The Effects of Folic Acid and Zinc Supplementation in Men on Semen Quality and Live Birth

Sunni L Mumford, PhD

Epidemiology Branch, DIPHR
Eunice Kennedy Shriver National Institute of Child Health and Human Development
I have no conflicts of interest to disclose.
FAZST Participants - 2370!

Enrique Schisterman PhD, Co-PI
Lindsey Sjaarda PhD
Neil Perkins PhD
James Mills MD
Pauline Mendola PhD
Zhen Chen PhD

Matthew Peterson MD
Erica Johnstone MD
Ahmad Hammoud MD
Denise Lamb
Douglas Carrell MD
Jim Hotaling MD

Traci Clemons PhD
Kayla Cheney

Brad VanVoorhis MD
Ginny Ryan MD
Karen Summers MPH

Jared Robins MD
Bruce Campbell MD

Joyce Southey MD
Jun Liu PhD
Jana Davis PhD
Carrie Herbert MS
Motivation

Infertility affects 7-15% of couples

Male factor major contributor to couple infertility
- 40% of etiology remains unknown
- Low-cost interventions entirely lacking for male

Treatments are stressful, expensive & frequently lack insurance coverage
- First line (OI/IUI) → 7-12% success rate per cycle
  - Specialty REI clinics
  - OI common among OBGyn general providers
- Advanced technologies (IVF) cost $12K+ per cycle
Motivation

Dietary supplements = potentially promising low-cost intervention with tremendous population-level impact

U.S. dietary supplement industry rapidly growing & FDA regulation is limited

Supplements for male fertility are common: folic acid & zinc

Efficacy largely unproven
Biologic rationale

Folate key component of DNA
\[\rightarrow\] purine & pyrimidine biosynthesis

Zinc is co-factor for > 80 metalloenzymes involved in DNA transcription

Folic acid & zinc are antioxidants
\[\rightarrow\] Sperm DNA sensitive to oxidative stress due to small cytoplasm
Small prior trials

Combination of folic acid and zinc → increased sperm concentration in subfertile men

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n=25)</th>
<th>Zinc + folic acid (n=24)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-intervention</td>
<td>Post-intervention</td>
</tr>
<tr>
<td>Sperm concentration</td>
<td>7.5 (0.7-110)</td>
<td>9 (0.8-80)</td>
</tr>
<tr>
<td></td>
<td>7.5 (0.3-130)</td>
<td>12 (0.5-180)*</td>
</tr>
<tr>
<td>Motility</td>
<td>30 (2-70)</td>
<td>30 (5-80)</td>
</tr>
<tr>
<td></td>
<td>33 (5-80)</td>
<td>35 (5-70)</td>
</tr>
<tr>
<td>WHO (abnormal)</td>
<td>80 (53-98)</td>
<td>79 (61-98)</td>
</tr>
<tr>
<td></td>
<td>80 (54-100)</td>
<td>84 (60-100)*</td>
</tr>
<tr>
<td>Strict criteria (normal)</td>
<td>2 (1-9)</td>
<td>2 (1-8)</td>
</tr>
<tr>
<td></td>
<td>2 (1-12)</td>
<td>2 (1-13)</td>
</tr>
<tr>
<td>Total normal sperm count</td>
<td>6.4 (0.3-113)</td>
<td>6.3 (0.1-80)</td>
</tr>
<tr>
<td></td>
<td>5.1 (0-92)</td>
<td>8.9 (0-106)*</td>
</tr>
</tbody>
</table>

*indicates significant difference, pre- vs post-intervention

- Folic acid alone or zinc alone did not impact measures
- No intervention improved parameters of fertile men

Modified from Wong et al., 2002 Fertil Steril
Research Gap

2017 meta-analysis: positive effects of folic acid & zinc in sub-fertile men
- Primarily on sperm concentration
- Noted heterogeneity of included studies (4 RCTs for sperm concentration analysis)
- All very small trials, < 30 men per group
- None examined pregnancy/live birth outcome

