

Supplementary Online Content

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This supplementary material has been provided by the authors to give readers additional information about their work.

eTable 1. Semen quality after six months of trial intervention in the Folic Acid and Zinc Supplementation Trial by intervention group, using non-parametric testing

	Folic Acid & Zinc Median (IQR)	Placebo Median (IQR)	Median Difference (CI)^a	Stratified Wilcoxon p-value^b	Weighted stratified Wilcoxon p-value^{b, c}
Overall^d	N = 794	N = 835			
Concentration, M/mL	56.0 (103)	69.0 (94.0)	-4.2 (-10, 0.6)	0.12	0.10
Motility, %	57.5 (26)	57.0 (27.0)	0.0 (-2.0, 1.8)	0.71	0.66
Morphology, % normal	5.0 (5.5)	5.0 (6.0)	0.0 (-0.5, 0.5)	0.35	0.30
Volume, mL	3.2 (2.1)	3.3 (2.3)	0.0 (-0.1, 0.2)	0.70	0.75
DNA fragmentation index, %	24.8 (21.2)	22.9 (19.9)	1.5 (0.1, 2.9)	0.05	0.05
Total motile sperm count, M	100 (227)	118 (232)	-4.4 (-15, 2.9)	0.29	0.30
IVF^d	N=124	N=135			
Concentration, M/mL	51.1 (105.5)	51.5 (90.0)	0.4 (-13, 13)	0.98	0.73
Motility, %	57 (25.8)	56.8 (26.8)	0.3 (-4.5, 4.8)	0.81	0.56
Morphology, % normal	4.5 (5.5)	4.8 (5.3)	0.0 (-1.0, 1.0)	0.71	0.56
Volume, mL	3.2 (2.2)	3.2 (1.9)	0.0 (-0.4, 0.3)	0.85	0.81
DNA fragmentation index, %	22.9 (19.3)	20.6 (22.5)	0.7 (-2.7, 4.2)	0.80	0.84
Total motile sperm count, M	84 (202)	106.1 (186)	-0.2 (-23, 20)	0.82	0.68
Other infertility treatment onsite^d	N=566	N=590			
Concentration, M/mL	56 (101)	72.3 (92.0)	-7.2 (-14, -0.5)	0.05	0.06
Motility, %	57.1 (25.8)	57.3 (26.5)	-0.8 (-3.0, 1.3)	0.50	0.50
Morphology, % normal	5.0 (5.5)	5.5 (5.8)	-0.5 (-1.0, 0.0)	0.10	0.10
Volume, mL	3.3 (2.2)	3.3 (2.3)	0.0 (-0.2, 0.2)	0.57	0.59
DNA fragmentation index, %	25.8 (21.7)	23.1 (18.5)	1.8 (0.0, 3.5)	0.03	0.02
Total motile sperm count, M	99.9 (236)	119.1 (239)	-8.8 (-22, 1.4)	0.19	0.23
Other infertility treatment offsite^d	N=104	N=110			
Concentration, M/mL	73.8 (108)	67.7 (100)	2.0 (-14, 19)	0.71	0.82
Motility, %	61.1 (25.4)	56.1 (32.8)	3.0 (-2.8, 8.5)	0.42	0.31
Morphology, % normal	6.0 (7.5)	5.0 (7.0)	1.0 (-0.3, 2.0)	0.08	0.10
Volume, mL	3.2 (2.1)	3.3 (2.3)	0.0 (-0.4, 0.5)	0.95	0.89
DNA fragmentation index, %	24.4 (22.3)	23.7 (24.9)	1.2 (-2.9, 5.5)	1.00	0.82
Total motile sperm count, M	125 (221)	107 (239)	4.7 (-19, 33)	0.69	0.71

^aDifference is the Hodges-Lehmann estimation of the location shift between the active and placebo groups. Asymptotic confidence intervals are for the estimate of the location shift. Confidence intervals are calculated using pre-specified 0.049 confidence level for concentration, motility, and morphology (prespecified for interim analysis), and 0.05 confidence level for DNA fragmentation index and total motile sperm count.

^bWilcoxon two-sample test two-sided $P > Z$, normal approximation, stratified by infertility treatment stratum (IVF, other infertility treatment onsite, and other infertility treatment offsite) and study site, as appropriate.

