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REPRODUCTIVE PHYSIOLOGY AND DISEASE

# The relationship of plasma antioxidant levels to semen parameters: the Males, Antioxidants, and Infertility (MOXI) randomized clinical trial

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## Abstract

**Purpose** The understanding of the role of plasma antioxidant levels in male fertility in the USA is limited. In a secondary analysis of the Males, Antioxidants, and Infertility (MOXI) randomized clinical trial, we sought to determine whether serum levels of vitamin E (α-tocopherol), zinc, and selenium were correlated with semen parameters and couple fertility outcomes.

**Methods** This study is a secondary analysis of the MOXI clinical trial. The primary endpoints in this secondary analysis include semen parameters, and DNA fragmentation and clinical outcomes including pregnancy and live birth. Analyses were completed using Wilcoxon's rank-sum test and linear regression models.

**Results** At baseline, the analysis included plasma labs for vitamin E ($n=131$), selenium ($n=124$), and zinc ($n=128$). All baseline plasma values were in the normal ranges. There was no association between selenium, zinc, or vitamin E levels and semen parameters or DNA fragmentation. Baseline antioxidant levels in the male partners did not predict pregnancy or live birth among all couples. Among those randomized to placebo, baseline male antioxidant levels did not differ between those couples with live birth and those that did not conceive or have a live birth.

**Conclusions** Among men attending fertility centers in the USA, who have sufficient plasma antioxidant levels of zinc, selenium, or vitamin E, no association was observed between vitamins and semen parameters or clinical outcomes in couples with male infertility. Higher levels of antioxidants among men with circulating antioxidants in the normal range do not appear to confer benefit on semen parameters or male fertility.

**Keywords** Male infertility · Infertility · Antioxidants · Selenium · Zinc · Vitamin E · Semen parameters · Sperm DNA fragmentation · Plasma levels

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## Introduction

The relationship of specific levels of antioxidants for male fertility has yet to be elucidated in a North American population. Dietary patterns in men that include high intakes of fruits, vegetables, fish, and whole grains have been associated with better sperm quality [1, 2]. Such diets are relatively high in antioxidants which include arginine, carnitine, carotenoids, coenzyme Q10, cysteine, vitamin E, vitamin C, and the micronutrients folate, zinc, and selenium. It is not known per se if other aspects of these healthy dietary patterns lead to the improved fertility of men who consume them.

Subfertile men have been reported to have increased reactive oxidative species (ROS) and lower levels of antioxidants [3–5]. Sperm are susceptible to oxidative damage, and are generators of ROS. Pathologic conditions that have an increase in ROS include cancer, infection, varicoceles, and environmental exposures. Specifically related to fertility, ROS in semen may interfere with the acrosome reaction due to changes in lipid peroxidation affecting the sperm plasma membrane, impairing the process of fertilization [5]. These changes in the acrosome reaction may lead to decreased fertilization and clinical pregnancy rates. Furthermore, ROS can damage deoxyribonucleic acid (DNA), and if damaged DNA is transmitted to the embryo by the sperm, it could impair early embryonic development, which could decrease pregnancy rates and potentially increase pregnancy loss [6–8].

Herein, we focus on three antioxidants that have known effects on testicular and sperm function: vitamin E, zinc, and selenium. Alpha-tocopherol, the bioactive form of vitamin E, is the most potent lipid-soluble antioxidant and is known for its ability to protect cell membranes from oxidation [4, 9]., It plays a role in spermatogenesis in rats [10]. Zinc is involved in protein synthesis and DNA transcription, and is an antioxidant with a role in the development of testes and spermatogenesis [11, 12]. Selenium involved in normal spermatogenesis and is indirectly involved as an antioxidant as it increases the activity of glutathione peroxidase, an antioxidant enzyme [13, 14].