![Fig 5. Effects of folic acid & zinc supplementation in subfertile men](Modified from Irani et al., 2007 Urol J)
Research Gap

2017 meta-analysis: positive effects of folic acid & zinc in sub-fertile men
  ◦ Primarily on sperm concentration
  ◦ Noted heterogeneity of included studies (4 RCTs for sperm concentration analysis)
  ◦ All very small trials, < 30 men per group
  ◦ None examined pregnancy/live birth outcome

- Fertility supplements are growing market
- Great potential for impact, but also great vulnerability for couples with infertility

→ Tremendous need for proven scientific efficacy & safety

Fig 5. Effects of folic acid & zinc supplementation in subfertile men
Modified from Irani et al., 2007 *Urol J*
Aim

Double-blind, randomized, placebo-controlled clinical trial to:

Assess the effects of folic acid and zinc supplementation in males on semen quality and live birth among couples seeking infertility treatment.
Trial Design

2370 couples

Supplement: 5 mg folic acid + 30 mg elemental zinc taken orally daily

Randomization stratified by type of planned infertility treatment:
1. IVF treatment
2. OI/IUI treatment (On-site clinics)
3. OI/IUI treatment (Off-site clinics)

6 months of male study visits (2, 4, 6 months)

Up to 18 months of couple follow-up (live birth)

CT.gov: [https://clinicaltrials.gov/ct2/show/NCT01857310](https://clinicaltrials.gov/ct2/show/NCT01857310)
Tablet formulation

Unlike other studies using off-the-shelf formulations

Designed and manufactured active and placebo tablets

5 mg folic acid + 30 mg elemental zinc
  ◦ Folic acid similar to prior studies
  ◦ Within upper tolerable limit (40 mg) of zinc

Stability testing ensured quality
Semen quality
Quality Control for Semen Analysis

Semen analysis is highly complex and procedurally difficult to standardize

- Assessment of concentration, motility, morphology involves counting a limited number of sperm which are presumed to represent the whole sample
- Even in a well mixed sample, there is considerable variability
- QC is essential, especially across labs
Quality Control for Semen Analysis

All labs performing semen analysis for FAZST were centrally trained and completed standardized inter-lab QA testing throughout the study

- QC samples with known sperm concentration, motility, and morphology were measured each month, by each technician, at each lab
  - Slides of fixed semen smears used for morphology from good, medium, and poor quality semen

- Variation was assessed using Westgard rules and ongoing training was done as needed
Example $X_{\text{bar}}$ chart for sperm concentration

[Chart showing $X_{\text{bar}}$ chart with data points and control limits for sperm concentration]
Study endpoints

Primary

• Semen quality
• Live Birth

Secondary

• hCG-detectected pregnancy
• Clinical intrauterine pregnancy
• Pregnancy loss
• Pregnancy & birth outcomes/complications
• Embryo parameters (among IVF)

Biospecimen repository: blood, urine, semen, saliva, toenails
Couple Eligibility Criteria

**Couples Inclusion Criteria:**

- Male-female couples, female (18-45 years) & male (18+ years)
- Couples actively trying to conceive and seeking infertility treatment at participating reproductive endocrinology & infertility clinics
- Couples who are planning OI, IUI, or natural fertility optimization methods, willing to be on the study supplement for ≥ 3 weeks before next treatment cycle or attempting pregnancy.

**Couples Exclusion Criteria:**

- Female partner unwilling to participate
- Couples using donor, cryopreserved sperm, or sperm obtained via microsurgical or percutaneous epididymal sperm aspiration.
Male Exclusion Criteria:

- Unwilling to abstain from use of non-study approved supplements or medications containing folic acid or zinc
- Diagnosis of Vitamin B_{12} deficiency or anemia at screening
- Known Vitamin B_{12} deficiency or anemia within the last 5 years that has not been successfully treated
- Consuming a vegan diet
- Genetic cause of male factor subfertility, including chromosomal disorders related to subfertility
- Current use of drugs known to interact with folic acid or interfere with the biosynthesis of folic acid
- History of vasectomy without reversal, obstructive azoospermia such as CBAVD, or ejaculatory duct obstruction.
- Physician diagnosed:
  - Current poorly controlled chronic diseases (incl'd heart disease, diabetes mellitus, hypertension, cancer, and more)
  - Crohn’s disease, celiac disease, ulcerative colitis, gastric bypass surgery, lap band surgery or history of intestinal surgery to remove a portion of small bowel.
  - History of diseases/symptoms that require folic acid supplementation, such as megaloblastic anemia, homocystinemia, and homocystinuria
  - History of alcohol/drug dependency disorder
Male Participant Flow

0 2 4 6
Month

Safety
Questionnaires
Medication Review
Semen/biospecimens
FFQ
Anthropometrics

Safety
Questionnaires
Medication Review
Semen/biospecimens
24 Hour Diet Recall
Adherence

Safety
Questionnaires
Medication Review
Semen/biospecimens
24 Hour Diet Recall
Adherence
Toenail Collection

Safety
Questionnaires
Medication Review
Semen/biospecimens
FFQ
Adherence
Anthropometrics
Overall Participant Flow

Men: Folic Acid & Zinc or Placebo

Abstracted Medical Record

OI/IUI/IVF

Females

Questionnaires

Anthropometrics

Biospecimens

FFQ

Non-pregnancy Verification

Male Visits

Female Monthly Online Check-in

Pregnancies conceived within 9 months, followed to outcome (loss, birth).
Analysis: primary endpoints

**Intention to treat for all outcomes**

**Live birth:**
- Mantel-Haenszel analysis

**Semen quality:**
- Comparison at 6-months post-randomization
- Two-sided t-tests
  - Concentration
  - Motility
  - Morphology
- Total motile count, overall quality marker
- DNA fragmentation index
- Unadjusted & adjusted for study center and (for overall) infertility treatment strata
Analysis: Secondary endpoints

Intention to treat, one outcome per randomized couple

Binary outcomes: standard Z-score (using the normal approximation to the binomial distribution)

Continuous outcomes: t-test

Embryo outcomes
- Among IVF stratum only
- GLM and GEE to account for multiple cycles per woman, multiple embryos per cycle

hCG-detected pregnancy

Clinical intrauterine pregnancy

Pregnancy loss
- Early pregnancy loss (< 20 weeks’ gestation)
- Stillbirth (≥ 20 weeks gestation)

Pregnancy & birth outcomes/complications
- Preeclampsia/gestational hypertension
- Gestational diabetes
- Cesarean delivery
- Preterm delivery & gestational age
- Small-for-gestational-age & birthweight
- Chromosomal & structural abnormalities
- Serious maternal or neonatal morbidity or mortality

Embryo parameters (among IVF)
Interim analysis

Interim analysis for harm conducted after 50% men completed
  ◦ Sperm concentration
  ◦ Sperm morphology
  ◦ Sperm motility

Analysis done by DCC under direction of DSMB
  ◦ One-sided t-test for harm
  ◦ Boundary for harm -3.0452

Spent alpha of 0.0012

Confidence intervals adjusted to 95.1% confidence level
Interim analysis

Boundary for harm: -3.0452

Complete 6-mo. data:
- Concentration: T-statistic: -1.05
- Morphology: T-statistic: -0.04
- Motility: T-statistic: 0.95

Imputed missing 6-mo. data:
- Concentration: T-statistic: -0.82
- Morphology: T-statistic: -0.08
- Motility: T-statistic: 1.17
Analysis: sensitivity analysis

Inverse probability weighting, secondary analysis

Account for potential bias of dropping out before final study visit (semen parameters only)

Variables in weights:
- Baseline characteristics: age, BMI, education, ethnicity, income, insurance, marital status, employment
- Common side effects (GI symptoms)
- Pregnancy status at 6-month timepoint
- Study center
- Infertility treatment strata
Results
Recruitment