^cSemen parameters were weighted by stabilized inverse probability of completing six-month clinic visit (based on treatment assignment, age, pregnancy, gastrointestinal adverse events, education, income, insurance, marital status, stratum, site, employment, and body mass index).

^dInfertility treatment stratum was the planned infertility treatment ascertained at the time of randomization and included either *in vitro* fertilization (IVF stratum), as well as other infertility treatment strategies including ovulation induction, intrauterine insemination, and natural fertility optimization methods administered through one of the reproductive endocrinology and infertility specialist study centers (other infertility treatment onsite), or with a community provider (other infertility treatment offsite). If sperm concentration was rare/too few to count, no sperm in sample, or retrograde, then concentration, motility, and morphology were set to zero, and DNA fragmentation was calculated as $[(100 -$

max)/sqrt(2)]+max, where the “max” measured value for DNA fragmentation index was 98.4%. Motility includes % progressive motile and % non-progressive motile. Total motile sperm count calculated as volume * concentration * motility. WHO 5 criteria definition for normal morphology is ≥ 4 % normal forms.

eTable 2. Semen quality after six months of trial intervention in the Folic Acid and Zinc Supplementation Trial by intervention group, treating samples with inadequate sperm concentration (no sperm in sample, rare/too few to calculate, retrograde) as missing

	Folic Acid & Zinc, Mean (SD)	Placebo, Mean (SD)	Adjusted mean difference (95% CI)	Adjusted, weighted mean difference (95% CI)
Overall^{a,b,c}	N = 761	N = 815		
Concentration, M/mL	88.5 (85.2)	91.2 (84.9)	-2.5 (-10.9, 5.8)	-3.3 (-11.8, 5.2)
Motility, %	54.7 (18.9)	54.2 (18.9)	0.5 (-1.4, 2.3)	0.4 (-1.5, 2.3)
Morphology, % normal	5.9 (4.1)	6.2 (4.8)	-0.2 (-0.7, 0.2)	-0.3 (-0.7, 0.1)
Volume, mL	3.5 (1.7)	3.5 (1.8)	0.0 (-0.2, 0.2)	0.0 (-0.1, 0.2)
DNA fragmentation index, %	26.5 (14.6)	25.8 (14.8)	0.7 (-0.8, 2.2)	0.3 (-1.1, 1.8)
Total motile sperm count, M	191 (228)	186 (213)	5.8 (-15.8, 27.3)	5.0 (-16.6, 26.7)
IVF^{a,b,d}	N = 119	N = 132		
Concentration, M/mL	85.2 (97.0)	77.8 (78.6)	8.3 (-12.8, 29.5)	9.2 (-11.8, 30.2)
Motility, %	54.3 (19.0)	52.5 (19.1)	1.6 (-3.1, 6.3)	1.3 (-3.5, 6.1)
Morphology, % normal	5.4 (4.3)	5.6 (4.7)	-0.2 (-1.3, 0.9)	-0.4 (-1.5, 0.8)
Volume, mL	3.5 (1.5)	3.5 (1.7)	-0.1 (-0.5, 0.3)	0.0 (-0.4, 0.4)
DNA fragmentation index, %	24.6 (14.5)	25.6 (17.5)	-1.5 (-5.5, 2.6)	-1.3 (-5.3, 2.8)
Total motile sperm count, M	173 (224)	156 (189)	16.4 (-33.2, 66.0)	17.0 (-32.3, 66.3)
Other infertility treatment onsite^{a,b,d}	N = 544	N = 578		
Concentration, M/mL	88.5 (83.0)	94.1 (84.6)	-5.4 (-15.2, 4.4)	-6.8 (-16.9, 3.3)
Motility, %	54.3 (19.1)	54.7 (18.5)	-0.4 (-2.5, 1.8)	-0.4 (-2.6, 1.8)
Morphology, % normal	5.8 (3.9)	6.4 (4.9)	-0.5 (-1.0, 0.0)	-0.6 (-1.1, 0.0)
Volume, mL	3.5 (1.7)	3.5 (1.8)	0.0 (-0.2, 0.2)	0.0 (-0.2, 0.2)
DNA fragmentation index, %	27.1 (14.6)	25.8 (14.1)	1.2 (-0.5, 2.9)	0.6 (-1.1, 2.3)
Total motile sperm count, M	193 (228)	192 (207)	2.4 (-22.8, 27.7)	1.1 (-24.5, 26.6)
Other infertility treatment offsite^{a,b,d}	N = 98	N = 105		
Concentration, M/mL	92.6 (82.6)	91.9 (92.6)	1.8 (-23.0, 26.6)	1.8 (-22.8, 26.3)
Motility, %	57.2 (18.1)	53.4 (20.9)	3.9 (-1.6, 9.4)	4.5 (-1.1, 10.0)
Morphology, % normal	7 (4.8)	5.8 (4.3)	1.3 (0.1, 2.6)	1.3 (0.1, 2.6)
Volume, mL	3.5 (1.7)	3.4 (1.7)	0.0 (-0.4, 0.5)	0.0 (-0.4, 0.5)
DNA fragmentation index, %	26.1 (14.6)	25.5 (14.7)	-0.3 (-4.6, 3.9)	0.0 (-4.3, 4.2)
Total motile sperm count, M	204 (235)	192 (264)	12.8 (-57.9, 83.6)	13.7 (-56.9, 84.3)

