FAZST

FOLIC ACID AND ZINC SUPPLEMENTATION TRIAL

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Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD)
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UPM Pharmaceuticals, Inc.

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This study is being conducted in compliance with the protocol, FDA regulations (21 CFR Parts 50, 54, 56 and 312 when applicable), Good Clinical Practices, applicable local regulations and the Declaration of Helsinki.

INVESTIGATOR STATEMENT OF APPROVAL

FAZST Protocol Version 11.0

I have read the Protocol and agree to follow the procedures as outlined in this Protocol.

I will not start the study until I have obtained written approval by the governing Institutional Review Board/Ethics Committee. I will obtain written informed consent from all study participants prior to conducting any study procedures.

I understand that my electronic or handwritten signature or that of a co-investigator on a case report form indicates that the data contained on that form have been reviewed and accepted as accurate by the signatory.

I agree to conduct this study in compliance with the applicable local regulations, FDA regulations as applicable (21 CFR 50, 54, 56, 312), Good Clinical Practices, and the Declaration of Helsinki.

I understand and am aware of my responsibilities as an Investigator as described in the applicable Good Clinical Practices guidelines.

I agree to protect the confidentiality of my patients when allowing the Sponsor of this clinical trial, their authorized monitors and/or relevant regulatory authorities access to my medical records for FAZST participants.

_____

Principal Investigator, Printed Name

_____ _____

Address: Telephone #

_____ _____

Principal Investigator, Signature Date

Upon signing, send this page (original) to the DCC for their files and keep a copy for your Regulatory Binder.

FAZST Data Coordinating Center
c/o Kayla Chaney 401 N. Washington Street, Suite 700
Rockville, MD 20850-1785

Glossary of Abbreviations

AE	Adverse Event
AMH	Anti-Müllerian Hormone
ART	Assisted Reproductive Technology
CDC	Centers for Disease Control and Prevention
DCC	Data Coordinating Center
DFI	DNA Fragmentation Index
DNA	Deoxyribonucleic Acid
DSMB	Data and Safety Monitoring Board
EC	Ethics Committee
eCRF	Electronic Case Report Forms
EPL	Early Pregnancy Loss
ER	Egg Retrieval
ET	Embryo Transfer
FDA	Food and Drug Administration
FET	Frozen Embryo Transfer
FF	Follicular Fluid
GCP	Good Clinical Practice
hCG	Human Chorionic Gonadotropin
HEPT	Hamster Egg Penetration Test
HSG	Hysterosalpingogram
ICH	International Conference on Harmonization
ICSI	Intracytoplasmic Sperm Injection
IEC	Independent Ethics Committee
IRB	Institutional Review Board
IND	Investigational New Drug
ITT	Intent to Treat
IUI	Intrauterine Insemination
IVF	In Vitro Fertilization
MOP	Manual of Procedures
MSM	Marginal Structural Models
NICHD	*Eunice Kennedy Shriver* National Institute of Child Health and Human Development
NIH	National Institutes of Health

OHRP	Office of Human Research Protection
OI	Ovulation Induction
OPIM	Other Potentially Infectious Materials
ORI	Office of Research Integrity
OSHA	Occupational Safety and Health Administration
P4	Progesterone
PHS	Public Health Service
PI	Principal Investigator
PLB	Probability of Live Birth
PPE	Personal Protection Equipment
RCT	Randomized Controlled Trial
ROC	Receiving Operating Characteristic
RR	Rate Ratio
SA	Semen Analysis
SAE	Serious Adverse Event
SART	Society for Assisted Reproductive Technologies
SCPS	Specimen Collection, Processing and Storage
SCSA	Sperm Chromatin Structure Assay
SDI	Sperm Deformity Index
SGA	Small for Gestational Age
SOP	Standard Operating Procedures
TCC	Trial Coordinating Center
TZI	Teratozoospermic Index
US	Ultrasound

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PROTOCOL SYNOPSIS

Study Title	Folic Acid and Zinc Supplementation Trial (FAZST).
Objectives	To evaluate the effects of folic acid and zinc dietary supplementation in males on semen quality parameters, and indirectly on fertility and pregnancy outcomes among couples attempting to conceive and seeking assisted reproduction.
Study Design	This is a multi-center, double-blind, block randomized placebo-controlled trial which randomizes men of couples attempting to conceive and seeking assisted reproduction either to study dietary supplements or to placebo to assess the effects of folic acid and zinc dietary supplementation on semen quality and fertility outcomes. The trial is sponsored by the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD).
Cohorts and Sample Size	A total of 2,400 male participants will be randomized equally (1:1) either to active study dietary supplements (5 mg folic acid and 30 mg elemental zinc daily) or to matching placebo. Only the males will be randomized to study dietary supplement or placebo. Randomization will be stratified by site and assisted reproduction technique (IVF, non-IVF/receiving fertility treatment at a study site, non-IVF/receiving fertility treatment at a non-study site).
Supplement Duration	6 months
Participant Selection	Male participants will be recruited from couples seeking assisted reproduction. Additional inclusion/exclusion criteria are described in Section 3.2.
Primary Hypothesis	Folic acid and zinc dietary supplementation will maintain and improve normal semen quality parameters and increase live birth rate.
Study Endpoints	Primary a) Semen quality – Standardized quantification of volume, concentration, motility, morphology, sperm count, and deoxyribonucleic acid (DNA) fragmentation index. b) Live birth – based on hospital records. Secondary c) Human Chorionic Gonadotropin (hCG) detected pregnancy (implantation) – a quantitative hCG evaluation in serum > 5 mIU/mL. d) Clinical intrauterine pregnancy – visualized gestational sac in the uterus on ultrasound. e) Ectopic pregnancy – either visualization of no gestational sac in the uterus with a suspicious mass in the adnexa on ultrasound, an hCG level more than 1500 mIU/mL without visualization of an intrauterine gestational sac on ultrasound, or a slowly rising or plateauing serum hCG level without visualization of an intrauterine gestation on ultrasound. f) Early pregnancy loss (EPL) – hCG pregnancy loss will be defined as a serum hCG > 5 mIU/mL followed by a decline. Clinically recognized pregnancy losses will be identified by visualization of an intrauterine gestational sac followed by a loss prior to 20 weeks gestation. g) Specific pregnancy outcomes – including, but not limited to Cesarean section, pre-eclampsia, gestational diabetes, growth restriction, gestational age, preterm birth, birth weight (small for gestational age), and major neonatal complications including death, and severe post-partum maternal morbidity. Outcomes will be determined based on hospital records and medical chart abstraction. h) Early embryonic development parameters – Fertilization rates, method of fertilization, number of cells and embryo morphology on day 3 and day 5, number of good quality embryos on day 5, proportion of good quality embryos on day 5, number of embryos transferred, quality of embryos transferred, number of embryos cryopreserved, and

Study Title	Folic Acid and Zinc Supplementation Trial (FAZST).
	sperm penetration assay results. When available, information regarding the chromosomal complement of embryo will be assessed. i) Reproductive hormones and other measured biomarkers – Measured urinary, serum, and salivary concentrations of reproductive hormones, particularly androgens, proteomic analysis of human sperm and cardiometabolic risk factors and markers of oxidative stress, as well as measures of trace elements in toenails.
Plans for Data Analysis	The primary analysis will be an intent to treat (ITT) analysis based on the total cohort of randomized participants. This approach will be applied to the primary endpoints (semen parameters, live births) as well as designated secondary endpoints (i.e., early pregnancy loss, specific pregnancy outcomes and early embryonic development parameters). The difference between the measures of semen quality parameters, including concentration, motility, and morphology, and sperm DNA fragmentation for the two study cohorts will be examined by two-sided t-tests. The common null hypothesis states that the effect of folic acid/zinc on the outcomes is null compared to the placebo. Multivariate techniques will also be employed to consider the effects of folic acid/zinc on overall semen quality. The semen parameters will also be assessed using analysis of covariance (ANCOVA) models with each semen parameter as the dependent variable, clinical center, IVF strata and treatment group as factors. The outcome of live birth will also be assessed using a Mantel-Haenszel analysis to evaluate effects across fertility treatment strata. The secondary objectives with binary endpoints will be analyzed using the standard Z-score (using the normal approximation to the binomial distribution). For binary outcomes such as pregnancy loss, the time to event may be of interest. In this case, survival analysis methods will be applied with the log-rank test for comparison of the two study cohorts. A stratified log-rank test will be used to assess treatment effects across IVF strata. For continuous outcomes such as birth weight, either parametric or non-parametric methods will be used as appropriate. If transformations to approximate a normal distribution are feasible, they will be carried out prior to the parametric analysis. The normality of the data will be examined both graphically using Q-Q plots and by formal tests (Shapiro Wilks test). For data (either on original or transformed scale) assumed to be normally distributed, the comparison of the two cohorts will be carried out by a t-test. Alternatively, if a non-parametric test is required, the Wilcoxon-Mann-Whitney test will be used. Stratified analyses will also be performed for secondary outcomes. Specifically, continuous secondary outcomes stratified analyses will be conducted using ANCOVA models with IVF strata and clinical center as factors for the comparison of the two cohorts. If a non-parametric test is required, van Elteren tests will be used to compare the two cohorts, accounting for strata, to detect differences in the distributions of the secondary endpoints.

The trial is sponsored by the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD). The designated NICHD Study Project Officers for FAZST are Drs. Enrique Schisterman and Sunni Mumford. Drs. Schisterman and Mumford will be responsible for:

- General design and conduct of the trial and preparation of the essential study documents, including the protocol, Manual of Procedures (MOP), and data collection forms
- Obtaining necessary regulatory approvals including, if applicable, an Investigational New Drug (IND) application, Office of Management and Budget Clinical Exemption, and an Institutional Review Board (IRB) Reliance Agreement
- Monitoring and overseeing the contractors' performance
- Reviewing data collection practices and procedures

- Reviewing and implementing any recommendations from the Data and Safety Monitoring Board (DSMB)
- Changing study procedures as appropriate
- Appointing to, and disbanding of, study implementation subcommittees
- Reviewing study progress in achieving goals and taking necessary steps to enhance the likelihood of achieving those goals
- Overseeing all study-related analyses and publications

Drs. Schisterman and Mumford will have no contact with study participants nor will they have access to any personal identifying information.

Drs. Matthew Peterson and Erica Johnstone are the University of Utah (Salt Lake City, UT) Principal Investigators. The co-Principal Investigators at the University of Iowa are Dr. Ginny Ryan and Dr. Brad Van Voorhis. The Principal Investigator at Northwestern University is Dr. Jared Robins. Dr. Bruce Campbell is the Principal Investigator at the Center for Reproductive Medicine (Minneapolis, MN). They each are responsible for the following:

- Ensuring the study is conducted according to the protocol and MOP
- Identifying, recruiting, screening, enrolling, and retaining participants
- Protecting participants' rights
- Updating the IRB regarding changes in the study protocol and MOP
- Obtaining signed informed consent from each participant
- Collecting study data per protocol, and following participants through study completion, including on-site semen analysis and collection and storage of biospecimens
- Distributing the study dietary supplements to participants and assuring safety and integrity of the dietary supplements when stored at the study sites
- Retaining specific records (e.g., dietary supplement storage and distribution records, local participant information, etc.)
- Preparing and sending required reports to the DCC (e.g., recruitment, adverse event reports, documentation of IRB review and approval)
- Providing the DCC with de-identified data from the clinical questionnaire and SART databases for FAZST enrolled couples
- Communicating questions, concerns, and/or observations to the NICHD Project Officer and/or DCC

Dr. Traci Clemons is the Principal Investigator for the DCC at The EMMES Corporation. The roles and responsibilities of the DCC include:

- Developing and maintaining the study website and web-based data management system
- Developing and amending the study protocol and MOP
- Developing the randomization scheme and procedures as well as blinding/unblinding procedures
- Developing and implementing data management procedures, including data entry, edits, and error reporting
- Coordinating and managing data collection, processing, and tracking (data and biospecimens), and data quality control for the duration of the study
- Monitoring and reporting adverse events

- Communicating with clinical sites: scheduling of meetings and training sessions, responding to, and documenting ad hoc communications
- Conducting site visits to ensure adherence to the protocol and procedures
- Establishing and implementing quality control procedures
- Developing periodic reports: enrollment, participant status, study progress, adverse events, participant status (e.g., drop-outs), etc.
- Obtaining and distributing dietary supplements to the clinical sites
- Creating statistical analysis and presentation of the study data
- Providing resulting datasets and documentation to NICHD
- Organizing DSMB meetings: preparing reports for DSMB review and preparing summary reports of DSMB recommendations

1.0 STUDY DESCRIPTION AND OBJECTIVES

This is a multi-center, double-blinded, block randomized placebo-controlled clinical trial to assess the effects of folic acid and zinc dietary supplementation in males on semen quality, and fertility rates among couples trying to conceive and seeking assisted reproduction.

1.1 Primary Objective

The primary objective of the study is to assess the impact of folic acid and zinc dietary supplementation in males on semen quality and indirectly fertility outcomes (i.e., live birth rate) among couples attempting to conceive and seeking assisted reproduction. A double-blind, placebo-controlled block randomized trial of folic acid and zinc dietary supplementation will be conducted. Following a screening/randomization visit, participants will return at 2, 4, and 6 months. The rate of adverse events will be noted throughout the double-blind trial.

2.0 BACKGROUND

Two micronutrients fundamental to the process of spermatogenesis, folic acid (folate) and zinc, are of particular interest for fertility as they are of low cost and wide availability. Though the evidence has been inconsistent, small randomized trials and observational studies show that folate and zinc have biologically plausible effects on spermatogenesis and improved semen parameters. These results support the potential benefits of folate on spermatogenesis and suggest that dietary supplementation with folate and zinc may help maintain and improve semen quality, and perhaps, fertility rates. Healthy, "fertile" couples attempting to conceive face a normal success rate of only 15-20% per month. Couples who attempt conception for more than one year without achieving pregnancy are thought to have low fertility and are likely to be ideal candidates for the study of the effect of dietary supplementation on semen parameters and fertility rate. It is estimated that 10 to 15% of couples fall under this category.