Goal: 2400 couples

Began enrollment: June 2013
Enrollment complete: Dec 2017
Follow-up complete: June 2018

Final: **2370** couples randomized
## Baseline characteristics

<table>
<thead>
<tr>
<th></th>
<th>Folic acid &amp; Zinc (N=1185)</th>
<th>Placebo (N=1185)</th>
<th>Total (N=2370)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male age</td>
<td>32.52 (5.7)</td>
<td>32.65 (6.0)</td>
<td>32.59 (5.9)</td>
</tr>
<tr>
<td>Female age</td>
<td>30.63 (5.0)</td>
<td>30.82 (5.2)</td>
<td>30.72 (5.1)</td>
</tr>
<tr>
<td>BMI</td>
<td>30.05 (6.7)</td>
<td>29.61 (6.7)</td>
<td>29.83 (6.7)</td>
</tr>
<tr>
<td>White race</td>
<td>974 (82)</td>
<td>962 (81)</td>
<td>1936 (82)</td>
</tr>
<tr>
<td>Married/living with partner</td>
<td>1180 (99.6)</td>
<td>1179 (99.5)</td>
<td>2359 (99.5)</td>
</tr>
<tr>
<td>&gt; High school education</td>
<td>975 (82)</td>
<td>995 (84)</td>
<td>1970 (83)</td>
</tr>
<tr>
<td>Income &gt; $75K</td>
<td>513 (43)</td>
<td>510 (43)</td>
<td>1023 (43)</td>
</tr>
<tr>
<td>Employed</td>
<td>860 (73)</td>
<td>851 (72)</td>
<td>1711 (72)</td>
</tr>
</tbody>
</table>

Data are mean (SD) or n(%) unless otherwise noted.
Primary outcomes
% Live birth

Folic Acid and Zinc
Placebo

Overall | IVF | Non-IVF onsite | Non-IVF offsite
---|---|---|---
0.98 (0.88, 1.09) | 1.10 (0.89, 1.34) | 0.97 (0.84, 1.11) | 0.83 (0.58, 1.21)
-0.76 (-4.6, 3.1) | 4.6 (-5.6, 14.7) | -1.1 (-5.6, 3.4) | -4.6 (-13.8, 4.7)

Risk ratio: 0.98 (0.88, 1.09) 1.10 (0.89, 1.34) 0.97 (0.84, 1.11) 0.83 (0.58, 1.21)
Risk difference: -0.76 (-4.6, 3.1) 4.6 (-5.6, 14.7) -1.1 (-5.6, 3.4) -4.6 (-13.8, 4.7)
## Semen Quality at 6 months

<table>
<thead>
<tr>
<th></th>
<th>Folic Acid &amp; Zinc</th>
<th>Placebo</th>
<th>Difference (95% CI)</th>
<th>Difference, adj for site, strata (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Concentration, M/mL</strong></td>
<td>84.8 (85.2)</td>
<td>89.0 (85.0)</td>
<td>-4.2 (-12.5, 4.1)</td>
<td>-4.3 (-12.5, 3.9)</td>
</tr>
<tr>
<td><strong>Motility, % motile</strong></td>
<td>52.7 (21.2)</td>
<td>53.2 (20.1)</td>
<td>-0.5 (-2.5, 1.5)</td>
<td>-0.5 (-2.5, 1.5)</td>
</tr>
<tr>
<td><strong>Morphology, % normal</strong></td>
<td>5.7 (4.2)</td>
<td>6.0 (4.8)</td>
<td>-0.4 (-0.8, 0.1)</td>
<td>-0.4 (-0.8, 0.1)</td>
</tr>
<tr>
<td><strong>DFI, via SCSA</strong></td>
<td>22.0 (18.3)</td>
<td>20.4 (14.4)</td>
<td>1.5 (-0.1, 3.2)</td>
<td>1.6 (-0.1, 3.2)</td>
</tr>
<tr>
<td><strong>DFI, via COMET</strong></td>
<td>29.7 (20.5)</td>
<td>27.2 (17.8)</td>
<td><strong>2.5 (0.6, 4.4)</strong></td>
<td><strong>2.4 (0.5, 4.4)</strong></td>
</tr>
<tr>
<td><strong>Total motile sperm count</strong></td>
<td>183.2 (226.3)</td>
<td>181.7 (212.0)</td>
<td>1.5</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(-19.8, 22.8)</td>
<td>(-19.7, 22.5)</td>
</tr>
</tbody>
</table>