In this secondary analysis of the NIH/NICHD Cooperative Reproductive Medicine Network's Males, Antioxidants and Infertility (MOXI) randomized clinical trial, we sought to determine whether plasma levels of vitamin E (α-tocopherol), zinc, and selenium were correlated with semen parameters and couple fertility outcomes. The primary study investigated antioxidant supplementation in the subfertile male on semen parameters and couple fertility. This secondary analysis is novel as it examines the association between plasma levels of antioxidants, which likely reflect intake from diet and supplements, and male fertility. We hypothesized that higher plasma levels of the individual antioxidants would be correlated with higher pregnancy and live birth rates and semen parameters.

## Materials and methods

This study is a secondary analysis of the Males, Antioxidants, and Infertility (MOXI) clinical trial.

### MOXI clinical trial

The MOXI clinical trial was conducted by the *Eunice Kennedy Shiver* National Institute of Child Health and Human Development (NICHD) Cooperative Reproductive Medicine Network (RMN). The study design and description of the trial can be found on clinicaltrials.gov (NCT02421887) [15]. Briefly, the study included heterosexual couples with at least 12 months of male factor infertility. Females were 18 to 40 years old with ovulatory cycles, a normal uterine cavity, and at least one patent fallopian tube. Males had at least one abnormal semen parameter. Men enrolled in the trial provided a baseline blood and semen sample. They were subsequently randomized to a placebo or an antioxidant formulation containing 500 mg of vitamin C, 400 mg of vitamin E (in the form of α-tocopherol), 0.20 mg of selenium, 1000 µg of folic acid, 1000 mg of L-carnitine, 20 mg of zinc, 2000 IU of vitamin D, and 10 mg of lycopene. Men received the assigned treatment for up to 6 months. The participants returned for repeat semen sample and blood work at 3 months. Treatment continued until end of study (6 months), adverse reaction, or pregnancy was achieved. Couples timed intercourse using ovulation predictor kits for the first 3 months. In months 4 to 6 for those who had not conceived, the female partners were treated with clomiphene citrate with intrauterine inseminations.

### Plasma testing

Men provided a plasma sample at baseline prior to initiating treatment and following 3 months of treatment with the antioxidant or placebo. Male participants were instructed to fast for 12 h and abstain from alcohol for 24 h prior to their blood draw. After processing, plasma samples were stored frozen at − 80 °C per protocol and then shipped to ARUP laboratories (Salt Lake City, UT) for measurement of selenium, vitamin E α-tocopherol, and zinc. Selenium (ARUP reference number 0025023) was analyzed using quantitative inductively coupled plasma-mass spectrometry with references ranges from 23.0 to 190.0 µg/L. Zinc (ARUP reference number 0020097) was analyzed using quantitative inductively coupled plasma-mass spectrometry with references ranges from 60.0 to 120.0 µg/dL. Vitamin E (α-tocopherol)

(ARUP reference number 0080521) was analyzed using quantitative high-performance liquid chromatography with adult reference range of 5.5–18 mg/L.

### Semen testing

Semen samples were obtained at baseline prior to initiating treatment and after 3 months of treatment. Semen samples were analyzed at the local site using World Health Organization (WHO) 5 standards for volume, concentration, percent motility, and forward progressive motility. Sperm morphology was assessed using a semen smear and then shipped and analyzed at the University of Utah School of Medicine Andrology and IVF Laboratory, using standard 5th edition of the WHO Manual for analysis. A separate aliquot of semen was stored frozen and then also shipped to the University of Utah for analysis of DNA fragmentation by the sperm chromatin structure analysis (SCSA) test [16].

### Secondary analysis

All MOXI participants with at least one antioxidant level were included in this secondary analysis. The labs were drawn on all participants but the antioxidant level analysis was completed on only 75% (131 of 171) due to closure of the MOXI trial following conclusion of the pilot portion of the study. The primary outcome was semen parameters; secondary outcomes included couple pregnancy and live birth rate. The clinical outcome of live birth was defined as delivery of a live infant after 20 weeks' gestation. Pregnancy was defined by a positive home pregnancy test. The original study was designed to achieve 80% power assuming assume sperm motility would differ by 13% (95% CI:3.45–23.49%) between the antioxidant and placebo groups and DNA fragmentation would be $9.1 \pm 7.2\%$ in the antioxidant group and $22.1 \pm 7.7\%$ placebo group, a 20% dropout rate, and an $\alpha = 0.05$. [4, 17]