Folate and zinc are of particular interest with respect to spermatogenesis through a likely effect on sperm DNA structure. Folate is a critical micronutrient for several reasons. Regulation of DNA function takes place in part by methylation, and folate provides methyl groups that are transferred to the N5 position of cytosine in DNA via methionine.[1] Folate is involved in the methylation of proteins as well. In addition, folate plays a direct role in building DNA by providing carbon atoms for both purine and pyrimidine synthesis. These carbon atoms provide portions of the carbon rings of DNA and RNA.[2] Since folate is essential for DNA, transfer RNA, and protein synthesis, it is clear that folate is critical for germ cell development, spermatogenesis, and reproduction.[3]

Zinc is essential in spermatogenesis as a co-factor for more than 80 metalloenzymes involved in DNA transcription, expression of steroid receptors, and protein synthesis.[4, 5] It has anti-apoptotic[6] and antioxidant properties[7] by binding sulphydryl groups in proteins and by occupying binding sites for iron and copper in lipids, proteins, and DNA.[8] These two nutrients, zinc and folate, are dependent on one another for proper utilization. Zinc is reported to be necessary for the conversion of polyglutamylfolates to the monoglutamate form of folate which can be absorbed in the intestine.[9, 10] Moreover, the increase in methionine synthetase activity observed in zinc-deficient animals could induce alterations in folate metabolism further promoting their synergistic properties.[11]

The effects of these nutrients on spermatogenesis have not only biological plausibility but have also been reported in recent literature. In the most recently conducted randomized controlled trial

(RCT), 94 infertile men and 99 fertile men were divided into four groups receiving either folic acid (5 mg/day)/zinc sulfate (66 mg/day), folic acid/placebo, zinc sulfate/placebo, or placebo/placebo for 6 months.[12] Only concomitant administration of folic acid and zinc led to a significant 74% increase in sperm density and total count in subfertile patients (insignificant increase in fertile men). Furthermore, in a study of 251 men of couples undergoing IVF or ICSI, fertile men showed a significant inverse association between seminal plasma folate and sperm DNA fragmentation index (DFI).[13] In a study of 89 healthy men, high levels of folate intake were associated with lower frequencies of sperm disomies X, 21, sex nullisomy, and a lower aggregate measure of sperm aneuploidy, further confirming the beneficial effects of folate on spermatogenesis.[14]

2.1. Couples seeking pregnancy through assisted reproduction

An ideal population to study the effect of micronutrients in an important bodily function such as reproduction is patients seeking to conceive through assisted reproduction. In this population, patients are highly motivated to follow markers of reproductive function such as ovulation and early pregnancy, and to report pregnancy outcomes. Patients undergoing assisted reproduction may have multiple semen analyses, hormonal assays, and ultrasounds that would allow collection of information on important steps in human reproduction such as follicular growth, exact timing of ovulation, timing of implantation, and early pregnancy development.[15]

Furthermore, the effect of micronutrients on sperm DNA integrity is expected to improve the quality of human embryos and maintain or improve normal conception. The measurement of sperm DNA integrity is an advanced technique that holds promise in predicting pregnancy outcomes in couples undergoing assisted reproduction. Sperm DNA integrity is necessary for normal fertilization and transmission of paternal genetic material and significant differences in DNA integrity have been observed between fertile and infertile men.[16-18] Pilot studies showed that high levels of DFI (>27%) were associated with decreased fertility in patients using assisted reproductive technologies (ART),[19] even in men with completely normal standard semen parameters.[20] Among the numerous available tests of sperm DNA quality, the sperm chromatin structure assay (SCSA), which measures the stability of sperm chromatin in acid media with acridine orange, is proven to be a superior measurement of DNA integrity and useful for epidemiologic studies.[21, 22] Additional knowledge of the causes of sperm DNA fragmentation and decreases in sperm quality are crucial for an improved understanding of the effect of dietary supplementation on fertility.

Tests of sperm DNA quality generally correlate well with other semen parameters, including concentration and motility,[17, 23] and time-to-pregnancy.[18, 24] However, the associations between DNA damage and pregnancy outcomes in assisted reproduction are less consistent. A recent literature review of work completed on this association from 1997 to present revealed little association between DNA integrity and fertilization rates; however, there was a strong association evident between DNA damage, embryo/blastocyst quality, and pregnancy rates.[22] While tests of DNA damage have been associated with pregnancy rates, their utility as a sensitive diagnostic tool for pregnancy outcomes for couples seeking assisted reproduction is questionable.[25] Given the conflicting evidence to date, large prospective studies are needed to further examine the predictive ability of traditional semen parameters along with DNA fragmentation on human reproduction.

Studies looking at the role of micronutrients on early embryonic development are practically nonexistent because of lack of access to the early developing human embryo in vivo. Patients undergoing in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) represent a

unique opportunity as they represent a highly motivated population that would allow access to descriptive information regarding early embryonic development. Possible parameters accessible in this population are rate of oocyte fertilization, quality of early embryo development assessed using the SART embryo scoring system, and ability of embryos to implant resulting in pregnancies and live births. Current technology will also allow the study of the chromosomal complement in the developing human embryo without affecting its chance of a successful implantation.[26] Such outcomes would be of utmost importance in the study of effects of dietary supplementation with folic acid and zinc in males on human reproduction.

In summary, preliminary evidence from the aforementioned studies suggests that dietary supplementation with folic acid and zinc may help maintain or improve semen quality, and perhaps, downstream fertility rates. Large prospective studies are needed to further elucidate the effects of folic acid/zinc dietary supplementation on semen quality, fertility rates, and early embryonic development parameters.

3.0 STUDY DESIGN

3.1. Introduction

This is a multi-center, double-blind, block randomized placebo-controlled trial to assess the effects of folic acid and zinc dietary supplementation on semen quality and reproductive outcomes among 2400 male partners of couples attempting to conceive and seeking assisted reproduction.

FAZST will be conducted according to the guidelines described in the International Conference on Harmonisation (ICH) E6 Industry Guidance for Good Clinical Practice (CGP). These guidelines can be found at the following website:
http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E6_R1/Step4/E6_R1__Guideline.pdf

3.2. Eligibility Criteria

Couples Inclusion Criteria:

a. Heterosexual couples in a committed relationship with a female partner aged 18-45 years and male partner aged 18 years and older attempting to conceive and seeking assisted reproduction at participating fertility clinics.
b. Couples actively trying to conceive.
c. Couples who are planning ovulation induction (OI), natural fertility optimization methods, or intrauterine insemination (IUI) should be willing to be on the study dietary supplement for at least 3 weeks before starting the next assisted reproduction cycle. Women with regular periods may initiate their fertility therapy at the start of the woman's menstrual cycle following randomization if randomization occurred within the first 10 days of the cycle, but must wait one menstrual cycle if the visit occurred after day 10 of the cycle). For women with irregular periods or amenorrhea, the male must be on the study supplement for 3 weeks prior to initiation of any ovulation induction medication (e.g., clomid, letrozole, gonadotropins).

Couples Exclusion Criteria:

a. Female partner unwilling to participate (e.g., no abstraction of her assisted fertility treatment record or unwilling to complete baseline visit).
b. Couples using donor, cryopreserved sperm, or sperm obtained via microsurgical or percutaneous epididymal sperm aspiration.
c. Couples attempting to conceive with a gestational carrier (surrogate).
d. Positive urine pregnancy test at screening.

Male Inclusion Criteria:

a. Willing to provide semen samples according to the proposed schedule at baseline, 2, 4, and 6 months of follow-up.
b. Able to complete regular study questionnaires and daily journals aimed at capturing ejaculation, sexual intercourse and lifestyle factors considered to affect male fecundity (e.g., cigarette smoking, fever, high temperature environment and other environmental exposures) and other data collection instruments (e.g., physical activity, food frequency questionnaire, stress).

Male Exclusion Criteria:

a. Age <18 years.
b. Unwilling to abstain from use of non-study approved dietary supplements or medications containing folic acid or oral preparations containing zinc throughout the study.
c. Unwilling to abstain from use of testosterone supplementation throughout the study.
d. Diagnosis of Vitamin B_{12} deficiency or pernicious anemia.
e. Consuming a vegan diet.
f. A known genetic cause of male factor subfertility, including chromosomal disorders related to subfertility (e.g., Y chromosome deletions).
g. Current use of drugs known to interact with folic acid or interfere with the biosynthesis of folic acid including all dihydrofolate reductase inhibitors, including but not limited to Trimethoprim, Triamterene, Bactrim, Iclaprim, and Sulfonamides including diuretics, such as Hydrochlorothiazide (HCTZ), Metolazone, Indapamide, Lasix, Bumex, Torsemide, Chlorthalidone, Acetazolamide, Mefruside, Xipamide; Sulfonylureas, such as Glipizide and Glyburide; Cox-2 inhibitors, such as Celecoxib; Probenecid; Sulfasalazine; Sumatriptan; Mafenide; Acetazolamide; Ethoxzolamide; Sulfiram; Zonisamide; optic agents including Dorzolamide and Dichlorphenamide; Valproic acid; Fluorouracil; Capecitabine; Methotrexate, or other drugs known to interact with folic acid.
h. History of organ transplantation
i. Physician diagnosed:
    i. Current poorly controlled chronic diseases such as heart disease, diabetes mellitus, hypertension, cancer, inflammatory diseases, autoimmune, thyroid disease, endocrine dysfunction, liver disease, kidney disease, or HIV/AIDS or other immune-insufficient related illnesses.
    ii. 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 dietary supplementation, such as megaloblastic anemia, homocystinemia, and homocystinuria.

iii. History of alcohol dependency disorder and/or other drug/substance dependency in the past 180 days.

iv. History of psychoses or other mental conditions that would result in cognitive impairment and inability to participate in any part of this study including the informed consent process, as diagnosed by a physician within the past year.

j. History of vasectomy without reversal, obstructive azoospermia such as CBAVD, or ejaculatory duct obstruction.

k. Known allergy to folic acid or zinc dietary supplements.

Female Exclusion Criteria:

a. Age <18 or >45 years.

3.3. Study Dietary Supplement Formulation

Male participants will be randomized (1:1) into two groups receiving one of the following:

- Folic acid 5 mg plus elemental zinc 30 mg (minimum, range 30-40 mg) taken orally daily
- Placebo taken orally daily

Randomization will be stratified by site and assisted reproduction technique (IVF, non-IVF receiving fertility treatment at a study site, and non-IVF receiving fertility treatment at a non-study site). Folic acid plus zinc and placebo tablets will be identical in appearance and weight to maintain blinding. The duration of dietary supplement regimens will be six months. For the remainder of the protocol, the term "Study Supplement" will be used to refer to folic acid and zinc dietary supplements.

4.0 OUTCOME MEASURES

Semen parameters

Four fresh semen samples, collected at baseline and after 2, 4, and 6 months of study supplement or placebo, will be assessed for the following parameters (outcome measures) utilizing the World Health Organization (WHO) semen analysis procedure 5th edition[27]: volume, concentration, total sperm count, total motile count, percent motility, percent forward motility, percent normal morphology, and sperm DNA integrity. For sperm DNA integrity testing, sperm chromatin structure assay (SCSA) and other testing will be used.[16] This assay measures the ability of sperm DNA to resist denaturation under acidic conditions. It is widely regarded as superior sperm DNA integrity test for epidemiologic studies given its high repeatability, efficiency, and prognostic value for men's fertility potential.[28] The test yields several parameters indicative of DNA denaturation and DNA fragmentation found to be predictive of fertility potential.

Early embryonic development parameters

Fertilization and embryo transfer will be based upon standard protocols within the trial clinical sites. Oocytes will be assessed 16–18 hours after insemination or microinjection to determine

whether fertilization occurred. Fertilization will be considered normal if two pronuclei and two polar bodies are identified. Oocytes without visible pronuclei will be considered unfertilized. Oocytes with more than two pronuclei will be considered abnormally fertilized, and will thus be discarded. Embryos will be scored three days after fertilization according to the size and shape of blastomeres and to their degree of fragmentation.[29] For couples who meet criteria for blastocyst culture, embryos will be graded 5 days after fertilization based on Society for Assisted Reproductive Technologies (SART) morphology criteria. When available, information from preimplantation genetic screening will be collected.

hCG recognized pregnancy (implantation)

For couples undergoing IVF/ICSI, serum quantitative hCG level will be measured at scheduled clinic visits at approximately 12 days post embryo transfer for day 5 embryo transfers, and 14 days post embryo transfer for day 3 embryo transfers in accordance with the usual care that the woman is receiving. For couples undergoing other assisted reproduction techniques, self-report of a positive home pregnancy test or a missed menstruation at least 14 days after ovulation will be followed by measurement of serum hCG at the laboratory of the patient's choice.

Clinically recognized pregnancy

Clinical recognition will be determined by the presence of a gestational sac on ultrasound scans. For the majority of patients, this will occur at the study site. For patients who opt out of this element of standard care, documentation of intrauterine gestational sac or fetal heart tones as assessed by US or Doppler at the initial prenatal care visit will serve to confirm clinical pregnancy.

Early (biochemical) pregnancy loss (EPL)

Pregnancy loss is a measure of impaired human fecundity and encompasses two types of losses: 1) EPL or hCG detected pregnancy loss, and 2) clinically recognized pregnancy loss. Approximately one-third of all pregnancies are spontaneously aborted, of which two-thirds are hCG pregnancy losses.[30] Serum hCG will be used to detect early pregnancy and subsequent EPL. EPL will be defined as a decrease in serum hCG between any 2 sequential serum hCG levels, followed by a decrease in hCG to <5 mIU/mL without intervention.

