc supplementationby men.

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