## Statistical analysis

Data are expressed as median (interquartile range (IQR)). Bivariate analyses were completed using Wilcoxon's rank-sum test. Linear regression models along with correlation statistics were performed to determine the correlation between antioxidant levels and semen parameters at baseline and following 3 months of treatment. All models included male: age, race, BMI, smoking history, alcohol use; and female: age, BMI; and couples: duration of infertility, previously pregnancy, annual household income, insurance coverage, and marital status as covariates. Subsequently, baseline antioxidant levels in the male partner were compared by pregnancy outcomes using Wilcoxon rank-sum testing. In a sensitivity analysis, this analysis was repeated among only those men randomized to placebo. Statistical significance was defined as a two-sided $p$ value less than 0.05. All analyses were conducted using SAS, version 9.4 (SAS Institute).

## Results

### Analytical sample

Cumulatively, there were 766 results for the three antioxidants. In the sample, we completed 383 analyses on plasma samples collected at baseline (prior to initiating supplementation) and 383 analyses on plasma samples collected 3 months after initiating antioxidants or placebo. In the primary study, the change in treatment versus placebo group was analyzed and there was a significant change in plasma levels in the treatment versus placebo groups. [12] There were no plasma levels outside the normal ARUP reference ranges for all vitamins.

### Baseline sample

There were 131 results for vitamin E, 124 results for selenium, and 128 results for zinc (75%, 72%, and 74% of 174 male original participants, respectively) at baseline. Bivariate analyses are presented in Table 1. Baseline antioxidant levels did not vary by age, ethnicity, race, BMI, or treatment group. Men with higher education level had lower selenium levels ($P = 0.001$) and men with intermediate duration of infertility had higher levels of selenium ($P = 0.03$). Social influences including current smoking, marijuana use, or alcohol use also did not affect the antioxidant levels. Antioxidant levels did not differ by marital status, insurance coverage, or household income. Male partner vitamin E, selenium, and zinc levels were not associated with any female partner characteristics (Supplemental Table 1).

### Baseline antioxidants and semen parameters

There was no correlation between baseline selenium, zinc, or α-tocopherol levels and semen parameters of concentration, motility, total motile count, or DNA fragmentation (Table 2). After treatment of 3 months, there was also no correlation between antioxidant levels and semen parameters or DNA fragmentation (Table 3).

### Baseline antioxidants and pregnancy outcomes

Baseline selenium, zinc, and vitamin E levels in the male partners did not predict pregnancy or live birth among all couples enrolled in the trial, regardless of treatment

**Table 1** Antioxidant baseline levels stratified by covariates for males from both groups. Data presented a median (interquartile range). References ranges from ARUP, selenium from 23.0 to 190.0 μg/L; zinc from 60.0 to 120.0 μg/dL; and Vitamin E (α-tocopherol) from 5.5 to 18 mg/L