Clinically recognized spontaneous pregnancy loss

Clinically recognized pregnancy loss is defined as a pregnancy loss before 20 weeks after a diagnosis of clinically recognized pregnancy as determined by presence of a gestational sac on ultrasound scan. Clinically recognized pregnancy losses affect approximately 10-15% of women with recognized pregnancies.

Ectopic pregnancy

Ectopic pregnancy will be defined as either visualization of no gestational sac in the uterus with a suspicious mass in the adnexa on ultrasound, an hCG level more than 1500 mIU/mL without visualization of an intrauterine gestational sac on ultrasound, or a slowly rising or plateauing serum hCG level without visualization of an intrauterine gestation on ultrasound.

Multiple gestation

Multiple gestations will be identified by ultrasound scan at the study site or at the participant's prenatal visits.

Pregnancy outcomes

The outcome of live birth will be defined as birth of a fetus with any sign of life (e.g., pulsation of umbilical cord, purposeful movements).

Stillbirth will be defined as intrauterine fetal demise occurring at or after 20 completed weeks gestation or as the complete expulsion or extraction from its mother, after at least 20 completed weeks pregnancy, of a product of conception in which, after such expulsion or extraction, there is no breathing, beating of the heart, pulsation of the umbilical cord, or purposeful movement of voluntary muscle.

For IVF patients, the gestational age will be based on the date of embryo transfer. For all other patients, gestational age will be based on the LMP using the guidelines defined in the table below.

Method of estimating gestational age	**Variability (2 standard deviations)**
Days from oocyte retrieval or co-incubation in in vitro fertilization + 14 days	±1 day
Days from estimated ovulation in Ovulation induction + 14 days	±3 days
Days from artificial insemination + 14 days	±3 days
Days from known single sexual intercourse + 14 days	±3 days
Days from estimated ovulation by basal body temperature record + 14 days	±4 days
First-trimester physical examination	±2 weeks
Second-trimester physical examination	±4 weeks
Third-trimester physical examination	±6 weeks
First-trimester obstetric ultrasonography (crown-rump length)	±8% of the estimate
Second-trimester obstetric ultrasonography (head circumference, femur length)	±8% of the estimate
Third-trimester obstetric ultrasonography (head circumference, femur length)	±8% of the estimate

As a general rule, the official gestational age should be based on the actual beginning of the last menstrual period, unless any of the above methods gives an estimated date that differs more than the variability for the method, in which case the difference cannot probably be explained by that variability alone. For example, if there is a gestational age based on the beginning of the last menstrual period of 9.0 weeks, and a first-trimester obstetric ultrasonography gives an estimated gestational age of 10.0 weeks (with a 2 SD variability of ±8% of the estimate thereby giving a variability of ±0.8 weeks), the difference of 1.0 weeks between the tests is larger than the 2 SD variability of the ultrasonography estimate, indicating that the gestational age estimated by ultrasonography should be used as the official gestational age. (Reference: A Simple Solution to Dating Discrepancies: The Rule of Eights Hunter, L. A. (2009). "Issues in Pregnancy Dating: Revisiting the Evidence". *Journal of Midwifery & Women's Health* **54** (3): 184–190. doi:10.1016/j.jmwh.2008.11.003)

Small for gestational age will be defined when infant birth weight is ≤ 10% for gestational age (weight < 10th percentile), as defined by the standards of Kramer, et al. (http://www.pediatrics.org/cgi/content/full/108/2/e35).

Preterm birth will be defined as delivery prior to 37 weeks of completed gestation. Early preterm (up to <34 weeks) will be distinguished from late preterm 34 up to 37 weeks. Spontaneous preterm labor and delivery and preterm premature rupture of membranes will be distinguished from indicated preterm birth due to maternal or fetal conditions (for example, preeclampsia or small for gestational age (SGA) fetus.

Fetal intolerance of labor will be defined as Cesarean delivery due to abnormal fetal heart rate tracing.

Abruption or vaginal bleeding will be defined as abruption or vaginal bleeding that result in hospitalization, Cesarean delivery, or pre-term birth.

Biospecimen analysis

Reproductive hormones and other biomarkers will be measured from stored urine, serum, and saliva samples for assessment of reproductive hormone concentrations, particularly androgens, cardiometabolic risk factors, markers of oxidative stress, and measures of trace metals in toenails. A proteomic analysis of human sperm will also provide valuable information regarding potential mechanisms.

5.0 PARTICIPANT MANAGEMENT

5.1. Recruitment

Potential study participants will be recruited from heterosexual couples seeking fertility evaluation and/or assisted reproduction at fertility clinics or other participating practices providing reproductive care. Recruitment methods will include attempts to enroll couples of various racial and socioeconomic backgrounds. This recruitment will happen in the clinical setting and may also occur within the community such as health fairs, radio ads, brochures and flyers, standardized phone messages while patients are “on hold” at the study site clinics, social media, community health clinics, student health centers, and holistic care centers; such as fertility yoga, fertility acupuncture and fertility massage etc. Couples seeking assisted conception will be approached regarding the study by their clinician, study coordinator or member of the research staff before, during, or after their initial consult or clinical visit. They will be given information regarding the study, and the opportunity to enroll on that day or given the opportunity to schedule an enrollment visit at a later time.

Patients attending clinic visits who request information regarding the trial will obtain information via phone calls, email, posted mail or meet with research staff, who will then further explain the study and invite them to participate if appropriate. Couples seeking assisted conception at participating study centers may be given a brief information form regarding the study and the opportunity to schedule an enrollment visit. Whenever possible, clinic patients will be provided basic study information and a consent form to review in the days prior to their clinic visit. This basic study information will be given, explained, and questions answered via phone, mail, or email. When discussing the study information with interested couples, the males will be asked to abstain from ejaculation for 2-5 days prior to presentation for their clinic visit so that, if eligible, the male partner can provide a semen sample for analysis and complete enrollment as well as randomization

on that first visit. Because of couple time constraints of work or home, the couple may need to return to clinic on another day to complete the enrollment/baseline visit, semen analysis, and randomization.

Men presenting to the primary clinical sites for semen analysis without their partner will be provided with study and contact information for research staff. Research staff will contact potentially eligible and willing participants who have agreed to be contacted to determine eligibility and provide information regarding study participation. Eligible couples who agree to participate will be scheduled for baseline data collection. Study information materials and contact information will be provided to outside and community clinics allowing recruitment of patients seen in these clinics for New Consultations. Study site websites will provide information regarding study participation as well as contact information for research staff. Patients attending clinic visits who request information regarding the trial will be provided with contact and study information. The study coordinator, research staff and principal investigator (PI) may provide inclusion/exclusion and enrollment criteria to local physicians and clinic nurse managers and be available to discuss the study parameters for potential study integration into their clinics. Facebook, web and radio announcements can be developed as needed.

Couples seeking assisted reproduction will be approached at their first clinic visit. Potential participants planning to undergo a care protocol other than IVF will be counseled that they may participate in the study if the male partner is willing to be on study dietary supplements for at least 3 weeks prior to initiating their assisted reproduction method. Women with regular periods may initiate their therapy at the start of the woman's menstrual cycle following the visit if randomization occurred within the first 10 days of the cycle, or to skip one menstrual cycle if the visit occurred after day 10 of the cycle). For women with irregular periods or amenorrhea, the male must be on the study supplement for 3 weeks prior to initiation of any ovulation induction medication (e.g., clomid, letrozole, gonadotropins).

5.2. Screening Process

Couples will be asked screening questions by study personnel, and those that meet the initial inclusion/exclusion criteria will be invited to participate in the study. These screening questions for inclusion and exclusion criteria can take place via phone, email, or in person. Testing for anemia (Hemoglobin <13 gm/dL) in males will be done using a point-of-care, hemoglobin meter, as a preliminary screen for men who require additional evaluation to rule out B12 deficiency. Men who screen positive for anemia (hemoglobin <13) will have blood drawn for a serum B12 with reflex to methylmalonic acid (MMA), but may still be enrolled and randomized. They will be advised of their abnormal lab result, and to discuss this with their primary care provider. Written informed consent will be obtained prior to the initiation of any study-specific procedures. Female partners will provide informed consent for the baseline collection of study-related biospecimens (blood and urine) and a urine pregnancy test, medical chart abstraction, study questionnaires, anthropometric body measurements, vital sign measurement (blood pressure and pulse rate) and email or online contact for study related surveys. If a female is pregnant at the screening visit, the couple will be excluded from participation in the study. During the screening procedures, both the female and male participants must confirm that the female partner is not currently pregnant and a urine pregnancy test will be completed. If participants remain interested, they will either be immediately enrolled (if they meet study inclusion and abstinence criteria), or will be scheduled for a later enrollment visit and reminded to abstain from ejaculation for 2-5 days. Participants will

be contacted by phone, text, mail, or email to remind them of their study visits, and study staff will provide instruction on study eligibility criteria.

For men who screen positive for anemia, results of further testing will be available within 3 business days. If serum B12 is ≥ 300 pg/mL, no further testing is needed and the male will continue study participation. If serum B12 is <300 pg/ml, MMA will be determined from the same serum sample. If MMA is > 0.4 µmol/L, the study supplement will be discontinued, and the participant will be advised to discuss likely B12 deficiency with his primary care provider. Other aspects of study participation (questionnaires, semen analysis, and biospecimens) will continue.

5.3. Baseline Study Visit

Baseline data collection for males and females will consist of questionnaires (i.e., diet, physical activity, medication, and reproductive history), and anthropometric and vital sign (blood pressure and pulse rate) measurements. The baseline study visit will include an additional review of inclusion/exclusion criteria in the event of any changes during the intervening time period. The baseline interview will be conducted at study centers. Urine, blood, saliva, and semen samples will be collected from male participants, and urine and blood samples will be collected from female participants. Male participants will also be given access and instructed in the completion of an online daily journal. This will be the only study visit for the female participants. After all baseline data are collected, study personnel will randomize male participants to one of the two study cohorts.

5.4. Randomization

A computer-generated randomization schedule that assigns participants to the study supplement and placebo cohorts will be created by the Data Coordinating Center (DCC). A total of 2,400 participants will be randomized (1:1) to active study supplements or matching placebo.

Randomization will be stratified (with random sequences of block sizes) by site and assisted reproduction technique (IVF; non-IVF/receiving fertility treatment at a study site; and non-IVF/ receiving fertility treatment at a non-study site) to ensure that balance between groups is maintained within these factors over the enrollment period. The two Non-IVF strata will include natural fertility optimization methods, OI, and IUI. Participants who meet all criteria for randomization will be formally randomized during the baseline visit by entering enrollment information into the Advantage Electronic Data Capture (AdvantageEDC^{SM}) system. Cohort assignments will be generated automatically by the AdvantageEDC^{SM} system.

Subsequent to randomization, male participants will be given bottles that will have a sufficient number of tablets for daily intake until the next scheduled visit (two and a half months). Participants will be counseled on how to take study supplements and will be instructed to begin taking study supplements starting on the day of randomization.

5.5. Active Follow-up

Male participants will have scheduled follow-up visits at 2, 4, and 6 months (± 1 week). Study personnel will collect anthropometric measurements, urine specimens, weigh remaining supplements, perform venipuncture for blood collection, and collect additional biospecimens including semen, saliva and, when appropriate, toenail clippings. During these scheduled follow-up visits participants will be asked about their journal use to ensure appropriate recording of information. Additional supplement bottles will be provided as needed at the visits (i.e.,

participants turn in previous bottle and receive a new one at the end of the visit). Participants will respond to questionnaires including, but not limited to, dietary intake, physical activity, reproductive health, and medication use, and basic anthropometrics will be measured. Prior to the follow-up visits, study staff will issue reminders via text message, email or phone to reinforce study participation, and to remind participants to abstain from ejaculation for 2-5 days before each study visit. A summary of assessments at each visit is described in Table 1.

5.6. Female Partner Follow-up

Female partners will complete a baseline questionnaire, anthropometric and vital sign measurements, and provide blood and urine specimens, and then will be passively followed by medical record abstraction at each visit to the clinic during assisted reproduction cycles for a period of up to 9 months after study enrollment. In addition, females will be asked to complete a short monthly questionnaire via online, phone or email to update their pregnancy status, and reproduction cycles progress. Once (if during this initial 9-month period) a female partner becomes pregnant, pregnancy outcomes will also be followed by chart abstraction and monthly updates. If a female partner has one or more pregnancies, followed by a loss, all pregnancies will be followed through the initial 9-month period. Couples initiating an IVF cycle in the sixth or seventh month after randomization will continue to be followed if pregnancy occurs for pregnancy outcomes during the initial 9-month period. A summary of assessments at each visit is described in Table 2.

5.7. Specimen Collection

All biospecimens collected during the FAZST study will be initially processed and stored at the local trial coordinating center sites in –80°C freezers. Eventually ALL specimens collected from the clinical sites will be sent to the NIH Tissue Repository (Fisher Bioservices: 627 Lofstrand Lane, Rockville, MD 20850; 301-762-1772). All samples will be tracked by the DCC from the clinic to the repository.

In general, biospecimens will be collected from male participants at each clinic visit in the following order: blood, urine, semen, saliva, and toenail (at one follow-up visit). For women partners, blood and urine are collected, and they will be obtained only at the baseline visit.

Blood collection

Each male participant will have blood collected by research personnel at their study visits. In general, participants will be seated for several minutes prior to blood draw to allow fluid shifts to equilibrate. Male participants will have blood collected at each study visit. Female partners will have blood collected at the baseline study visit only. A total of up to 50 mL of blood will be drawn at each blood draw using the following tubes:

2 X 4.5 mL Light Blue Top, citrate anticoagulant
2 X 10 mL Red Top, no anticoagulant
1 X 10 mL Green Top, Sodium Heparin (freeze dried), 72 USP Units
1 X 10 mL Lavender Top, Liquid K_3 EDTA, 15% additive solution

Blood samples will be processed into serum, aliquoted into cryovials, and frozen for future use.