Data are mean (SD) or n(%) unless otherwise noted
### Semen Quality at 6 months

<table>
<thead>
<tr>
<th></th>
<th>Folic Acid &amp; Zinc</th>
<th>Placebo</th>
<th>Difference (95% CI)</th>
<th>Difference, adj for site, strata (95% CI)</th>
<th>Reweighted difference, adj for site, strata (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration, M/mL</td>
<td>84.8 (85.2)</td>
<td>89.0 (85.0)</td>
<td>-4.2 (-12.5, 4.1)</td>
<td>-4.3 (-12.5, 3.9)</td>
<td>-5.2 (-13.6, 3.1)</td>
</tr>
<tr>
<td>Motility, % motile</td>
<td>52.7 (21.2)</td>
<td>53.2 (20.1)</td>
<td>-0.5 (-2.5, 1.5)</td>
<td>-0.5 (-2.5, 1.5)</td>
<td>-0.6 (-2.7, 1.4)</td>
</tr>
<tr>
<td>Morphology, % normal</td>
<td>5.7 (4.2)</td>
<td>6.0 (4.8)</td>
<td>-0.4 (-0.8, 0.1)</td>
<td>-0.4 (-0.8, 0.1)</td>
<td>-0.4 (-0.9, 0.0)</td>
</tr>
<tr>
<td>DFI, via SCSA</td>
<td>22.0 (18.3)</td>
<td>20.4 (14.4)</td>
<td>1.5 (-0.1, 3.2)</td>
<td>1.6 (-0.1, 3.2)</td>
<td>1.9 (0.2, 3.6)</td>
</tr>
<tr>
<td>DFI, via COMET</td>
<td>29.7 (20.5)</td>
<td>27.2 (17.8)</td>
<td><strong>2.5 (0.6, 4.4)</strong></td>
<td><strong>2.4 (0.5, 4.4)</strong></td>
<td><strong>2.3 (0.3, 4.3)</strong></td>
</tr>
<tr>
<td>Total motile sperm count</td>
<td>183.2 (226.3)</td>
<td>181.7 (212.0)</td>
<td>1.5</td>
<td>1.4</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Data are mean (SD) or n(%) unless otherwise noted.
Secondary outcomes
## Couple-based outcomes

<table>
<thead>
<tr>
<th></th>
<th>Folic Acid &amp; Zinc</th>
<th>Placebo</th>
<th>Relative Risk (95% CI)</th>
<th>Difference (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>hCG pregnancy</td>
<td>478 (40%)</td>
<td>485 (41%)</td>
<td>0.99 (0.90, 1.09)</td>
<td>-0.59 (-4.55, 3.36)</td>
</tr>
<tr>
<td>Clinical pregnancy</td>
<td>446 (38%)</td>
<td>456 (38%)</td>
<td>0.98 (0.89, 1.08)</td>
<td>-0.84 (-4.75, 3.07)</td>
</tr>
<tr>
<td>Any indication of</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pregnancy</td>
<td>514 (43%)</td>
<td>531 (45%)</td>
<td>0.97 (0.89, 1.06)</td>
<td>-1.43 (-5.43, 2.56)</td>
</tr>
<tr>
<td>Complications</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PE/gHTN</td>
<td>44 (4%)</td>
<td>48 (4%)</td>
<td>0.02 (0.62, 1.37)</td>
<td>-0.34 (-1.89, 1.22)</td>
</tr>
<tr>
<td>GDM</td>
<td>26 (2%)</td>
<td>34 (3%)</td>
<td>0.77 (0.46, 1.27)</td>
<td>-0.68 (-1.94, 0.59)</td>
</tr>
<tr>
<td>Cesarean</td>
<td>138 (12%)</td>
<td>124 (10%)</td>
<td>1.11 (0.89, 1.40)</td>
<td>1.18 (-1.34, 3.71)</td>
</tr>
<tr>
<td>Preterm</td>
<td>67 (6%)</td>
<td>44 (4%)</td>
<td><strong>1.52 (1.05, 2.21)</strong></td>
<td><strong>1.94 (0.24, 3.64)</strong></td>
</tr>
<tr>
<td>Singleton PTB</td>
<td>38 (3%)</td>
<td>29 (2%)</td>
<td>1.31 (0.80, 2.14)</td>
<td>0.76 (-0.57, 2.09)</td>
</tr>
<tr>
<td>SGA</td>
<td>56 (5%)</td>
<td>58 (5%)</td>
<td>0.97 (0.68, 1.38)</td>
<td>-0.17 (-1.89, 1.55)</td>
</tr>
</tbody>
</table>