^aInfertility treatment stratum was the planned infertility treatment ascertained at the time of randomization and included either *in vitro* fertilization (IVF stratum), as well as other infertility treatment strategies including ovulation induction, intrauterine insemination, and natural fertility optimization methods administered through one of the reproductive endocrinology and infertility specialist study centers (other infertility treatment onsite), or with a community provider (other infertility treatment offsite). Motility includes % progressive motile and % non-progressive motile. Total motile sperm count calculated as volume * concentration * motility. WHO 5 criteria definition for normal morphology is $\geq 4\%$ normal forms.

^bConfidence intervals for concentration, motility, and morphology represent 95.1% confidence intervals to properly account for the alpha spent in the interim analysis.

^cAdjusted for infertility treatment stratum (IVF, other infertility treatment onsite, and other infertility treatment offsite) and study site.

^dAdjusted for study site.

eTable 3. Live birth frequency in couples participating in the Folic Acid and Zinc Supplementation Trial by intervention group, restricted to men with male factor infertility diagnoses and/or poor baseline semen quality measures

Live birth	Folic Acid & Zinc N (%)	Placebo N (%)	Risk Ratio (95% CI)	Risk Difference (95% CI)
Any male subfertility indicator at baseline (N=1185)	191 (32%)	179 (31%)	1.04 (0.88, 1.24)	1.3 (-3.9, 6.6)
Male factor infertility diagnosis history ^a	52 (33%)	61 (37%)	0.88 (0.65, 1.19)	-4.5 (-14.8, 5.9)
Baseline Count \leq 39 M	49 (24%)	58 (28%)	0.88 (0.63, 1.22)	-3.4 (-11.8, 5.1)
Baseline Motility \leq 40%	78 (30%)	56 (23%)	1.27 (0.95, 1.71)	6.3 (-1.4, 14.0)
Baseline Morphology < 4% normal forms	137 (32%)	119 (31%)	1.04 (0.85, 1.28)	1.3 (-5.1, 7.8)
Baseline Total Motile Count < 20 M	60 (25%)	63 (26%)	0.97 (0.71, 1.31)	-0.8 (-8.6, 7.0)

^aMale factor infertility diagnosis self-reported on questionnaire at enrollment.

eTable 4. Semen quality after six months of trial intervention in the Folic Acid and Zinc Supplementation Trial by intervention group, restricted to men with male factor infertility diagnoses and/or poor baseline semen quality measures