Urine collection

Male participants will be asked to provide a urine sample in a collection cup at each visit. Female partners will be asked to provide a urine sample only at the baseline study visit.

Semen collection

Men will be instructed to abstain from ejaculation for at least 2 full days and not more than 5 days before attending the baseline clinic visit. Samples will be collected by masturbation into 4.5-oz. Specimen Containers with screw cap. Sample volume will be measured after which semen aliquots will be removed for determination of motility, morphology, and frozen for later DNA fragmentation analysis (see Outcomes Section 4.0 for specifics). If a participant is unable to provide an adequate sample (e.g., due to spillage, forgetting abstinence, etc.), he will be asked to schedule another clinic visit to provide an additional sample.

Saliva collection

Male participants will be asked to donate a bolus sample (1 mL) of whole saliva at each clinic visit. Samples will be collected using the passive drool technique.[31]

Toenail collection

Toenails will be self-collected by the male participant at the four month follow-up visits to measure trace metals and minerals that have been implicated in the physiology of reproduction such as selenium and chromium.[32, 33] Selenium is an essential trace element of importance to human biology and health. Increasing evidence suggests that this mineral plays an important role in normal growth and reproduction in animals and humans. Toenail levels of these measures reflect long-term (on average, up to 1 year) exposures which usually cannot be captured using blood and urine samples.

Table 1: Schedule of Events: Male Participant

	Screening	Baseline	Month 2	Month 4	Month 6
Assessments					
Informed Consent process	X				
Inclusion/Exclusion criteria review	X	X			
Demographic Collection	X				
Concomitant Medication Review	X	X	X	X	X
Anthropometrics (body measurements)	X				X
Vital Signs (blood pressure and pulse)	X				
Screening for Anemia with reflex B12 and MMA testing	X				
Medical History		X			
Lifestyle Questionnaire		X			
Food Frequency Questionnaire		X			X
Soy/Caffeine Questionnaire		X			X
24-Hour Dietary Recall			X	X	
Questionnaire Baseline		X			
Questionnaire month 2			X		
Questionnaire month 4				X	
Questionnaire month 6					X
Daily Journal		X	X	X	X
Dietary Supplement Randomization		X			
Dietary Supplement Adherence Assessment			X	X	X
Dietary Supplement Safety Questionnaire		X	X	X	X*
Biospecimen Collection					
Semen		X	X	X	X
Blood		X	X	X	X
Urine		X	X	X	X
Saliva		X	X	X	X
Toenail				X	

*The Dietary Supplement Safety Questionnaire will also be administered via email 7 days after dietary supplement discontinuation.

Table 2: Schedule of Events: Female Partners

Assessments	Screening	Baseline Visit Collection and Instruction	During Assisted Conception	Pregnancy Follow Up
	Ideally screening and baseline visit will be on the same day			
Informed Consent Process	**X**			
Inclusion/Exclusion Criteria Review	**X**	**X**		
Demographic Collection	**X**			
Food Frequency Questionnaire		**X**		
Soy/Caffeine Questionnaire		**X**		
Lifestyle Questionnaire		**X**		
Anthropometrics (body measurements)		**X**		
Vital Signs (blood pressure and pulse)		**X**		
Baseline Questionnaire		**X**		
New Couples Questionnaire – Clinic Baseline	**X**			
Monthly Questionnaire			**X**	**X**
Medical Chart Abstraction		**X**	**X**	**X**
Biospecimen Collection				
Urine Pregnancy Test	**X**			
Blood		**X**		
Urine		**X**		

5.8. Retention Plan and Compensation

Retention of the research participants will be one of the most difficult tasks of this study. There are multiple clinical visits each requiring blood, semen, saliva, and urine collection. Timing of these visits is important. As such, participant burden should be minimized as much as possible. Retention begins at the time of recruitment, and it is essential to establish a positive rapport with the research participants. This includes providing information in a friendly manner, being responsive to their concerns and time commitments of work and home, providing potential participants with enough detail to understand the requirements of participation, and stressing the importance of the potential research findings. The importance of timing and frequency of study visits and sample collection need to be stressed clearly. A gender-specific participant pamphlet will be provided to each participant with FAQs, instructions for filling out questionnaires and contact information of research staff at the baseline visit. The male participant will be provided with an ASA 24 Dietary Recall instruction sheet at his month 2 visit. Awareness of the participants' time commitment will be integral. Participants will be compensated for their participation in the trial.

A total compensation amount of $350 will be provided to participants who complete all of the required procedures at the following visits:

1. Baseline visit $50
2. 2nd visit $50 Participants will receive an additional $15 if 80% of the daily journals have been submitted since the previous visit
3. 3rd visit $75 Participants will receive an additional $15 if 80% of the daily journals have been submitted since the previous visit
4. 4th visit $125 Participants will receive an additional $20 if 80% of the daily journals have been submitted since the previous visit

Required for Baseline visit participant reimbursement are Baseline and Study Questionnaires in AdvantageEDC[SM]. For subsequent visits this will also include the ASA24. Compensation checks will be mailed to participants after they have completed the visit and signed the compensation form. Participants will also be required to complete a W9 form for personal tax purposes. The original form will remain in their research chart. If a participant is unable or unwilling to provide a social security number on the W9 form, he can still be in the study if interested but cannot be compensated monetarily. Females will not receive reimbursement for their participation.

Exit Questionnaire (Withdrawal)

As in any study, participants may choose not to follow the study protocol fully until its completion. Even with the utmost effort to retain study participants, some drop out is expected. To remove the possible bias, information on reasons for study withdrawal, non-compliance, and drop out will be collected whenever possible.

Study Supplement Retrieval

All attempts will be made to retain participants at any level they feel comfortable participating in this study. In general, study staff will continue to work with participants as long as they are responsive, to find the best way to retrieve the supplement bottles dispensed to each participant. If the participant is not responsive after three attempts, the supplement bottles will be considered lost. On a case-by-case basis, the number of attempts may vary to ensure that we do not unnecessarily burden or disturb participants. All forms of attempted communication to recover IP bottles will be documented. If the participant cannot be contacted by email, phone, or text a certified letter can be sent to the participant requesting the return of the dispensed supplement by whichever method is most convenient for the participant:

- Staff may offer to pick up the bottle from physician's office or participant's home
- Staff may send a stamped, self-addressed envelope to the participant if the current address is known.

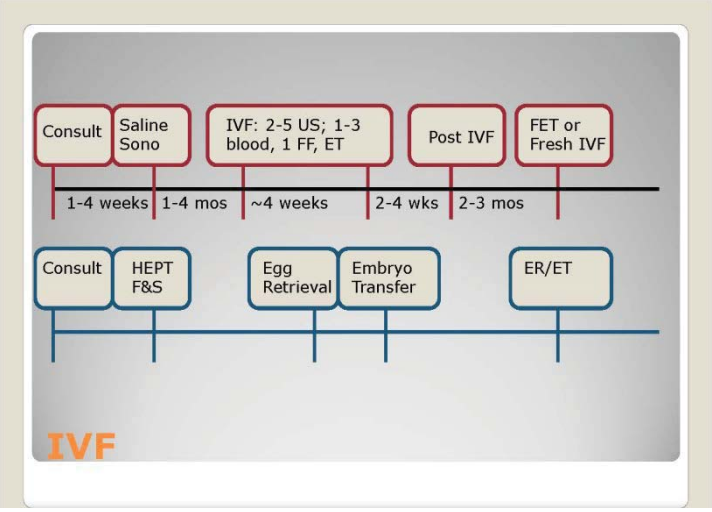
When the dispensed bottle is determined to be lost or thrown away, this will be documented in AdvantageEDC, on the last completed visit Supplement Accountability (ACT) Form.

6.0 STUDY TIMELINE

The following figures display an overview of the study design and timelines and data collection scheduled throughout FAZST. Male participants will complete study visits at baseline and 2, 4, and 6 months of follow-up. Both questionnaires and biospecimens will be collected at each study visit. The duration of study supplementation or placebo will be 6 months. Female partners will complete a study visit at baseline and then be passively followed throughout the 6 months that the male partners are on the study supplement and an additional 3 months after supplement discontinuation. If she becomes pregnant during the 9 months of her follow up, her pregnancy and delivery will be followed and data collected via medical chart abstraction tools. Information regarding assisted reproduction methods and medications from female partners will be abstracted from medical records.

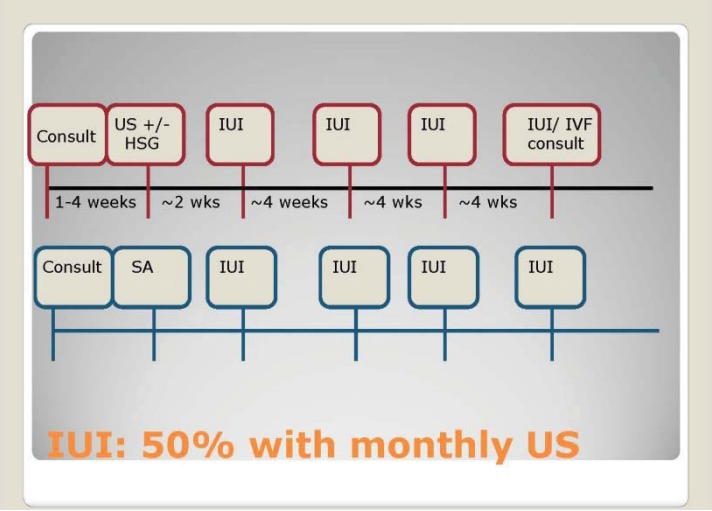
The typical timelines for men and women undergoing IVF assisted conception are shown in Figure 1 (female timeline is shown on the top in red, male timeline is shown on the bottom in blue). After the initial consultation, women may have a saline sonohysterogram and/or an ultrasound (US) completed. Within 1 to 4 months, the first IVF assisted conception cycle is initiated. During the IVF cycle she will undergo 2 to 5 US and blood samples to assess uterine lining, follicular development and maturity. Egg retrieval (ER) is done and 3 or 5 days later embryo transfer (ET) is completed. For the men, a SA is completed, along with a hamster egg penetration test (HEPT). Male partners collect a fresh semen sample on the day of ER. Depending on the pregnancy outcome, an additional frozen embryo transfer (FET) or fresh cycle may be initiated within the following months. During the six months of study follow-up it is anticipated that couples may complete between 1 and 3 IVF assisted reproduction cycles.

Figure 1. Recruitment and subsequent clinic (non-study related) visits for new IVF consults



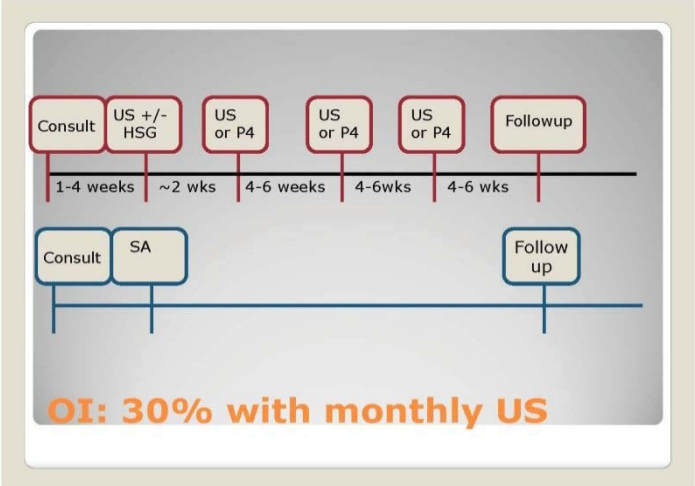
The typical timelines for men and women initiating therapy with IUI are shown in Figure 2 (female timeline is shown on the top in red, male timeline is shown on the bottom in blue). After the initial consultation women usually undergo an US, hysterosalpingogram (HSG), or saline sonohysterogram. Once IUI cycles are initiated they can be done for several months in a row as required. If unsuccessful after approximately 3 to 4 IUI assisted conception cycles, the patients may then undergo an IVF consultation. The men undergo a SA after the initial consultation and then are usually present at each IUI assisted conception. It is anticipated that couples may undergo up to 4 IUI cycles over the 6-month course of the study protocol.

Figure 2. Recruitment and subsequent clinic (non-study related) for patients initiating therapy with IUI



The typical timelines for men and women undergoing OI are shown in Figure 3 (female timeline is shown on the top in red, male timeline is shown on the bottom in blue). After the initial consultation women usually undergo an US, HSG, or saline sonohysterogram. OI cycles are then followed-up by US and/or estrogen and progesterone (P4) measurements. If unsuccessful after approximately three OI assisted conception, the women may then undergo an additional consultation for further assisted conception methods. The men undergo a SA after the initial consultation. It is anticipated that couples may undergo up to three OI cycles over the 6-month course of the study protocol.

Figure 3. Recruitment and subsequent clinic (non-study related) for patients undergoing OI



7.0 STATISTICAL ANALYSIS

7.1. Overview

The FAZST trial is designed as a multi-center double-blinded block randomized controlled clinical trial with two cohorts: folic acid and zinc compared to placebo. The primary objectives are to compare the cohorts on four quantitative assessments of sperm quality (concentration, motility, morphology, and DFI) and live births in their partners. The study is designed with a sample size of 2,400 randomized participants based on obtaining adequate power to detect meaningful differences in the live birth rate between cohorts. Since the comparison of sperm parameters are differences between continuous assay measurements, this sample size will be more than sufficient for the primary sperm parameter comparisons.

The primary analysis plan is based on an "intention-to-treat" (ITT) approach comparing the two cohorts based on the randomized assignment, ignoring later changes in dietary supplement consumption. This analysis is described in Section 7.2. The DCC will perform periodic safety analyses and present interim reports to the Data and Safety Monitoring Board (DSMB) as requested, during the recruitment phases of the trial. It is anticipated that safety analyses will be performed every 6-12 months. The final analysis will be performed upon completion of data collection and editing in the follow-up and close-out phase of the trial. Also, one full formal interim analysis is planned and the power calculations with considerations for the choice of optimal time for the analysis are given in Section 7.3.