PE/gHTN, preeclampsia and gestational hypertension; GDM, gestational diabetes mellitus; PTB, preterm birth; SGA, small for gestational age

Data are mean (SD) or n(%) unless otherwise noted.
### Gestational Age & Birth Weight

<table>
<thead>
<tr>
<th></th>
<th>Folic Acid &amp; Zinc</th>
<th>Placebo</th>
<th>Difference (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gestational Age, wks</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>38.6 (2.5)</td>
<td>38.8 (2.2)</td>
<td>-0.20 (-0.53, 0.13)</td>
</tr>
<tr>
<td>IVF</td>
<td>38.0 (3.0)</td>
<td>38.3 (2.3)</td>
<td>-0.23 (-1.01, 0.55)</td>
</tr>
<tr>
<td>Non-IVF onsite</td>
<td>38.8 (2.4)</td>
<td>38.9 (2.2)</td>
<td>-0.10 (-0.49, 0.29)</td>
</tr>
<tr>
<td>Non-IVF offsite</td>
<td>38.6 (1.6)</td>
<td>39.2 (2.1)</td>
<td>-0.57 (-1.36, 0.23)</td>
</tr>
<tr>
<td><strong>Birthweight, g</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>3078 (708.4)</td>
<td>3130 (656.4)</td>
<td>-52.38 (-143.81, 39.05)</td>
</tr>
<tr>
<td>IVF</td>
<td>2906 (804.9)</td>
<td>3060 (628.1)</td>
<td>-153.4 (-350.85, 44.10)</td>
</tr>
<tr>
<td>Non-IVF onsite</td>
<td>3137 (666.5)</td>
<td>3143 (660.0)</td>
<td>-5.75 (-116.17, 104.67)</td>
</tr>
<tr>
<td>Non-IVF offsite</td>
<td>3144 (638.7)</td>
<td>3198 (692.5)</td>
<td>-53.74 (-332.94, 225.47)</td>
</tr>
</tbody>
</table>

Data are mean (SD) or n(%) unless otherwise noted.
## Embryonic Parameters

<table>
<thead>
<tr>
<th></th>
<th>Folic Acid &amp; Zinc N=185</th>
<th>Placebo N=188</th>
<th>Difference (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cycle level</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fertilization rate</td>
<td>75.34 (2.25)</td>
<td>77.67 (1.74)</td>
<td>-2.34 (-7.90, 3.23)</td>
</tr>
<tr>
<td># good quality embryos, d5</td>
<td>2.66 (0.09)</td>
<td>2.98 (0.07)</td>
<td>0.89 (0.72, 1.11)</td>
</tr>
<tr>
<td>% good quality embryos, d5</td>
<td>17.17 (2.05)</td>
<td>18.48 (1.81)</td>
<td>-1.31 (-6.66, 4.05)</td>
</tr>
<tr>
<td># embryos transferred</td>
<td>1.49 (0.04)</td>
<td>1.48 (0.03)</td>
<td>1.01 (0.91, 1.12)</td>
</tr>
<tr>
<td># embryos cryopreserved</td>
<td>4.22 (0.08)</td>
<td>4.35 (0.07)</td>
<td>0.97 (0.79, 1.19)</td>
</tr>
<tr>
<td>Sperm penetration assay</td>
<td>62.7 (12.97)</td>
<td>74.8 (10.96)</td>
<td>-12.1 (-45.36, 21.19)</td>
</tr>
</tbody>
</table>