| Male | Vitamin E<br>*N* = 131<br>9.5 (8.0, 10.7) | Selenium<br>*N* = 124<br>156.5 (146.0, 174.0) | Zinc<br>*N* = 128<br>88.0 (81.5, 96.0) |
|---|---|---|---|
| Age (categories) | | | |
| ≤ 30 | 9.4 (7.7, 9.8)<br>*N* = 36 | 151.0 (143.0, 173.0)<br>*N* = 35 | 88.0 (83.0, 95.0)<br>*N* = 36 |
| 31–39 | 9.6 (8.3, 11.3)<br>*N* = 72 | 161.0 (151.0, 176.0)<br>*N* = 66 | 88.0 (82.0, 96.5)<br>*N* = 68 |
| ≥ 40 | 9.6 (7.3, 11.1)<br>*N* = 23 | 150.0 (142.0, 166.0)<br>*N* = 23 | 88.0 (80.0, 94.5)<br>*N* = 24 |
| *P* values | 0.19 | 0.25 | 0.56 |
| Ethnicity | | | |
| Hispanic or Latino | 9.7 (8.1, 10.1)<br>*N* = 7 | 163.0 (146.0, 169.0)<br>*N* = 7 | 99.0 (93.0, 104.0)<br>*N* = 7 |
| Non-Hispanic | 9.4 (7.8, 10.8)<br>*N* = 120 | 156.0 (144.0, 176.0)<br>*N* = 112 | 88.0 (81.0, 95.0)<br>*N* = 116 |
| Unknown | 9.7 (9.6, 12.2)<br>*N* = 4 | 166.0 (156.0, 169.0)<br>*N* = 5 | 87.0 (82.0, 93.0)<br>*N* = 5 |
| *P* values | 0.67 | 0.91 | 0.11 |
| Race | | | |
| White | 9.5 (7.8, 10.7)<br>*N* = 103 | 157.0 (146.0, 178.0) *N* = 95 | 88.0 (81.0, 96.0)<br>*N* = 99 |
| Black | 8.7 (7.0, 11.2)<br>*N* = 10 | 148.0 (142.0, 160.0) *N* = 9 | 89.0 (81.0, 102.0)<br>*N* = 10 |
| Asian | 9.6 (9.0, 13.9)<br>*N* = 8 | 145.0 (140.0, 154.0) *N* = 9 | 86.5 (82.0, 93.0)<br>*N* = 8 |
| Other | 9.7 (9.3, 9.9)<br>*N* = 10 | 166.0 (151.0, 169.0) *N* = 11 | 93.0 (82.0, 103.0)<br>*N* = 11 |
| *P* values | 0.44 | 0.23 | 0.64 |
| BMI | | | |
| Underweight | – | – | – |
| Normal | 9.3 (8.2, 10.2)<br>*N* = 41 | 160.0 (146.0, 169.0)<br>*N* = 37 | 87.5 (79.0, 97.5)<br>*N* = 40 |
| Overweight | 9.5 (7.6, 10.6)<br>*N* = 44 | 155.0 (142.0, 170.0)<br>*N* = 43 | 87.5 (82.0, 95.0)<br>*N* = 44 |
| Obese | 9.7 (7.8, 11.1)<br>*N* = 45 | 159.0 (150.0, 181.0)<br>*N* = 43 | 89.0 (82.0, 96.0)<br>*N* = 43 |
| *P* values | 0.61 | 0.49 | 0.44 |
| Current smoking | | | |
| Yes | 9.2 (7.3, 10.1)<br>*N* = 15 | 151.5 (145.0, 169.0)<br>*N* = 14 | 87.0 (81.0, 95.0)<br>*N* = 14 |
| No | 9.5 (8.1, 10.8)<br>*N* = 116 | 157.0 (146.0, 174.0)<br>*N* = 110 | 88.0 (82.0, 96.0)<br>*N* = 114 |
| *P* values | 0.89 | 0.72 | 0.61 |
| Marijuana | | | |
| Yes | 8.3 (7.3, 10.7)<br>*N* = 6 | 164.0 (140.0, 185.0)<br>*N* = 6 | 88.0 (86.0, 88.0)<br>*N* = 5 |
| No | 9.5 (8.1, 10.6)<br>*N* = 125 | 156.0 (146.0, 174.0)<br>*N* = 118 | 88.0 (81.0, 96.0)<br>*N* = 123 |
| *P* values | 0.80 | 0.55 | 0.81 |
| Alcohol (current) | | | |
| Yes | 9.6 (8.1, 10.8)<br>*N* = 120 | 156.0 (146.0, 175.0)<br>*N* = 112 | 88.0 (81.5, 96.0)<br>*N* = 116 |
| No | 9.0 (7.8, 9.5)<br>*N* = 11 | 158.5 (147.0, 169.5)<br>*N* = 12 | 91.5 (81.0, 101.5)<br>*N* = 12 |
| *P* values | 0.13 | 0.54 | 0.35 |