7.2. Study Endpoints and ITT Analysis

Study endpoints: operational definition and verification

Study endpoints will be determined based on laboratory evaluation including standardized SA, clinical pregnancy tests, and medical record evaluations (including routine pregnancy follow-up, ultrasound). Whenever possible, endpoints will be verified using different sources of information. For example, self-reports of pregnancy loss will be ascertained by medical records (in case of a clinically recognized pregnancy) or by blood.

Operational definitions for study outcome events are detailed in Section 4.0. The sources of information for study endpoints ascertainment are described below:

Primary

a) Semen quality – Standardized quantification of volume, concentration, motility, morphology, sperm count, and DFI fragmentation.
b) Live birth – based on hospital records

Secondary

c) hCG detected pregnancy (implantation) – a quantitative hCG evaluation in serum > 5 mIU/mL.
d) Early pregnancy loss (EPL) – hCG pregnancy loss will be defined as a serum hCG > 5 mIU/mL followed by a decline. Clinically recognized pregnancy losses will be identified by visualization of an intrauterine gestational sac followed by a loss prior to 20 weeks gestation.
e) Clinical intrauterine pregnancy – visualized gestational sac in the uterus on ultrasound.

f) Ectopic pregnancy – either visualization of no gestational sac in the uterus with a suspicious mass in the adnexa on ultrasound, an hCG level more than 1500 mIU/mL without visualization of an intrauterine gestational sac on ultrasound, or a slowly rising or plateauing serum hCG level without visualization of an intrauterine gestation on ultrasound.
g) Specific pregnancy outcomes - including but not limited to Cesarean section, preeclampsia, gestational diabetes, growth restriction, gestational age, preterm birth, birth weight (small for gestational age), major neonatal complications including death, and severe post-partum maternal morbidity will be determined based on hospital records and medical chart abstraction.
h) Early embryonic development parameters - Proportion of ICSI, proportion of MII oocytes fertilized, number of cells and embryo morphology on day 3 and day 5, number of good quality embryos on day 5, proportion of good quality embryos on day 5, number of embryos transferred, quality of embryos transferred, number of embryos cryopreserved, sperm penetration assay results.
i) Reproductive hormones and other measured biomarkers – Measured urinary, serum, and salivary concentrations of reproductive hormones, particularly androgens, proteomic analysis of human sperm and cardiometabolic risk factors and markers of oxidative stress, as well as measures of trace elements in toenails.

All events will be reviewed and verified by a central outcome review that will be performed by the NICHD Project Officers and the Principal Investigator at the study clinical sites and the DCC.

ITT analysis

The primary analysis of the clinical trial will be ITT analysis based on the total cohort of randomized participants. This approach will be applied to the two primary endpoints (semen parameters and live birth rate) as well as designated secondary endpoints (number of follicles, number and proportion of oocytes fertilized). The difference between the measures of semen quality parameters, including concentration, motility, and morphology, and sperm DNA fragmentation for the two cohorts will be examined by two-sided t-tests. The common null hypothesis states that the effect of folic acid/zinc on the outcomes is null compared to the placebo. Multivariate techniques will also be employed to consider the effects of folic acid/zinc on overall semen "quality". Specifically, a permutation test based on the sum of t-statistics across the four quality measurements will be conducted. The frequency of live births across cohorts using the standard Z-score (using the normal approximation to the binomial distribution) will be compared. Both of the primary analyses will be done with two-sided tests conducted at the 0.05 level. The semen parameters will also be assessed using analysis of covariance (ANCOVA) models with each semen parameter as the dependent variable, clinical center, IVF strata and treatment group as factors. The outcome of live birth will be assessed using a Mantel-Haenszel analysis to evaluate treatment effect across IVF strata.

The secondary objectives with binary endpoints will be analyzed using the standard Z-score. For binary outcomes such as live births, the time to event may be of interest. In this case, survival analysis methods will be applied with the log-rank test for comparison of the two study cohorts. A stratified log-rank test will be used to assess treatment effect across IVF strata. For continuous outcomes such as birthweight either parametric or non-parametric methods will be used as appropriate. If transformations to approximate a normal distribution are feasible, they will be carried out prior to the parametric analysis. The normality of the data will be examined both

graphically using Q-Q plots and by formal tests (Wilks-Shapiro test). For data (either on original or transformed scale) assumed to be normally distributed, the comparison of the two cohorts will be carried out by a t-test. Alternatively if a non-parametric test is required, the Wilcoxon-Mann-Whitney test will be used. For continuous secondary outcomes stratified analyses will be conducted using ANCOVA models with IVF strata and clinical center as factors for the comparison of the two cohorts. If a non-parametric test is required, van Elteren tests will be used to compare the two cohorts, accounting for strata, to detect differences in the distributions of the secondary endpoints.

7.3. Sample Size and Power Determinations

Table 3 presents results of power calculations for FAZST. An alpha level of 0.05 and a sample size of 2,400 couples divided equally to the folic acid/zinc and placebo arms of the trial are assumed throughout the trial. Previously published studies suggest that for the control group, a probability of about 0.60 - 0.70 for live birth (PLB) can be expected, assuming up to three cycles of assisted conception.

In the following, a range of probabilities for the placebo arm of 0.60, 0.63 and 0.65 is considered along with effect sizes of 1.05, 1.10 and 1.15. The effect of the intervention will be measured as the rate ratio (RR) of live birth in the folic acid/zinc arm compared to placebo. The calculations were performed using R statistical software based on a two-sample test of populations. Table 3 presents the resulting power with no interim reviews.

Table 3. Power for comparing live birth rates in the two cohorts with no interim looks based on an alpha level of 0.05 and a sample size of 2,400.

PLB in placebo arm	Live Birth RR to be Detected		
	1.05	1.10	1.15
0.60	0.33	0.86	0.99
0.63	0.36	0.90	0.99
0.65	0.39	0.93	1.00

The FAZST study is comprised of three strata (couples receiving IVF, couples receiving non-IVF at a FAZST site, couples receiving non-IVF at a non-FAZST site), which are block randomized. The power and sample size calculations for the analysis of the overall trial are presented previously. The following calculations are to demonstrate adequate statistical power when stratified analysis is to be performed. In Table 4, we provide the results in sample size distributions among the strata and their corresponding live birth RRs that can be detected at 80% statistical power, with an alpha level of 0.05 and a total sample size of 2400 couples divided among the folic acid/zinc and placebo arms of the trial. These calculations assume that the cumulative live birth rate in the placebo arm for the IVF stratum is 0.63 and 0.50 for the placebo arms of the other two strata. For example, when the IVF stratum is expected to enroll 1000 participants and the two non-IVF strata is each expected to enroll 700 participants, the detectable effect sizes in live birth RR are 1.13, 1.21, and 1.21, respectively. The lower effect size in the IVF stratum than that in the non-IVF strata is consistent with clinical knowledge in that enhanced live birth rates attributable to folic acid/zinc supplementation is likely to be greater in the non-IVF strata assuming it is generally more fecund than the IVF strata and the IVF treatments are highly effective leaving less room for improvement. When multiple comparisons are corrected, we see slight elevation of effect sizes

(1.15, 1.24, and 1.24, respectively) that can be detected at the same 80% statistical power and with the same sample sizes.

Table 4. Stratified power analysis for comparing live birth rate in treatment and placebo arms with no interim looks based on a total sample size of 2400, 80% statistical power, and an alpha level of 0.05. The three strata are: A-IVF stratum, B-non-IVF FAZST site stratum and C-non-IVF non-FAZST site stratum. The PLB in placebo in these strata are 0.63, 0.50 and 0.50 respectively.

n per arm			RR			RR adjusting for multiple comparisons		
A	B	C	A	B	C	A	B	C
300	450	450	1.17	1.19	1.19	1.19	1.21	1.21
400	400	400	1.15	1.20	1.20	1.17	1.23	1.23
500	350	350	1.13	1.21	1.21	1.15	1.24	1.24
600	300	300	1.12	1.23	1.23	1.14	1.26	1.26

While three strata have been conservatively block randomized by design, the Non-IVF FAZST and Non-IVF non-FAZST site are anticipated to be similar populations and could potentially be collapsed during analysis. The positive power and sample size of this collapsing of strata are displayed in Table 5. The gain of statistical power in the non-IVF strata allows us to shift some enrollment to the IVF stratum which is believed to have a smaller effect size to detect. For example, with a 700 and 500 sample sizes per arm for the IVF and non-IVF strata respectively, we are able to detect a live birth RR of 1.11 and 1.18, respectively. When multiple comparisons are corrected, the detectable live effect sizes are 1.12 and 1.19 respectively.

If the strata are deemed collapsible while recruitment is still active then a re-alignment of sample size goals will allow for the detection of smaller effect sizes in the live birth RR, in the IVF stratum. This can be achieved by periodically assessing the distribution of characteristics of enrolled participants between non-IVF FAZST stratum and non-IVF non-FAZST stratum. On the other hand, if the strata are deemed collapsible after recruitment is completed, the sample size of the Non-IVF strata will obviously double resulting in a substantial reduction in effect estimates detectable with 80% statistical power and an alpha level of 0.05.

Table 5. Stratified power analysis for comparing live birth rate in treatment and placebo arms with no interim looks based on a total sample size of 2400, 80% statistical power, and an alpha level of 0.05, when the two non-IVF strata are collapsed. The two strata are: A-IVF stratum, B-non-stratum. The PLB in placebo in these strata are 0.63 and 0.50 respectively.

n per arm		RR		RR adjusting for multiple comparisons	
A	B	A	B	A	B
300	900	1.17	1.13	1.18	1.14
400	800	1.15	1.14	1.16	1.15
500	700	1.13	1.15	1.15	1.16
600	600	1.12	1.16	1.13	1.18
700	500	1.11	1.18	1.12	1.19
800	400	1.10	1.20	1.11	1.22

7.4. Interim Analysis

For a multi-year trial such as this one it is useful to examine the data as it accumulates in order to see if the study should be ended early or to catch any surprising developments. One of the most widely used procedures for interim analysis of a clinical trial is the alpha spending function approach of Lan and DeMets.[49] This method uses a function of time to specify the rate at which the total Type I error probability will be spent during the trial interim reviews so that this quantity does not exceed the desired overall alpha level. For FAZST, a two-stage interim analysis plan will be implemented. The following group sequential two-stage test plan describes how alpha (α) will be spent across the interim and final analyses of the data.

The interim analyses will be performed based first on the sequential interim monitoring of the semen quality parameters (sperm concentration; total sperm motility; and sperm morphology (abnormal form)) after 50% of the FAZST participants have completed their six months follow-up. The analyses will be unadjusted by any covariates, in line with the ITT approach. The overall Type I error rate will be controlled at a pre-specified level, accounting for both multiple semen outcomes and the interim analysis. As the effects of the nutritional supplementation are not clearly understood, the study should be stopped early only if there is strong evidence of harm. Given this a one-sided alpha spending function, which approximates the O'Brien-Fleming[50] boundary, will be developed. First, a Bonferroni adjustment will be used to distribute the (sometimes called experiment-wise) Type I error alpha among the multiple semen parameters. A $\alpha=0.025$ (one-sided) is to be assigned to the experiment-wide alpha level for the assessment of semen parameters, where $\alpha_3=\alpha/3 = 0.0083$ will be used to compute the boundary for each individual test. For each semen parameter, the amount of α_3 available for the interim analysis performed at time t is $\alpha_3(t)$ and will be obtained from the O'Brien-Fleming spending function. Time t for the first stage of the FAZST interim analysis will be once 1200 (50% of the participants) have completed six months of follow-up. Using these assumptions the test statistic for lower bound of harm will be 3.5547 for each of the 3 comparisons with $\alpha_3(t) = 0.00019$ where t represents 50% of the participants completing 6 months of follow-up. The DSMB will consider the interim analysis results as a resource to evaluate the risk of study treatment. When a stopping boundary is crossed, there will be an indication that the treatment has an increased risk of harm for at least one of the semen

parameters. The DSMB will consider the consistency of all semen analysis data to determine whether the second stage of the interim analysis should be considered.

If the DSMB determines that the second stage of the interim analysis is applicable, it will be conducted based on the available data and the outcome of live birth. For this stage, the estimated information fraction will be defined as the total number of participants completing 6 months of follow-up at the time of database closure for the second stage interim analysis. Similarly, the alpha spending function approach will be applied to the sequential monitoring of live birth. An overall alpha of 0.05 and a one-sided procedure in data monitoring will be used. The hypothesis will be tested by a one-sided O'Brien-Fleming alpha spending design. Assuming PLB = 0.63 in the placebo arm and a RR of 1.100, interim test boundaries using the O'Brien-Fleming alpha spending design based on various values of the information fraction are provided in the table below. When a stopping boundary is crossed, there will be an indication that the treatment has an increased risk of harm for live birth. The DSMB will consider this result and all live birth data to determine whether a suspension of the trial intervention should be considered.

Table 6. Examples of Critical Values of the Test Statistics for Live Birth

Information Fraction	Lower Bound
0.20	4.8769
0.25	4.3326
0.30	3.9286
0.35	3.6128
0.40	3.3569
0.50	2.9626

7.5. Prediction Models and Secondary Analyses

In addition to the ITT analysis the data collected in FAZST will be utilized to develop prediction models for various endpoints for couples undergoing IVF and/or ICSI procedures. These models will enhance understanding of these processes and the factors that may affect outcomes. Thus, the study will provide important information beyond the evaluation of the effects of folic acid/zinc on semen quality alone.

The prediction models approach is described here only briefly.