	Folic Acid & Zinc Mean (SD)	Placebo Mean (SD)	Difference, adjusted for site, strata (95% CI)	Weighted difference adjusted for site, strata (95% CI)
Any male subfertility indicator at baseline^a	n=425	n=428		
Concentration, M/mL	54.4 (70.1)	63.4 (78.7)	-9.5 (-19.5, 0.5)	-12.5 (-23.1, -1.9)
Motility, %	45.2 (23.0)	45.1 (21.6)	-0.1 (-3.1, 2.9)	-0.2, (-3.2, 2.9)
Morphology, % normal	4.1 (3.8)	4.4 (4.5)	-0.4 (-0.9, 0.2)	-0.4 (-1.0, 0.2)
Volume, mL	3.5 (1.7)	3.5 (1.7)	0.0 (-0.2, 0.2)	0.0 (-0.2, 0.2)
DNA fragmentation index, %	33.0 (24.0)	30.0 (20.3)	3.1 (0.0, 6.3)	3.1 (-0.1, 6.4)
Total motile sperm count, M	106.6 (181.8)	113.6 (158.2)	-8.6 (-31.3, 14.1)	-12.6 (-36.2, 11.1)
Male factor infertility diagnosis history^{a,b}	n=127	n=125		
Concentration, M/mL	43.2 (55.9)	52.0 (62.4)	-8.7 (-23.4, 6.1)	-8.2 (-23.0, 6.6)
Motility, %	41.0 (22.8)	43.4 (21.0)	-2.5 (-8.0, 3.0)	-2.5 (-8.1, 3.1)
Morphology, % normal	3.7 (3.9)	4.3 (4.9)	-0.7 (-1.9, 0.4)	-0.9 (-2.0, 0.3)
Volume, mL	3.7 (1.7)	3.6 (1.7)	0.1 (-0.3, 0.5)	0.1 (-0.3, 0.5)
DNA fragmentation index, %	37.9 (26.3)	32.8 (23.8)	5.3 (-1.3, 11.8)	5.6 (-1.1, 12.3)
Total motile sperm count, M	78.2 (112.8)	86.7 (112.2)	-7.9 (-35.7, 20.0)	-7.4 (-35.3, 20.5)
Baseline Count ≤ 39 M^a	n=149	n=139		
Concentration, M/mL	22.5 (64.4)	23.4 (42.8)	-0.9 (-14.0, 12.3)	-1.7 (-14.5, 11.2)
Motility, %	33.2 (24.7)	35.6 (23.5)	-2.9 (-8.6, 2.9)	-1.8 (-7.7, 4.0)
Morphology, % normal	3.2 (4.2)	3.1 (3.6)	-0.1 (-1.1, 0.8)	-0.1 (-1.1, 0.9)
Volume, mL	3.1 (1.7)	3.5 (1.8)	-0.2 (-0.6, 0.2)	-0.2 (-0.6, 0.2)
DNA fragmentation index, %	48.8 (31.0)	39.7 (26.6)	8.4 (1.1, 15.7)	7.3 (-0.3, 14.9)
Total motile sperm count, M	32.5 (111.4)	35.2 (66.5)	-1.9 (-23.8, 19.9)	-3.1 (-24.5, 18.3)
Baseline Motility ≤ 40%^a	n=198	n=184		
Concentration, M/mL	35.9 (56.5)	42.6 (56.6)	-6.9 (-18.4, 4.6)	-6.8 (-18.0, 4.5)
Motility, %	33.0 (22.3)	34.0 (21.0)	-0.9 (-5.3, 3.5)	-0.3 (-4.8, 4.1)
Morphology, % normal	3.2 (3.7)	3.5 (4.1)	-0.3 (-1.1, 0.5)	-0.3 (-1.1, 0.5)
Volume, mL	3.3 (1.7)	3.5 (1.8)	-0.2 (-0.5, 0.2)	-0.1 (-0.5, 0.2)
DNA fragmentation index, %	39.1 (29.2)	32.6 (22.1)	6.6 (1.1, 12.2)	6.3 (0.5, 12.1)
Total motile sperm count, M	44.2 (76.1)	63.0 (117.9)	-18.0 (-38.0, 1.9)	-17.5 (-37.0, 2.0)
Baseline Morphology < 4% normal forms^a	n=293	n=280		
Concentration, M/mL	53.0 (65.6)	68.7 (84.9)	-17.0 (-29.4, -4.6)	-20.9 (-34.4, -7.4)
Motility, %	45.6 (23.8)	45.4 (22.3)	-0.2 (-4.0, 3.6)	-0.5 (-4.4, 3.4)
Morphology, % normal	3.8 (3.4)	4.2 (4.4)	-0.6 (-1.2, 0.1)	-0.7 (-1.3, 0.0)
Volume, mL	3.4 (1.7)	3.4 (1.7)	0.0 (-0.2, 0.3)	0.1 (-0.2, 0.3)
DNA fragmentation index, %	32.8 (24.1)	28.6 (19.0)	4.2 (0.4, 8.0)	4.6 (0.6, 8.5)
Total motile sperm count, M	112.5 (197.7)	122.5 (166.5)	-13.6 (-43.3, 16.2)	-18.7 (-49.8, 12.5)
Baseline total motile count < 20 M^a	n=180	n=169		
Concentration, M/mL	24.9 (59.6)	26.4 (44.4)	-2.0 (-13.3, 9.3)	-2.7 (-13.7, 8.4)
Motility, %	32.5 (23.5)	33.6 (21.8)	-1.5 (-6.4, 3.4)	-0.6 (-5.5, 4.3)
Morphology, % normal	3.2 (3.9)	3.2 (3.6)	-0.2 (-1.0, 0.7)	-0.2 (-1.0, 0.7)
Volume, mL	3.3 (1.8)	3.4 (1.8)	-0.1 (-0.5, 0.2)	-0.1 (-0.5, 0.3)
DNA fragmentation index, %	43.4 (30.0)	36.5 (24.8)	6.5 (0.3, 12.7)	5.6 (-0.8, 12.1)
Total motile sperm count, M	33.7 (103.1)	34.0 (63.0)	-0.7 (-19.0, 17.7)	-1.3 (-19.2, 16.5)