**Table 1** (continued)

| Male | Vitamin E<br>*N* = 131<br>9.5 (8.0, 10.7) | Selenium<br>*N* = 124<br>156.5 (146.0, 174.0) | Zinc<br>*N* = 128<br>88.0 (81.5, 96.0) |
|---|---|---|---|
| Education level | | | |
| High school | 9.4 (7.6, 10.7)<br>*N* = 23 | 168.5 (160.5, 184.5)<br>*N* = 20 | 88.0 (82.0, 89.0)<br>*N* = 23 |
| College | 9.6 (8.3, 10.3)<br>*N* = 48 | 160.0 (150.0, 177.0)<br>*N* = 47 | 91.5 (80.0, 97.0)<br>*N* = 46 |
| Graduate | 9.6 (8.4, 12.1)<br>*N* = 34 | 151.0 (135.0, 156.0)<br>*N* = 33 | 87.5 (82.0, 95.0)<br>*N* = 34 |
| *P* values | 0.39 | 0.001 | 0.35 |
| Duration of infertility | | | |
| 12–24 months | 9.5 (8.2, 10.7)<br>*N* = 74 | 155.0 (146.0, 169.0)<br>*N* = 70 | 87.0 (81.0, 95.0)<br>*N* = 74 |
| 25–36 months | 9.7 (7.8, 11.1)<br>*N* = 22 | 169.5 (156.5, 185.5)<br>*N* = 20 | 88.0 (80.0, 97.0)<br>*N* = 21 |
| 37 + months | 9.2 (7.4, 10.5)<br>*N* = 29 | 155.5 (146.5, 173.0)<br>*N* = 28 | 88.5 (83.5, 95.5)<br>*N* = 28 |
| *P* values | 0.41 | 0.03 | 0.94 |
| Previous pregnancies | | | |
| Yes | 9.7 (7.3, 10.7)<br>*N* = 53 | 156.0 (146.0, 179.0)<br>*N* = 47 | 88.0 (82.0, 97.0)<br>*N* = 50 |
| No | 9.5 (8.4, 10.5)<br>*N* = 78 | 157.0 (145.0, 169.0)<br>*N* = 77 | 88.0 (81.0, 96.0)<br>*N* = 78 |
| *P* values | 0.59 | 0.26 | 0.79 |
| Marital status | | | |
| Married | 9.5 (7.9, 10.8)<br>*N* = 124 | 156.0 (146.0, 174.0)<br>*N* = 117 | 88.0 (82.0, 96.0)<br>*N* = 121 |
| Not married | 9.1 (8.2, 9.7)<br>*N* = 7 | 160.0 (153.0, 169.0)<br>*N* = 7 | 84.0 (70.0, 93.0)<br>*N* = 7 |
| *P* values | 0.42 | 0.72 | 0.19 |
| Annual household Income | | | |
| < 49,999 | 8.5 (8.1, 10.2)<br>*N* = 13 | 160.0 (154.0, 168.0)<br>*N* = 13 | 86.5 (82.0, 95.0)<br>*N* = 14 |
| 50,000 to 100,000 | 9.6 (7.5, 10.5)<br>*N* = 58 | 159.5 (148.0, 182.0)<br>*N* = 54 | 91.0 (81.0, 99.0)<br>*N* = 55 |
| > 100,000 | 9.6 (8.1, 11.4)<br>*N* = 48 | 155.0 (141.0, 171.0)<br>*N* = 47 | 87.0 (82.0, 93.0)<br>*N* = 47 |
| *P* values | 0.21 | 0.27 | 0.60 |
| Insurance coverage | | | |
| MCP/HMO/Private | 9.6 (8.1, 10.8)<br>*N* = 120 | 156.0 (146.0, 176.0)<br>*N* = 116 | 88.0 (82.0, 96.0)<br>*N* = 117 |
| Medicaid/Medicare | 9.5 (9.4, 9.5)<br>*N* = 2 | 165.0 (157.0, 173.0)<br>*N* = 2 | 87.5 (82.0, 93.0)<br>*N* = 2 |
| Self-pay/Uninsured | 8.2 (7.2, 10.2)<br>*N* = 7 | 146.0 (143.0, 160.0)<br>*N* = 5 | 78.0 (78.0, 88.0)<br>*N* = 7 |
| *P* values | 0.26 | 0.44 | 0.39 |
| Treatment group | | | |
| Antioxidant | 9.6 (8.1, 11.1)<br>*N* = 64 | 154.5 (143.0, 170.0)<br>*N* = 62 | 88.0 (82.0, 96.0)<br>*N* = 65 |
| Placebo | 9.5 (7.8, 10.5)<br>*N* = 67 | 160.0 (148.0, 176.0)<br>*N* = 62 | 88.0 (80.0, 96.0)<br>*N* = 63 |
| *P* values | 0.80 | 0.24 | 0.85 |