Prediction of live birth

Various prediction models can be built for this binary event considering baseline and follow-up information (including, though not limited to, sperm concentration, motility, morphology, sperm DNA fragmentation, female age, female anti-müllerian hormone (AMH) and/or antral follicular count, female diagnoses, female assisted conception type, the number of embryos transferred, and embryo quality). Three methods can be used to build a prediction model: (a) logistic regression, (b) neural networks, and (c) regression trees. While logistic regression is a standard technique, the

other two methods will allow consideration for more complicated associations between risk factors and outcomes (e.g., interactions and optimal cut-off points) as well as discrete outcomes with several possible categories (e.g., early pregnancy loss, pregnancy complications, and birth).

Validation methods will be used to compare these approaches whereby the data will be divided randomly into training (e.g., 2/3 of the data) and validation sets (e.g., 1/3 of the data). The models will be fit using the training set and their predictive ability will be compared on the validation set. Receiving operating characteristic (ROC) curves will be used to compare the different models and determine which is preferred.[34, 35] The optimal probability threshold for the logistic and neural network models will be chosen using the Youden Index.[36-38]

7.6. Safety Analyses

All reported adverse events will be listed by term, frequency, severity, and cohort. Standard statistical tests using normal theory will be readily applicable for assessing these data. Adverse events will be collected in the males receiving study supplements though important female associated study endpoints, including adverse pregnancy outcomes and pregnancy loss, will be collected throughout the study and reported as adverse events via questionnaire and medical chart abstraction.

7.7. Evaluation of Drop Outs and Adherence

The DCC will co-develop with the Project Officer statistical methods for evaluation of drop out and compliance for future analyses of the study results. Both drop-outs and non-compliance introduce a problem when the processes do not occur at random. Drop-outs potentially lead to selectively missing data that may bias crude ITT comparisons. Non-compliance to assigned study supplements can also be influenced by intermediate study outcomes and therefore introduces additional bias. Marginal structural models (MSM) suggested in the causal inference framework[39-41] have been used in observational studies to account for time-dependent covariates that are on the pathway to disease outcome or self-selection into treatment.[42, 43] MSMs have also been useful in clinical trial for handling non-compliance to provide unbiased estimates of the causal effect of cohort assignment building upon the theory of causal inference and the idea of 'potential outcomes'.[44, 45] A key component to these models is inverse probability weighting to model the exposure (e.g., folic acid and zinc supplementation) with the end result providing an estimate of the relative risk of the outcome of interest among the actual dietary supplements compared to placebo, with appropriate confidence intervals and appropriately adjusting for time-dependent confounders affected by prior exposure.

Compliance

All bottles, both study supplement and placebo bottles, will be weighed as a measure of participant adherence at the 2-, 4-, and 6-month study visits. Participants will be asked to bring all bottles (both unopened and opened) and unused tablets to each study visit. The original weight of the bottles when distributed should be recorded on the appropriate visit checklist. The bottles will be weighed using a calibrated scale. The bottles should be weighed outside the room where the participant is located, when possible.

7.8. Implementation of Statistical Analysis

Descriptive data analysis will be performed by the DCC. Questions related to data analysis and methodological issues will be discussed periodically at staff meetings at the DCC. Interim results will be summarized and presented at the DSMB meetings and discussed with the Project Officer.

Holding regular discussions of study analysis is crucially important to ensure that study aims are met and that the interpretation of data is valid. Publication quality summaries of the results of the statistical analyses will be prepared by the DCC team for interim and final reports; presentations and manuscripts in conjunction with statistical investigators at NICHD.

8.0 DATA MANAGEMENT

8.1. Web-based Clinical Trials

The DCC will develop an internet-based system for the FAZST in close collaboration with the investigators and staff members at the TCC and clinical sites.

Over the last decade increasing numbers of RCTs as well as other clinical multi-center studies are performed using internet-based technologies. Some of the advantages of such systems include minimization of errors, real-time data reporting, saving in resources for recording of the data, less paper and less storage space required.[46, 47]

8.2. FAZST Web-based System

Data collection

Source Documents: Source documents are defined as original documents, data and records. They may include hospital records, clinical and/or office charts, laboratory data/information, participants' journals or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, microfiches, photographic negatives, microfilm or magnetic media and x-rays. Study staff will clearly define the various source documents used to support the study as part of their local data management support.

The investigator(s)/institution(s) will permit study-related monitoring, audits, IRB/ Independent Ethics Committee (IEC) review, regulatory inspection(s), and will provide direct access to all source data documents at the time of monitoring and/or upon request by the DCC.

Web-based data collection and management system

Data collection will occur via a web-based data entry system (AdvantageEDC for study specific needs such as enrollment and participant ID assignment) provided by the DCC for FAZST to allow easy access to enrollment 24 hours a day, seven days a week. Upon enrollment in AdvantageEDC, a form submission schedule is generated for each participant and displayed as a grid of forms by study visit that permits direct access to each electronic case report form for data entry. As data are entered, they are validated through range and within-form consistency checks.

Data storage and quality control

Data for individual participants will be recorded on electronic case report forms (eCRF) in the AdvantageEDC system. All participants screened for the study, including the screen failures, must be entered into AdvantageEDC. Participants will be randomized in the AdvantageEDC system, and the eCRFs must be current to reflect participant status at each phase during the course of the study. Participants will not be identified on the eCRFs by name or initials; instead, once participants are entered into AdvantageEDC, the system will assign them unique participant

identification numbers. Investigators are required to keep a separate log of participant names and addresses.

Because of the potential for errors and inaccuracies in entering data into eCRFs, laboratory and other test results must be kept on file with the participant's study dossier. Study records should be maintained for at least three years post completion of the final study report or publication of the primary endpoints or per local site IRB requirements (whichever is longer).

Data processing and data management

Clinical data processing and management will be employed based on the procedures developed in conjunction with the DCC. All of the data entered into the AdvantageEDC system will be checked for valid values and ranges, between item logical consistency, and within-participant variation. Prior to any analyses the distributions of the measures will be examined to aid in selecting appropriate statistical techniques and data transformations. Data transformations (e.g., log transformations) will be used in an attempt to normalize non-normally distributed data so as to still be able to use statistical techniques appropriate for normal data. In addition, nonparametric and semi-parametric techniques may be used with non-normally distributed data.

Data plan for participants with incomplete data

Participants will be included in the data analysis provided that they complete the testing procedures listed and have completed at least one follow-up visit, and do not receive additional interacting medications. The DCC will work with site staff to ensure that the study records for all participants who terminate early are as up to date and as possible, with field and form exceptions reviewed and accepted to account for all required data.

8.3. Questionnaires and Other Data Forms

All necessary forms and documentation material will be available online through the study website and can also be printed on location at the clinical sites as needed. The availability of information online ensures that all are working with the same most updated version

Forms will be designed to be clear, concise, and convenient to use and for the web-based forms also include imbedded coding and initial error checking mechanisms. Data collection for the FAZST trial will begin with screening, continue through the baseline visit, active follow-up (with daily journals and biospecimens collections) for six months, and end with passive follow-up during pregnancy. The principal mode of data collection for FAZST will be web-based.

Questionnaires

The male study questionnaires include the baseline questionnaire, 24-hour dietary recall, food frequency questionnaires, and 2-, 4-, and 6-month follow-up questionnaires and daily journals. Refer to Table 1 for specific questionnaire content. Briefly, baseline questionnaires will elicit socio-demographic information, stress, medical and reproductive history and health-related habits. Follow-up visits and questionnaires will obtain information on adherence to and safety of study supplement and brief behavior and lifestyle information. Questionnaires are self-administered and entered into the AdvantageEDC system. All questionnaires, except the 24-hour dietary recall, will be programmed by the DCC and incorporated into the AdvantageEDC system. For the 24-hour

dietary recall, the ASA24 Automated Self-administered 24-hour Recall (v. 2011 or 2014) developed by the National Cancer Institute will be used. The ASA24 is a freely available, secure, Web-based tool that enables self-administered 24-hour recalls (http://riskfactor.cancer.gov/tools/instruments/asa24/). All questionnaires will be reviewed by the study nurse/staff to verify understanding and completion.

The female study questionnaires include the baseline questionnaire, food frequency questionnaires, and monthly questionnaires. Refer to Table 2 for specific questionnaire content. Briefly, baseline questionnaires will elicit socio-demographic information, stress, medical and reproductive history and health-related habits. Monthly on-line follow-up questionnaires will obtain information on any current fertility treatment she has received and pregnancy outcomes. Questionnaires are self-administered and entered into the AdvantageEDC system. Questionnaires will be reviewed by the study nurse/staff to verify understanding and completion.

Daily journals

These will be completed daily via the internet, smartphone, or a telephone system by each male participant during the six months on study dietary supplements. These forms, eliciting information on dietary supplement compliance, intercourse, exposures to alcohol and stress, and possible adverse effects are designed to be simple and convenient to encourage response.

Abstraction forms

Information on the reproductive history and demographics of the female partner and the course of fertility treatments she may have received, pregnancy and delivery will be obtained through abstraction of medical records from routine pre-natal clinic visits, routine tests (e.g., blood tests, ultra-sound, amniocentesis) and any hospitalization, OBGYN, other primary medical doctor, or emergency room visits.

Biospecimen tracking

For each visit where specimens are collected, pre-printed labels that include barcodes will be provided and placed on tubes and containers per the Manual of Procedures (MOP). Each specimen sample after processing will be labeled with a barcode and dot matrix code, and all biospecimens will be entered and tracked in the Global Trace System, a component of the AdvantageEDC System. The barcodes contain the participant identification number, the visit number and the type of specimen. Mapping of the repository boxes in Global Trace will allow the location of any specific specimen as needed. Shipment from the local repository to the central study repository will be documented and performed according to the Standard Operating Procedures (SOP) for the lab specimen and in collaboration with the central repository. The specimen tracking information will be saved in Global Trace which is maintained by the DCC.

8.4. Adverse Experience Reporting

Definitions of adverse event (AE) and serious adverse event (SAE)

Standard definitions for AEs and SAEs, their identification, characterization regarding severity and relationship to study interventions, and processing are included in Appendix A.

- AE collection period and follow-up

  All AEs will be recorded from the first day the study supplement was taken through seven days following the last dose of the supplement. All reportable events and all suspected adverse reactions will be followed until resolution or medically stable.

  All AEs, either observed by the Investigator or one of his/her medical collaborators, or reported by the participant spontaneously, or in response to direct questioning, will be reported. Any SAE regardless of severity or potential association with the study supplements must be documented in study records by the site Investigator and promptly reported to the DCC (within 24 hours of learning about the event). Non-serious adverse events can be collected in a routine manner using case report forms. Adverse events will be collected in the males receiving study supplements. Important female associated study endpoints, including adverse pregnancy outcomes and pregnancy loss, will be collected throughout the study and reported as adverse events via questionnaires and medical record abstraction forms.

- Obligations of Site Investigators

  FAZST requires the site investigators to report all AEs, regardless of their severity or potential association with the study supplements. When submitting adverse event information the DCC, a site investigator may not delegate someone other than a listed study physician the responsibility for reviewing the accuracy of the contents of the adverse event report. When reporting an AE, the site investigator must assign a severity grade to each event and also declare an opinion on the relatedness of the event to the study supplements.

  For any SAE, the DCC must be notified within 24 hours of when the site investigator first learns of the occurrence of the event. Adequate information must be collected with supporting documentation and entered in the AdvantageEDC data collection system. This data entry serves as notification of the DCC. The 24-hour reporting deadline is necessary to provide adequate time to investigate the report and determine if the SAE requires further reporting. The DCC and/or NICHD would then prepare materials to submit, if necessary, to regulatory authorities and IRBs within the timeframes required by regulation for expedited safety reports.

  For AEs considered non-serious, timely reporting is considered acceptable within seven days of learning about the event.

- SAE Reporting Responsibilities of the Site

  When an SAE is identified, the site investigator (or the study coordinator) shall promptly:

  1. Notify the site PI (if a different person) in person or by telephone about the SAE.
  2. Use the AdvantageEDC system to prepare the available SAE information on a study adverse event case report form. If supplementary information is to be supplied, this may be attached to the case report form in AdvantageEDC. If the data system is not available, use a MedWatch Form FDA 3500A and fax to the DCC. The event must be entered into AdvantageEDC as soon as the system is available.
  3. The site PI (or another designated study physician) is responsible for reviewing and approving the report contents (including the event description, grading of event severity, and relatedness to study supplements).

4. Submit the initial report to the DCC within 24 hours of recognizing the event. Submission to the local IRB is based on the site's responsibilities per the site's IRB.
5. The adverse event report and each page of any attached materials must describe the study participant only by their coded study identifier(s). Any personally identifying information (e.g., name, telephone number, address, etc.) must be removed or obscured before delivery to the DCC. Transmission may be done electronically via the AdvantageEDC system or by facsimile transmission to the DCC. Any information that cannot be submitted via the AdvantageEDC system to the DCC must be sent by facsimile transmission (1-800-576-5790).
6. If the DCC requests additional information or if further pertinent details become available (e.g., laboratory reports, follow-up evaluations, discharge summaries, autopsy reports, etc.), promptly submit them to the DCC.

- Submitting an expedited Safety Report to the Local IRB

  When the DCC or NICHD informs a site that expedited safety reporting to regulatory authorities is required, the PI should review and update any previously reported materials as needed.

  Each expedited safety report should routinely include a brief cover memorandum, the completed MedWatch Form FDA 3500A, and any additional pertinent information recommended by the DCC or the Medical Monitor. Once a report is assembled by the DCC and provided to each of the sites, the site PI must submit the expedited safety report to the local IRB within the required reporting timeframe. Follow-up reports will be submitted when required or when pertinent information becomes available. The PI must retain a complete copy of each expedited safety report as it was submitted to their IRB, and forward a copy of each report to the DCC. The DCC will be responsible for further dissemination of expedited safety reports as described below.