^aIf sperm concentration was rare/too few to count, no sperm in sample, or retrograde, then concentration, motility, and morphology were set to zero, and DNA fragmentation was calculated as $[(100 - \text{max})/\sqrt{2}] + \text{max}$, where the “max” measured value for DNA fragmentation index was 98.4%. Motility includes % progressive motile and % non-progressive motile. Total motile sperm count calculated as volume * concentration * motility. WHO 5 criteria definition for normal morphology is ≥ 4 % normal forms.

^bMale factor infertility diagnosis self-reported on questionnaire at enrollment.

eTable 5. Early embryonic parameters among participants in the IVF stratum in the Folic Acid and Zinc Supplementation Trial by intervention group

Continuous outcomes	N	Folic Acid & Zinc Mean (SD)	Placebo Mean (SD)	Mean Difference (95% CI)
Cycle level				
Fertilization rate, %	243	75.3 (2.25)	77.7 (1.74)	-2.34 (-7.90, 3.23)
# of good quality embryos on day 5 ^a	146	2.66 (0.23)	2.98 (0.21)	-0.11 (-0.33, 0.11)
% good quality embryos on day 5	243	17.2 (2.05)	18.5 (1.81)	-1.31 (-6.66, 4.05)
# of embryos transferred ^a	299	1.50 (0.06)	1.51 (0.05)	-0.01 (-0.11, 0.10)
# of embryos cryopreserved ^a	208	4.22 (0.32)	4.32 (0.31)	-0.02 (-0.23, 0.18)
Sperm penetration assay results, %	24	62.7 (13.0)	74.8 (11.0)	-12.1 (-45.4, 21.2)
Embryo level				
# of cells on day 3 ^a	2344	5.60 (0.20)	5.98 (0.19)	-0.07 (-0.16, 0.03)
Categorical outcomes				
		Predicted probability	Predicted probability	Risk Ratio (95% CI)
Cycle level				
Chromosomal complement	22			
Abnormal		0.75	0.32	2.35 (0.74, 7.43)
Normal		0.25	0.68	
Embryo level				
# of cells on day 3	2350			
<4		0.27	0.22	1.19 (0.88, 1.61)
≥4		0.73	0.78	
# of cells on day 5	2360			
<8		0.20	0.19	1.05 (0.77, 1.43)
≥8		0.80	0.81	
Method of fertilization	3470			
ICSI		0.74	0.79	0.93 (0.80, 1.09)
Other		0.26	0.21	
Embryo morphology on day 3	1880			
Excellent/good		0.66	0.68	0.98 (0.87, 1.09)
Fair/poor		0.34	0.32	
Embryo morphology on day 5	1977			
Excellent/good		0.28	0.35	0.82 (0.63, 1.06)
Fair/poor		0.72	0.65	
Quality of embryos transferred	352			
Excellent/Good		0.75	0.73	1.03 (0.88, 1.21)
Fair/poor		0.25	0.27	

Analyses for embryonic parameters were done using GEE to account for multiple cycles per couple or multiple embryos per cycle.

^aAnalysis for count outcomes done using Poisson regression with GEE.