**Table 2** Correlation between antioxidant levels and semen parameters adjusting for covariates at baseline (visit 1). Adjusted for covariates: Male: age, race, BMI, smoking, marijuana, alcohol; Women: age, BMI; Couple: duration of infertility, previous pregnancy, annual household income, insurance coverage, marital status. Partial correlation statistics were used to describe the relationship between two variables when controlling for the effects of one more variables in this relationship

| Antioxidant level | Concentration | Motility | DNA fragmentation | Total motile |
|---|---|---|---|---|
| Vitamin E | 0.14 ($P=0.15$)<br>$N=110$ | 0.02 ($P=0.84$)<br>$N=110$ | 0.08 ($P=0.49$)<br>$N=99$ | 0.09 ($P=0.37$)<br>$N=110$ |
| Selenium | −0.17 ($P=0.10$)<br>$N=106$ | −0.07 ($P=0.47$)<br>$N=106$ | −0.03 ($P=0.76$)<br>$N=95$ | −0.14 ($P=0.16$)<br>$N=106$ |
| Zinc | −0.01 ($P=0.89$)<br>$N=107$ | 0.02 ($P=0.83$)<br>$N=107$ | $N=97$ | −0.03 ($P=0.77$)<br>$N=107$ |

**Table 3** Correlation between antioxidant levels and semen parameters adjusting for covariates following 3 months of treatment (visit 3). Adjusted for covariates: Male: age, race, BMI, smoking, marijuana, alcohol; Women: age, BMI; Couple: duration of infertility, previous pregnancy, annual household income, insurance coverage, marital status. Partial correlation statistics were used to describe the relationship between two variables when controlling for the effects of one more variables in this relationship

| Antioxidant level | Concentration | Motility | DNA fragmentation | Total motile |
|---|---|---|---|---|
| Vitamin E | −0.08 ($P=0.41$)<br>$N=109$ | −0.13 ($P=0.22$)<br>$N=109$ | −0.14 ($P=0.19$)<br>$N=96$ | −0.04 ($P=0.72$)<br>$N=109$ |
| Selenium | −0.10 ($P=0.32$)<br>$N=105$ | 0.03 ($P=0.75$)<br>$N=105$ | −0.18 ($P=0.11$)<br>$N=93$ | −0.09 ($P=0.38$)<br>$N=105$ |
| Zinc | −0.13 ($P=0.21$)<br>$N=107$ | 0.13 ($P=0.19$)<br>$N=107$ | −0.09 ($P=0.40$)<br>$N=94$ | −0.02 ($P=0.87$)<br>$N=107$ |

**Table 4** Among all subjects, antioxidant levels and live birth or achieved pregnancy outcomes. Data presented as median (interquartile range)

| *Levels at visit 1* | Live birth<br>$N=34$ | No live birth<br>$N=137$ | *P* value | Pregnancy<br>$N=44$ | No pregnancy<br>$N=127$ | *P* value |
|---|---|---|---|---|---|---|
| Vitamin E | 9.3 (8.1, 10.5)<br>$N=27$ | 9.5 (7.7, 10.8)<br>$N=104$ | 0.84 | 9.3 (8.0, 11.8)<br>$N=35$ | 9.5 (7.9, 10.6)<br>$N=96$ | 0.61 |
| Selenium | 155.5 (142.0, 169.0)<br>$N=26$ | 157.0 (146.0, 174.0)<br>$N=98$ | 0.60 | 155.5 (141.0, 174.0)<br>$N=34$ | 158.0 (148.0, 174.0)<br>$N=90$ | 0.32 |
| Zinc | 88.0 (80.0, 91.0)<br>$N=26$ | 88.0 (82.0, 96.0)<br>$N=102$ | 0.23 | 88.0 (80.0, 92.0)<br>$N=34$ | 88.0 (82.0, 98.0)<br>$N=94$ | 0.36 |