- Obligations of Study Sponsor and Data Coordinating Center

  The NICHD, or the DCC on behalf of the NICHD, must immediately investigate each reported serious adverse event. Recommendations for reporting AEs for dietary supplements are described in the FDA "Guidance for Industry: Questions and Answers Regarding Adverse Event Reporting and Recordkeeping for Dietary Supplements as Required by the Dietary Supplement and Nonprescription Drug Consumer Protection Act" (2007) and if applicable, may be covered in the FDA regulations regarding drug products. FDA (and other relevant regulatory authorities), the DSMB and all participating investigators must be notified within 15 days of any adverse experience that is associated with the use of study supplements and that is both *serious* and *unexpected*. If the reported AE is an unexpected fatal or life-threatening experience associated with the use of the study supplements, the NICHD must notify the FDA and the DSMB as soon as possible but no later than seven calendar days after the sponsor's initial receipt of the information.

  All SAEs must be reported to the DCC through AdvantageEDC within 24 hours of the site becoming aware of the event. SAEs not requiring expedited reporting will be summarized in annual reports to regulatory authorities and disseminated to the DSMB, study investigators, and IRBs active in the study.

When the DCC or NICHD has determined that expedited reporting of an AE is required, the DCC will be responsible for performing the following procedures and for reporting the AE to regulatory authorities within the required timeframes:

1. Notify the site that expedited reporting will be required and request any additional information needed to complete an appropriate report. This may include completion or updating the information in AdvantageEDC and the submission of supporting documentation, laboratory reports, discharge summaries, etc. The DCC will prepare a cover memorandum for reporting the event to regulatory authorities.
2. When the cover memorandum, MedWatch Form FDA 3500A, and any pertinent attachments are ready, the DCC will submit a copy of the completed report, by fax or courier delivery before the regulatory reporting deadline, to the following persons:
    a. FDA (addressed to FDA, Center for Food Safety and Applied Nutrition, Office of Food Defense, Communication and Emergency Response, CAERS Team, HFS-11, 5100 Paint Branch Parkway, College Park, MD 20740, or if applicable, to the designated Medical Officer for any covering IND in effect for the study);
    b. Site PIs at each active participating site (who is responsible for forwarding the report to the local IRB);
    c. NICHD Project Officers;
    d. DSMB Chair.
3. If relevant follow-up information becomes available, the DCC will be responsible for obtaining the details from the site. This information will be reviewed by the Medical Monitor. A follow-up MedWatch form will be completed and forwarded to all parties that received the earlier safety report.
4. The DCC will also transmit copies of all expedited safety reports to the designated individual from any cross-referenced IND sponsors, if applicable. A copy of the safety sections of the annual FDA reports, if applicable, will be forwarded to the NICHD and will be provided to the designated individual from any cross-referenced IND sponsors.

- Terminology to Use for AE Description

When reporting an AE, the event description should use the best matching terminology describing the event as found in the "Common Terminology Criteria for Adverse Events" (CTCAE, v4.0). If an available CTCAE term fits the event well, no additional descriptors may be needed. However, necessary descriptions should be added in order to clarify the event or to place it in an appropriate context. The AE name should ideally be 1-3 words in length with additional description provided elsewhere on the adverse event report. A copy of the CTCAE is posted on the study website. Standardized terms from CTCAE are used the DCC and the study sponsor to categorize events for reporting to regulatory authorities using the "Medical Dictionary for Regulatory Activities" (MedDRA). In most cases, the CTCAE terms match the MedDRA coding terminology. If an appropriate term matching the AE cannot be found in the CTCAE and the preferred MedDRA term is unknown, the AE description should include a diagnosis, sign or symptom with additional information to facilitate subsequent categorizations into MedDRA coding terms.

8.5. Confidentiality Procedures

Strict confidentiality procedures ensure that information collected for the FAZST trial could not be linked to the participant's identifier except for cases where medical attention may be needed. Participants' personal information with exception of date of birth will be kept locally at each clinical center and kept separately from the data collected during the study where only the Participant ID for each participant will appear. The locator information will be kept in a secured local server with paper forms in a locked secure cabinet/area.

8.6. Quality Control Procedures

The DCC will perform ongoing checks of all data entered into the database. These will be part of the AdvantageEDC Suite (Integrity) and will include checks for discrepancy between information obtained through different forms and/or at different follow-up times. These procedures are essential for the success of the trial. Quality control processes will be established to identify any deviations and discrepancies in information obtained from various sources. Routine error and completeness reports will be sent to the clinical center and should be returned to the DCC in a timely manner to resolve identified problems and correction of errors. Concise summaries of the findings of the quality control procedures will be presented periodically on the study web-site to alert study personnel of common problems. The DCC will suggest resolutions as well as any modifications to the study protocol.

Quality control for clinical monitoring

The DCC with the NICHD will conduct annual field audits of the clinical sites to assure adherence to the study protocol and requirements. The audits will include examination of the data collection process, specimen collection and processing, data entry procedures, local data management and record keeping.

8.7. Reports and Newsletter

The DCC will be responsible for preparation and distribution of interim and final reports, annual newsletter, presentations and other publications in coordination with the Project Officers at the NICHD and in collaboration with the study investigators. Reports will include summaries of recruitment and follow-up status, by clinical site, graphical and tabular presentations and results of interim statistical analysis. The reports will have clear explanations of the methods used, interpretations and point out questions or problems that need special attention.

Special reports of AEs occurring during the course of the study will be prepared periodically and submitted to the Project Officer and the DSMB.

The annual newsletter will be made available on the study website and contain information on the progress and performance of the clinical centers.

8.8. Investigational Product

Identity of Investigational Product

The study supplement and matching placebo is supplied by UPM Pharmaceuticals Inc. Each tablet of the active study supplement contains 5 mg of folic acid and 30 mg elemental zinc (as zinc sulfate monohydrate). Inactive ingredients include silicified microcrystalline cellulose, isomalt, fumed silica, croscamellose sodium, fumed silica and magnesium stearate. The same inactive

ingredients are used to make the placebo tablets. All tablets are coated with hypromellose, polyethylene glycol, polysorbate 80 titanium dioxide for a uniform white appearance. The study supplement and placebo tablets are manufactured and tested to meet USP specifications and quality standards for nutritional and dietary supplements.

A sufficient supply of study supplement will be provided for each participant and will be sent to the study site. Study product labels will be printed with the protocol number, applicable storage conditions, lot number and the instruction to take one tablet each day. The label will also include space for participant number and visit number.

Blinding and placebo tablets

The AdvantageEDC system will be used for randomization and assignment of blinded study supplies. Matching placebo tablets will be supplied by UPM Pharmaceuticals Inc. to maintain the blinding of folic acid and zinc tablets and the placebo tablets.

If required, emergency unblinding of the participant can be completed by authorized site staff. Only a physician at the site will be authorized to unblind participants in emergent situations. In case of an emergency, the DCC should be notified before unblinding any participant. The DCC will keep a copy of the randomization code in a secure place. All unblinding events should be recorded on a protocol violation form in EDC within 24 hours of the unblinding.

Storage and Disposition of Supplies

A specified number of bottles containing 77 tablets of 5 mg folic acid and 30 mg elemental zinc or matching placebo will be shipped to the PI's designated pharmacy or other acceptable controlled storage location for inventory control and dispensing to study participants. All tablets will be stored at room temperature, in a tightly-closed container, away from heat and light, and in a locked storage area. Room temperature of 77°F is ideal; excursions permitted from 59° to 86°F (15 - 30°C). A temperature log will be kept on a weekly basis.

The dietary supplements and matching placebo products used in this study are designated specifically for and are to be used only within the context of this study under the supervision of the PI. Returned study supplies may not be re-dispensed. As specified in the Manual of Procedures, all used and expired study dietary supplements will be destroyed in accordance with the site SOP on study product destruction. Following study closeout, all other unused clinical supplies will be discarded.

Supplement Accountability

The PI, or a qualified designee, will verify that study supplies are received intact and in the correct amounts. An accurate inventory of study supplements will be kept by the site. Each site will be provided with electronic supplement accountability forms to document receipt of supplies from UPM Pharmaceuticals, Inc. Supplement dispensation and accountability will be verified by the DCC and study monitors throughout the study and at the site close out visit.

9.0 HUMAN SUBJECTS PROTECTION AND CONFIDENTIALITY PROCEDURES

This section details the issues relevant to protection of human subjects involved in the FAZST trial.

DSMB

This study will be overseen by a DSMB. The DSMB will receive tabulated data relating to safety of study participants. This evaluation will also assess data quality and timeliness, participant recruitment, accrual, and retention. These reviews will allow the DSMB to determine whether there is any change to the anticipated benefit-to-risk ratio of study participation. These reviews will also allow the DSMB to determine whether the study should: 1) continue as originally designed, 2) implement a protocol change, or 3) be terminated. If a recommendation is made to change the research study design, an adequate rationale for this decision must be provided. In addition, members of the DSMB will review data from individual study participants on a quarterly basis to evaluate the progress of the study and the safety and confidentiality of study participants. Any SAEs or breaches in confidentiality will be reviewed by the DCC and reported immediately to the DSMB for review throughout the study.

IRB review and informed consent

This protocol and the informed consent document and any subsequent modifications will be reviewed and approved by the IRB responsible for oversight of the study at each participating site. Four IRBs will review this protocol: 1) University of Utah, and 2) The Emmes Corporation 3) University of Iowa 4) Northwestern University. NICHD will be covered under a reliance agreement with the University of Utah.

The principles of informed consent in the current edition of the Declaration of Helsinki will be implemented before any protocol-specific procedures are carried out. Informed consent will be obtained in accordance with U.S. 21 CFR Part 50.2 and all other applicable regulatory requirements. A signed consent form will be obtained from the participant. The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy of the consent form will be given to the participants. Study participants will be informed he/she is free to refuse any aspect of study participation without repercussion or to withdraw at any time.

All investigators will have completed the federally required training in research ethics and will emphasize the voluntary nature of this study. Approval from the IRB will be in place prior to beginning recruitment.

Participant confidentiality

Raw data will be stored in locked cabinets in a locked office. Social security numbers may be collected for payment purposes only but will not be submitted to a central database. De-identified data will be submitted to a central database designed by the DCC. The database will be password protected. All laboratory specimens, evaluation forms, reports, and other records that leave the site will be identified only by the participant ID to maintain participant confidentiality. All records will be kept in a locked file cabinet. Clinical information will not be released without written permission of the participant, except as necessary for monitoring by the IRB, NICHD, or the NICHD's designee. Any information uncovered through the interviews that might indicate abuse or neglect requires the investigators to inform appropriate local agencies in accordance with State law. Otherwise, all information will remain confidential.

Statement regarding scientific misconduct

This study will be conducted using good clinical practice (GCP), as delineated in Guidance for Industry: E6 Good Clinical Practice Consolidated Guidance, and according to the criteria specified in this study protocol. Before study initiation, the protocol and the informed consent documents will be reviewed and approved by an appropriate IRB. Any amendments to the protocol or to the consent materials must also be approved before they are implemented.

Compliance with 42 CFR Part 93, Public Health Service (PHS) Policies on Scientific Misconduct is implicit in the application for this proposal. The academic institutions participating in this proposal have approved assurances and required renewals on file with the Office of Research Integrity (ORI) and compliance with these policies and procedures and the requirements of Part 93 are in place. The academic institutions participating in this proposal understand and abide by the definitions of research misconduct per PHS policies (fabrication, falsification, or plagiarism in proposing, performing, or reviewing research, or in reporting research results).

9.1. Regulatory Binders

9.2. Retention of Records

Each site will maintain a confidential regulatory binder that contains site-specific and study-wide documentation. The regulatory binder does not contain clinical records, CRFs or other source documents regarding individual participants. The site PI or designee is required to maintain this documentation and make it available for review by authorized study monitors, auditors and regulatory authorities. Both current and outdated study documents must be maintained. Older versions of documents may be stored elsewhere in a secure location, provided a reference to the actual storage location remains in the binder. If the binder contents become too voluminous, multiple volumes may be maintained. The regulatory binder may also be kept in electronic format; file structure will be provided by the DCC. If kept electronically, the binder must be stored on a secure network server.

The site PI is responsible for maintaining intact study records for a period of at least two years after the investigation is discontinued and the sponsor has notified any applicable regulatory authorities. Local policies for records retention may require longer periods, or the NICHD may request a longer retention period. The NICHD should inform the Investigator/Institution in writing when trial-related records are no longer needed.

9.3. Risks and Benefits

Procedure risks

Folic acid/zinc supplement and placebo: Any dietary supplement (including folic acid and zinc) can have harmful side effects, especially when mixed with other products or if the participant is allergic to folic acid or zinc. Participants who are allergic to the folic acid/zinc dietary supplements will be ineligible to participate. Research personnel will monitor participants' pill intake (including supplements and herbal remedies) through text/email reminders and the information provided in the daily journal. Folic acid usually has very few side effects, but symptoms reported with the use of folic acid include fever, general weakness or discomfort, reddened skin, shortness of breath, skin rash or itching, tightness in chest, trouble breathing, and wheezing. Although many people who take zinc do not experience side effects, nausea, vomiting and upset stomach may occur. Rare

but more severe symptoms reported with the use of zinc include fever, general weakness or discomfort, sore throat, reddened skin, shortness of breath, skin rash or itching, tightness in chest, trouble breathing, and wheezing. Although it is possible that folic acid supplementation may mask some symptoms of pernicious anemia, specifically the hematological disorder, but did not improve the associated neuropathy.[48] The neuropathy is reversible if treatment begins promptly, and the symptoms of anemia are not necessary to diagnose the deficiency and the brief length of study dietary supplement administration, 6 months, should ensure prompt treatment.

Blood Sample: This procedure is likely to involve discomfort or minimal pain as well as bruising. Rarely, blood draws may lead to clotting, infection, and fainting. Blood is drawn by certified healthcare personnel in research and/or medical facilities.