GEE used to account for multiple cycles per couple or multiple embryos per cycle.

Data are mean (SD) or n(%) unless otherwise noted.
Safety outcomes
## Couple-based outcomes

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Folic Acid &amp; Zinc</th>
<th>Placebo</th>
<th>Relative Risk (95% CI)</th>
<th>Difference (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ectopic</td>
<td>6 (&lt;1%)</td>
<td>5 (&lt;1%)</td>
<td>1.20 (0.37, 3.91)</td>
<td>0.08 (-0.46, 0.63)</td>
</tr>
<tr>
<td>Early preg loss (&lt;20 wks)</td>
<td>136 (11%)</td>
<td>149 (13%)</td>
<td>0.91 (0.73, 1.14)</td>
<td>-1.10 (-3.72, 1.52)</td>
</tr>
<tr>
<td>Stillbirth (≥ 20 wks)</td>
<td>1 (&lt;1%)</td>
<td>4 (&lt;1%)</td>
<td>0.25 (0.03, 2.26)</td>
<td>-0.25 (-0.62, 0.12)</td>
</tr>
<tr>
<td>Neonatal mortality</td>
<td>3 (&lt;1%)</td>
<td>2 (&lt;1%)</td>
<td>1.50 (0.25, 9.01)</td>
<td>0.08 (-0.29, 0.45)</td>
</tr>
<tr>
<td>Structural malformations</td>
<td>15 (1%)</td>
<td>14 (1%)</td>
<td>1.07 (0.52, 2.22)</td>
<td>0.08 (-0.80, 0.97)</td>
</tr>
<tr>
<td>Chromosomal anomalies</td>
<td>4 (&lt;1%)</td>
<td>5 (&lt;1%)</td>
<td>0.80 (0.21, 2.97)</td>
<td>-0.08 (-0.58, 0.41)</td>
</tr>
</tbody>
</table>

Malformations are among births and losses.

Data are mean (SD) or n(%) unless otherwise noted.
## Adverse Events in Men

<table>
<thead>
<tr>
<th></th>
<th>Folic Acid &amp; Zinc</th>
<th>Placebo</th>
<th>Relative Risk (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serious AEs</td>
<td>7 (0.59%)</td>
<td>5 (0.42%)</td>
<td>1.40 (0.45, 4.40)</td>
</tr>
<tr>
<td><strong>Gastrointestinal disorders</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abdominal discomfort/pain</td>
<td>66 (5.57%)</td>
<td>40 (3.38%)</td>
<td>1.65 (1.12, 2.42)</td>
</tr>
<tr>
<td>Nausea</td>
<td>50 (4.22%)</td>
<td>24 (2.03%)</td>
<td>2.08 (1.29, 3.37)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>32 (2.70%)</td>
<td>17 (1.43%)</td>
<td>1.88 (1.05, 3.37)</td>
</tr>
<tr>
<td><strong>Pyrexia</strong></td>
<td>66 (5.57%)</td>
<td>62 (5.23%)</td>
<td>1.06 (0.76, 1.49)</td>
</tr>
<tr>
<td><strong>Respiratory, thoracic disorders</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oropharyngeal pain</td>
<td>57 (4.81%)</td>
<td>60 (5.06%)</td>
<td>0.95 (0.67, 1.35)</td>
</tr>
<tr>
<td>Nasopharyngitis</td>
<td>32 (2.70%)</td>
<td>40 (3.38%)</td>
<td>0.80 (0.51, 1.26)</td>
</tr>
<tr>
<td><strong>Skin/subcutaneous tissue disorders</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythema</td>
<td>23 (1.94%)</td>
<td>8 (0.68%)</td>
<td>2.88 (1.29, 6.40)</td>
</tr>
<tr>
<td>Pruritus</td>
<td>20 (1.69%)</td>
<td>17 (1.43%)</td>
<td>1.18 (0.62, 2.23)</td>
</tr>
<tr>
<td>Rash</td>
<td>21 (1.77%)</td>
<td>12 (1.01%)</td>
<td>1.75 (0.87, 3.54)</td>
</tr>
</tbody>
</table>
Summary