**Table 5** Among subjects randomized to placebo, antioxidant levels and live birth or achieved pregnancy outcomes. Data presented as median (interquartile range)

| *Levels at visit 1* | Live birth<br>$N=21$ | No live birth<br>$N=65$ | *P* value | Pregnancy<br>$N=26$ | No pregnancy<br>$N=60$ | *P* value |
|---|---|---|---|---|---|---|
| Vitamin E | 9.3 (8.4, 10.2)<br>$N=18$ | 9.5 (7.6, 10.5)<br>$N=49$ | 0.94 | 9.3 (8.4, 10.5)<br>$N=22$ | 9.5 (7.3, 10.2)<br>$N=45$ | 0.54 |
| Selenium | 163.0 (146.0, 176.0)<br>$N=17$ | 160.0 (148.0, 174.0)<br>$N=45$ | 0.78 | 166.0 (146.0, 176.0)<br>$N=21$ | 160.0 (148.0, 171.0)<br>$N=41$ | 0.95 |
| Zinc | 88.0 (87.0, 91.0)<br>$N=17$ | 87.5 (80.0, 100.0)<br>$N=46$ | 0.76 | 88.0 (81.0, 91.0)<br>$N=21$ | 88.5 (80.0, 100.0)<br>$N=42$ | 0.43 |

allocation (Table 4). Among those randomized to placebo, baseline male antioxidant levels also did not differ between those couples that conceived and had a live birth and those that did not conceive or have a live birth (Table 5).

## Discussion

Herein, we demonstrate that plasma-circulating levels of key antioxidants believed to be related to spermatogenesis did not differ by most characteristics of the sample of subfertile men. Antioxidant levels were also not correlated with semen parameters or pregnancy outcomes. This finding was true regardless of the treatment assignment of the male partner. Compared to other studies of antioxidants and male fertility, our study had relatively larger numbers as discussed below and the men at baseline had antioxidant levels in the normal range. Thus, our overall negative findings may indicate that in the absence of a plasma vitamin deficiency state, antioxidant levels do not predict semen parameters. Of note in the primary MOXI study, plasma levels of all of the antioxidants studied increased in men in the active treatment group compared to placebo, and this increase did not result in any significant changes in semen parameters. [12] This analysis was not repeated in the current report.

The majority of previous studies on antioxidants and male infertility investigated the change in clinical outcomes with the use of supplementation [15, 18, 19]. In this study, the focus was on baseline plasma levels, and not on the effect of supplementation. The MOXI trial provides an important opportunity to investigate these relationships due to the large sample size, the multicenter design of the study, and the well-characterized sample.

We found no correlation between plasma antioxidant levels of zinc or vitamin E (α-tocopherol) and baseline patient characteristics. Selenium levels varied in two different populations, which are likely not clinically significant. Men with higher education level had lower selenium levels. One previous study using NHANES 1988–1994 data found an association with high school education and higher levels of selenium, whereas another NHANES 2003–2004 did not find a relationship in selenium levels with respect to education level [20, 21]. These studies were from different years with inconsistent findings. Men with intermediate duration of infertility had higher levels of selenium compared to those with shorter and longer duration of infertility. This finding of intermediate duration of infertility of 25–36 months and selenium levels may simply be due to chance and has not been reported in previous literature.