Anthropometric Measurements and vital signs: There are no anticipated physical risks associated with anthropometric measurements and the measurement of vital signs (i.e., blood pressure, pulse rate). However, there could be some discomfort and/or embarrassment when having the measurements taken. This procedure will be conducted by trained research personnel.

Urine Collection: There are no known risks associated with this procedure. This is a standard test.

Semen Collection: There are no known risks associated with this procedure. However, there could be some discomfort and/or embarrassment when providing semen samples.

Saliva Collection: There are no known risks associated with this procedure.

Toenail Collection: There are no known risks associated with this procedure.

Interview: Men will be interviewed at baseline, coinciding with initial evaluation at the fertility clinic, in order to obtain information on demographics, reproductive history, occupational history, stress, depression, medical history, and lifestyle factors that may be related to their fertility. This could be uncomfortable and or embarrassing. This interview will be conducted by trained personnel experienced in interviewing participants.

Daily journal: Recording information every day can be difficult and frustrating at times. However, the diary has been designed to collect information in a concise fashion to minimize the time spent on this component of the study. The risks associated with this procedure are minimal and include the accidental or incidental disclosure of sensitive personal information. This risk is minimized by adhering to strict confidentiality policies (see below).

Medical Records Review: The risks associated with this procedure are minimal and include the accidental or incidental disclosure of medical information. This risk is minimized by adhering to strict confidentiality policies (see below).

Benefits to participants and others

Couples enrolled in this study may not realize a direct benefit as a result of their participation. However, during the medical evaluations and interviews, information about their medical care and fertility enhancement may be discovered that may not have been without participating in this study. In this event, that information will be relayed to the couple and their referring physician so that management of these issues can be pursued.

It is possible that couples with nutritional deficiencies may have more positive fertility outcomes as a result of participation in this study. However, this cannot be guaranteed.

Information derived from this study may potentially identify whether folic acid and zinc dietary supplementation may significantly impact semen quality and fertility outcomes for many couples seeking assisted conception in the future.

Benefits and importance of the knowledge to be gained

Recent literature suggests that dietary supplementation with folic acid and zinc may be beneficial for maintaining semen quality, and perhaps, downstream fertility and pregnancy outcomes. Folic acid/zinc supplementation may be an ideal dietary supplement; it is safe, widely available, has few side effects, and is inexpensive. The possibility that these dietary supplements could have a major impact on semen quality and fertility is very important to study but there are still many unknowns. It is not clear which participants would benefit from folic acid/zinc supplementation, whether other factors that can influence semen parameters add to the effects of folic acid/zinc supplementation, and the potential side effects of this dietary supplementation. Earlier studies have only focused on specific effects of folic acid/zinc supplementation on semen parameters, and not necessarily with downstream fertility and pregnancy outcomes. As essential micronutrients in the spermatogenesis process, the effects of folic acid/zinc supplementation are expected to have an impact not only semen quality, but also may impact fertilization rates, embryo quality, implantation, and thus, increasing chance of conception.

The potential risks to the participants in this study are relatively low and are reasonable in relation to the importance of the knowledge gained. Procedures outlined in this protocol such as semen, blood and urine collection, anthropometric measurements, and interviews have minimal risk and it is not anticipated that the risks are above those in the course of a person's daily life.

Alternative procedures

Men and women may choose not to participate in this study and continue attempts at conceiving a pregnancy on their own or after consultation with a physician.

Protection of Human Subjects from Research Risks

DCC responsibilities

The DCC responsibilities to protect human subjects from research risk will focus on the information aspects. The potential risks involved in this study include clinical ones (e.g., adverse allergic reaction to the folic acid/zinc supplement), emotional ones (e.g., increased anxiety, embarrassment due to questions related to sexual behavior) and risks to privacy. The clinical sites, along with the DCC, will help develop a clear informed consent document to make sure that participants understand the study and know the risks involved, however minimal. Participants' personal information, test results and other data will be kept confidential throughout the trial and stored in secured data archives. Personal information will be kept in a locked file cabinet with limited access to specific study staff. Interviews (personal and by telephone) and specimen collection will be conducted in privacy. Personal information is never released without prior permission from participants. Locator information is stored separately from the data identified by

participants study ID number. Linking documents are kept secure by authorized staff members. Procedures to release information in case of an adverse event will be established in coordination with the Project Officers and the DSMB.

All DCC personnel will complete human subject protection training. Any harm suffered by a participant, either physical or emotional, or alleged infringement on privacy will be documented and reported to the IRB and to the DSMB.

NICHD responsibilities

The NICHD responsibilities to protect human subjects from research risk will focus on maintaining confidentiality for study participants by having access to no personally identifiable information. The NICHD will have no contact with study participants. All data that the NICHD will obtain will be de-identified. All information collected in the study will be kept strictly confidential and be used solely for research purposes. A Certificate of Confidentiality will be obtained.

All NICHD personnel will complete human subject protection training. Any harm suffered by a participant, either physical or emotional, or alleged infringement on privacy will be documented and reported to the IRB and to the DSMB.

The Division of Intramural Population Health Research, NICHD, is committed to reproducible research and fully supports the NIH data sharing (including biospecimens) policy. The Division has a protocol for requesting such information that was publicly posted in the Federal Register and approved by the NICHD. In short, potential collaborators submit a concept protocol using a standardized methodology that undergoes peer review. If found acceptable and contingent upon having the resources for the research, the Division prepares an analytic file and supporting biospecimens for distribution to the collaborating institution once IRB approval is in place at the destination. All information is de-identified, and may undergo additional security measures for ensuring confidentiality of data and participants' privacy, depending upon the research question. Every effort is afforded for the continued protection of study participants, their data and biospecimens during all aspects of research and beyond. There is no expiration of our commitment to full human subjects' protection through all phases of research including follow on studies relying on the biospecimen repository.

TCC responsibilities

The University of Utah's responsibilities to protect human subjects from research risk will focus on ensuring the informed consent, safety of participants, and confidentiality of data collection processes for its and subcontracted clinical sites.

All University of Utah personnel and personnel from subcontracted sites will complete human subject protection training from CITI or an equivalent documented training program. Any harm suffered by a participant, either physical or emotional, or alleged infringement on privacy will be documented and reported to the IRB and to the DSMB.

9.4. Study Population

Men and minorities

Couples attempting to conceive and seeking assisted conception at participating fertility clinics will be recruited for participation this study. No efforts will be made to exclude any men based on minority, racial, or ethnic status.

Participants will be requested to complete a brief questionnaire at the time of informed consent that identifies ethnicity and racial designation(s). The participant will be asked for ethnicity first and then will be able to identify one or more racial designation.
The trial is potentially open to all male partners of heterosexual couples attempting to conceive and seeking assisted conception at participating fertility clinics who fit the inclusion/exclusion criteria. The DCC will work with the clinical sites to identify potential sources of data to characterize as best as possible the race/ethnicity composition of the population to be screened for participation in the trial. Typically, trial populations do not accurately represent the target population. However, effort will be made to direct the screening to allow a heterogeneous group to be included. The DCC will generate monthly summaries to each site with the age and ethnic composition of the population screened and the group recruited. If an imbalance develops, the issue will be discussed with the clinical sites and with the project officer to come up with necessary changes in recruitment strategies to minimize exclusion of minorities for any reason. No sub-group analysis is planned but statistical modeling of the data will assess the impact of age and race/ethnicity on study endpoints.

Special populations and circumstances

The study will not be recruiting and enrolling special classes or participants such as children, prisoners, institutionalized individuals, or others who are likely to be vulnerable populations. This study does not include plans to include special conditions such as the use of recombinant DNA molecules, human embryonic germ cells, or human embryonic stem cells.

Pregnant women, fetuses, placenta

Women are excluded from enrolling in the study as partners if they are already pregnant. However, since this study is focused on improving the outcome of reproductive health, it will be necessary to follow women if they become pregnant during the course of the study. All stipulations outlined in 45 CFR 46.204 (Research Involving Pregnant Women and 45 CFR 46.206 (Research Involving, After Delivery, the Placenta, the Dead Fetus, or Fetal Material) will be followed to maintain research integrity and protection to the participants.

Exclusion if unable to consent

Although this is identified in the inclusion/exclusion above, it is important to reiterate that anyone who does not have the ability to provide effective informed consent will not be enrolled.

9.5. Confidentiality Procedures

To ensure the strict confidentiality of participants' information and data, the following procedures will be followed:

a) Participant IDs – upon recruitment, each participant will be assigned a study identification number (Participant ID). This ID will be used by project staff on all data forms, laboratory specimens and in the study's main databases. In this manner,

the identity of the participant will not be linked directly to the data collected during the study without a locator file. The assignment of ID numbers will be done by the DCC using random components that will make the sequence difficult to predict.

b) A separate locator file will have the identifying information for each participant (including name and address) linked to the Participant ID number. This file will be kept in a secure place and will only be available to the site personnel, not the DCC or sponsor.

c) Reports generated based on the collected data will not include any identifying information and thus preserve the confidentiality of the participants.

d) The only occasion where participant identity may be linked to the collected data is in case of an adverse event that requires medical intervention; these cases will be discussed with the Project Officer on a case-by-case basis.

e) The need to ensure strict confidentiality of the data collected from participants will be stressed as part of the training of the study personnel involved in data collection. The importance of preserving confidentiality as a moral duty of researchers will be explained. The data may include sensitive information about sexual history and contacts and other personal data such as medical history. The willingness of participants to give such information is based on the trust that this data will be used for the research purposes only. Therefore, it is essential that study staff understand this and protects the data collected not only through the study protocols but also information gathered through incidental observations of study participants.

f) The data collected during the study will be presented in an aggregated form as totals (means, medians, etc.) or percentages and will not include specific individual results.

g) Access to the database will be limited to authorized study staff and will be protected with a changing password.

The DCC will not have any street-level locator information in the study database. All locator information will be kept at the local clinical sites. The participant's ID number and identity will be kept in locked files in the research center of the study PIs. Only key staff and investigators will have access to the records. In order to monitor this research study, authorized representatives from the clinical sites' IRBs, and other federal agencies such as NIH (National Institutes of Health), FDA (Food and Drug Administration) and OHRP (Office of Human Research Protection), and qualified monitors from the DCC may inspect the research records. A Certificate of Confidentiality (#CC-HD-13-19) has been issued by the federal government that provides further confidentiality protection by authorizing study staff and contractors to protect the privacy of participants in this study.

10.0 APPENDICES

Appendix A: Safety Monitoring

Definitions

An **adverse event** (AE) is any untoward medical occurrence in humans, whether or not considered study supplement related which occurs during the conduct of a clinical trial. Any change in clinical

status, ECGs, routine labs, x-rays, physical examinations, etc., that is considered clinically significant by the study investigator is considered an adverse event.

Suspected adverse reaction is any adverse event for which there is a reasonable possibility that the study supplement caused the adverse event. A reasonable possibility implies that there is evidence that the study supplement caused the event.

Adverse reaction is any adverse event caused by the study supplement.

Serious Events (Serious Adverse Events, Serious Suspected Adverse Reactions or Serious Adverse Reactions)

A serious adverse event or serious suspected adverse reaction or serious adverse reaction as determined by the DSMB is any event that results in any of the following outcomes:

1. Death
2. Life-threatening AE (Life-threatening means that the study participant was, in the opinion of the investigator or sponsor, at immediate risk of death from the reaction as it occurred.)
3. Inpatient hospitalization or prolongation of existing hospitalization
4. Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5. Congenital abnormality or birth defect
6. Important medical event that may not result in one of the above outcomes, but may jeopardize the health of the study participant or require medical or surgical intervention to prevent one of the outcomes listed in the above definition of serious event.

Unexpected Adverse Event

Any adverse event, the specificity or severity of which is not consistent with the symptoms reported with the use of the study supplement or the informed consent.

AEs that can be associated with folic acid supplementation:

- Fever
- Weakness
- Reddened skin
- Shortness of breath
- Skin rash
- Itching

AEs that can be associated with zinc supplementation:

- Nausea
- Vomiting
- Upset stomach
- Fever

- Weakness
- Sore throat
- Reddened skin
- Shortness of breath
- Skin rash
- Itching
- Tightness in chest
- Trouble breathing
- Wheezing

Guidelines for assessing intensity of an adverse event

The Investigator should use the following definitions when assessing intensity of an AE:

- Mild: Transient (< 48 hours) or mild discomforts, no or minimal medical therapy or intervention required, hospitalization not necessary, no or little limitation in normal activities, nonprescription or single-use prescription therapy may be employed to relieve symptoms (e.g., aspirin for simple headache, acetaminophen with codeine for post-surgical pain). Mild adverse events may be listed as expected consequences of the therapy for any given protocol, and standard supportive measures for such an expected event do not necessarily elevate the event to a higher intensity.

- Moderate: Mild to moderate limitation in activity, some assistance may be needed; possibly none but usually minimal intervention/therapy required, hospitalization possible.

- Severe: Marked limitation in activity, some assistance usually required; medical intervention/therapy required; hospitalization possible or likely.

- Life-Threatening: Extreme limitation in activity, significant and immediate assistance required; significant medical/therapy intervention required to prevent loss of life; hospitalization, emergency treatment or hospice care probable. This grade is used when the participant was, in the view of the Investigator, at substantial risk of dying at the time of the adverse event, or it was suspected that use or continued use of the study supplements would have resulted in the participant's death. (This does not include a reaction that, had it occurred in a more serious form, might have caused death. For example, drug-induced hepatitis that resolved without evidence of hepatic failure would not be considered life-threatening even though drug-induced hepatitis can be fatal).

Guidelines for determining causality of an adverse event

The Investigator will use the following question when assessing causality of an adverse event to study supplement: Is there a reasonable possibility that the study supplement caused the event? Reasonable possibility implies that there is *evidence* that the specific adverse event observed in the specific subject is caused by the study supplement.

An affirmative answer designates the event as a suspected adverse reaction.

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