Folic acid and zinc supplementation not recommended for general infertility population

- More frequent adverse outcomes
- No improvements in semen quality
- No improvements in live birth or pregnancy health
Discussion: drop out

- Greater side effects could affect attendance at 6-month visit
  - Reweighted for probability of missing final study visit, including GI symptoms
  - No change in estimate
  - Null unlikely to be explained by drop out

<table>
<thead>
<tr>
<th></th>
<th>Overall visit completion (%)</th>
<th>Pregnant visit completion (%)</th>
<th>Loss visit completion (%)</th>
<th>Non-pregnant visit completion (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Month 2</td>
<td>2101 (88.6)</td>
<td>187 (87.4)</td>
<td>32 (86.5)</td>
<td>1882 (88.8)</td>
<td>0.75</td>
</tr>
<tr>
<td>Month 4</td>
<td>1834 (77.4)</td>
<td>337 (79.7)</td>
<td>84 (80.0)</td>
<td>1412 (76.7)</td>
<td>0.33</td>
</tr>
<tr>
<td>Month 6</td>
<td>1773 (74.8)</td>
<td>492 (78.1)</td>
<td>118 (76.1)</td>
<td>1163 (73.4)</td>
<td>0.06</td>
</tr>
</tbody>
</table>
Discussion: compliance

- Greater side effects could be marker of greater compliance
- Unlikely based on questionnaire estimates of missing doses

<table>
<thead>
<tr>
<th></th>
<th>% consuming dose &gt; 90% of days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Up to 2 month visit</td>
</tr>
<tr>
<td>Overall</td>
<td>87</td>
</tr>
<tr>
<td>Folic acid &amp; zinc</td>
<td>86</td>
</tr>
<tr>
<td>Placebo</td>
<td>88</td>
</tr>
</tbody>
</table>
Conclusions

Impression that supplements will at least ‘do no harm’ unfounded

Lack of efficacy & potential side effects can now be communicated
Implications

Relatively unregulated supplement industry potentially harmful

At best, non-efficacious male fertility supplements causing GI problems

Broader potential message for widespread dietary supplement use & regulation
Future opportunities

Still in need of safe and affordable options to improve fertility outcomes

Characterizing clinical subgroups who may benefit

<table>
<thead>
<tr>
<th>Semen parameters</th>
<th>DFI threshold</th>
<th>Novel biochemical or clinical markers?</th>
</tr>
</thead>
</table>

Future opportunities

Still in need of safe and affordable options to improve fertility outcomes

Characterizing clinical subgroups who may benefit

<table>
<thead>
<tr>
<th>Semen parameters</th>
<th>DFI threshold</th>
<th>Novel biochemical or clinical markers?</th>
</tr>
</thead>
</table>
Data & Biospecimen Repository

Largest RCT of couples seeking infertility treatment

Largest collection of longitudinal semen analyses

Longitudinal couple data & biospecimens for future research:

- Semen Analysis, to explore factors potentially related to:
  - Pregnancy and live birth rates
  - Embryo quality in IVF
  - Pregnancy/placental complications
- Anthropometrics
- Stress, Diet, Lifestyle
- Environmental contaminants
- Heat Exposures: Laptop Use, Sauna, etc.
- Sleep
Thank you