No correlation between plasma concentrations of selenium, zinc, or vitamin E and semen parameters or DNA fragmentation both at baseline and after 3 months of treatment with antioxidant supplements or placebo were found. In prior studies, selenium levels have been associated with semen parameters; however, this was only observed in regions where selenium deficiency was present. A study, from Scotland of men from an area with diets low in selenium, found that supplementation with selenium resulting in increased serum levels could improve sperm motility [22]. In a study from Turkey, serum levels of selenium were lower in men with oligozoospermia compared to normospermia [23]. In Western diets and soil, clinically low serum selenium concentrations are not common, which may account for the lack of a correlation between selenium concentration and sperm parameters in the present study. [24]

Studies of zinc and vitamin E plasma levels in relationship to male infertility are even fewer. In agreement with our findings, a small observational study of fertile versus infertile Italian men did not find a correlation between vitamin E concentration and semen parameters, but did report a correlation with oxidative stress biomarkers in the seminal plasma samples. This same study showed that the 31 men with infertility had decreased levels of α-tocopherol compared to fertile men (22 v 17 μmol/L). [25] For zinc, a case series similarly found no association between serum levels and semen parameters [26]. Another study of 20 obese men undergoing bariatric surgery showed a positive correlation between serum zinc and progressive motility of sperm [27]. Overall, the previous studies of these three serum antioxidant levels are small and lack rigorous design to allow for broad inference to different populations.

Clinical outcomes of pregnancy or live birth were not correlated with antioxidant levels in the present study. Our findings conflict with some studies; however, there is a lack of well-designed studies tracking clinical outcomes with change in serum antioxidant status. The Italian study as stated above reported that serum α-tocopherol vitamin E concentrations were lower in 31 infertile men than 12 fertile males [25]. For selenium in the Scotland study as stated above, a study of 64 men with low selenium status, supplementation to improve selenium levels improved conception [22]. For zinc, a case series of 11 men reported serum zinc levels were lower in infertile men compared to fertile men in California [26]. Compared to previous literature, our study has a larger sample size, is performed in the USA, and baseline antioxidant levels were in the normal range. However, none of these studies specifically examined the presence or absence of a true vitamin or antioxidant deficiency as a predictor of semen parameters or outcomes.

The lack of a correlation between plasma antioxidant levels and clinical outcomes observed in the present study could be due to the fact that all men had levels in the normal range. Specifically in relationship to the reference ranges listed by the ARUP laboratory, the plasma levels for participants in MOXI are in the upper part of the normal ranges for zinc and selenium. As antioxidants play a role in spermatogenesis, it is plausible that low levels could affect clinical outcomes. However, in the USA, true deficiencies

in selenium, zinc, and vitamin E are not common. [13, 28, 29] According to ARUP laboratories, plasma and serum selenium levels can both be used to determine deficiency, so serum versus plasma measurements should not be responsible for the discrepant findings between studies.

Strengths of this study include its prospective nature and the generalizability to men with subfertility in the USA. Participants in the MOXI trial were well characterized, treatment was standardized, and live birth rates were recorded. Additionally, antioxidant concentration measurements were performed at a single site with state of the art assays. DNA fragmentation assays were also performed at a single site. Limitations of this study are that all the antioxidant levels were in the normal ranges at baseline, possibly limiting the ability to detect an effect of supplementation. A separate power analysis was not completed for this secondary analysis; however, the original study was adequately powered to detect small differences in motility and DNA fragmentation between two groups. Thus, our lack of association should not be attributed to too small of a sample size. Also, not all MOXI participants had all three of the antioxidant labs drawn, leading to lower numbers of samples analyzed for some of the antioxidants, with a commensurate reduction in study power. Additionally, the MOXI study was not designed to address the question of whether abnormal antioxidant levels affect semen parameters or fertility outcomes, which may be important in selected populations with antioxidant deficiencies. Future studies aimed at antioxidant levels and clinical fertility outcomes should include a larger population with antioxidant deficiency.

## Conclusion

Among men attending fertility centers in the USA, plasma antioxidant levels of zinc, selenium, or vitamin E are not correlated with semen parameters or clinical outcomes in couples with male infertility. Higher levels of antioxidants among men with circulating antioxidants in the normal range do not appear to confer benefit on semen parameters or male fertility.

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## Declarations

**Conflict of interest** Dr. Diamond reports other support from Advanced Reproductive Care, LLC outside the submitted work. Dr. Steiner reports support from Prima-Temp outside the submitted work.

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