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The Effect of Antioxidants on Male Factor Infertility: The MOXI Randomized Clinical Trial

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CONFLICT OF INTEREST

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Abstract

Objective—To determine if antioxidants improve male fertility, as measured by semen parameters and DNA fragmentation at 3 months and pregnancy resulting in live birth after up to 6 months of treatment, among couples with male factor infertility.

Design—Multi-center, double blind, randomized, placebo-controlled trial with an internal pilot study [Males, Antioxidants, and Infertility (MOXI)]

Setting—Nine fertility centers in the United States from December 2015 to December 2018

Patients—Men (N=174) with sperm concentration ≤15M/ml, motility ≤ 40%, normal morphology ≤4%, or DNA fragmentation >25% were eligible. Female partners were ovulatory, ≤40 years old, and had documented tubal patency.

Interventions—Males were randomly assigned to receive an antioxidant formulation (N=85) containing 500 mg of Vitamin C, 400 mg of Vitamin E, 0.20 mg of selenium, 1000 mg of L-carnitine, 20 mg of zinc, 1000 mcg of folic acid, 10 mg of lycopene daily or placebo (N=86). Males were treated for a minimum of 3 months and maximum of 6 months. Couples attempted to conceive naturally during the first 3 months and with clomiphene citrate with intrauterine insemination of the female partner in months 4 through 6.

Main Outcome Measures—The primary outcome was live birth; secondary outcomes included pregnancy within 6 months of treatment. The primary outcomes for the internal pilot were semen parameters and sperm DNA fragmentation index after 3 months of treatment.

Results—After 3 months of treatment, change in sperm concentration differed between the antioxidant group [−4.0 (−12.0, 5.7) M/ml] and placebo group [+2.4 (−9.0, 15.5) M/ml] (p=0.03). However, there were no significant differences between the two groups in change in sperm morphology, motility, or DNA fragmentation. Among the 66 oligospermic men at randomization, sperm concentration did not differ at 3 months [8.5 (4.8, 15.0) M/ml versus 15.0 (6.0, 24.0) M/ml; p=0.08] between antioxidant and control groups. Of the 75 asthenospermic men, motility did not differ at 3 months (34±16.3% versus 36.4±15.8%; p=0.53). DNA fragmentation did not differ at 3 months among the 44 men with high DNA fragmentation [29.5 (21.6, 36.5)% versus 28.0 (20.6, 36.4)%; p=0.58]. In the entire cohort, cumulative live birth did not differ at 6 months between the antioxidant and placebo groups (15% versus 24%; p=0.14).

Conclusions—Antioxidants do not improve semen parameters or DNA integrity among men with male factor infertility. Although limited by sample size, this study suggests that antioxidant treatment of the male partner does not improve *in vivo* pregnancy or live birth rate

CAPSULE

Antioxidant treatment of the male partner does not improve semen parameters, DNA integrity, or *in vivo* pregnancy rates in couples with male factor infertility.

Keywords

Antioxidants; male factor infertility; randomized controlled trial

INTRODUCTION

Antioxidants are currently being marketed to treat male factor infertility. Indeed, biologic evidence supports the hypothesis that antioxidants would improve male fertility. A variety of pathologic conditions may increase oxidative stress in semen (1–3). Oxidative stress can cause lipid peroxidation, thereby producing structural modifications to the sperm plasma membrane, which have been shown to interfere with sperm motility, the acrosome reactions, and sperm-oocyte fusion. (4) Oxidative stress may also damage the nuclear and mitochondrial genome by causing single and double DNA breaks, chemical modifications of bases, DNA crosslinks, and DNA protein crosslinks. (5) In semen, antioxidants decrease oxidative stress (6), potentially improving sperm motility and reducing DNA fragmentation (7).

Studies of supplements have tended to show an improvement in semen parameters with the use of antioxidants. Benefits of Vitamin E (8), selenium (9), N-acetylcysteine (10), carnitine (7), on sperm motility have been seen after 3 months of treatment. Unfortunately, most of these studies have been small and heterogeneous. While most studies included only infertile men, some included those with normal baseline semen parameters and some with abnormal baseline semen parameters. Treatment with Vitamin C and Vitamin E has been shown to reduce DNA fragmentation compared to placebo (11).

A recent meta-analysis concluded that antioxidant supplementation taken by subfertile males may increase the chance of live birth; however, large randomized, well-designed, placebo-controlled trials were needed. (7) A number of the included trials used antioxidants in

combination with *in vitro* fertilization; it is certainly possible that the response to antioxidants would differ with *in vivo* fertilization. In addition, the meta-analysis included trials of "substances with antioxidant properties" (myo-inositol, polyunsaturated acids, resveratrol, vitamin B, and Vitamin D). A variety of antioxidant formulations are commercially available; however, trials using antioxidant formulations are limited by sample size and by use of secondary endpoints. The Males, Antioxidants, and Infertility (MOXI) trial was designed to test the hypothesis that antioxidants would improve male fertility without the use of assisted reproductive technology (ART).

METHODS

Study design

The Males, Antioxidants, and Infertility (MOXI) clinical trial was conducted by the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD) Cooperative Reproductive Medicine Network. The Collaborative Center for Statistics in Science at Yale University served as the data coordinating center. The trial was conducted at 9 clinical sites throughout the United States.

A full description of the trial with inclusion and exclusion criteria is listed on Clinicaltrials.gov (NCT02421887). This was a multicenter, randomized clinical trial involving couples with male factor infertility. Heterosexual couples with at least 12 months of infertility were eligible. Male partners were 18 years of age or older with at least one abnormal semen parameter on a semen analysis in the preceding 6 months: sperm concentration ≤15 Million/ml (oligospermia), total motility≤40% (asthenospermia), normal morphology ≤4% (teratospermia), or DNA fragmentation ≥25%. Female partners were between 18 and 40 years of age with regular menstrual cycles, defined as 25–35 days in duration, and evidence of ovulation by biphasic basal body temperature, ovulation predictor kits, or luteal serum progesterone level ≥3 ng/ml; and a normal uterine cavity with at least one patent Fallopian tube. Women over the age of 35 had normal ovarian reserve, defined as an early follicular phase FSH ≤10 IU/L, AMH ≥1.0 ng/ml, or antral follicle count >10. Male partners were excluded if they had a sperm concentration less than 5 million/ml on the screening semen analysis or if they were taking fertility medication or testosterone. Men were required to be off all vitamins for 4 weeks prior to randomization.

Approval for the study was obtained from the University of Pennsylvania, which served as the single IRB for each site with additional local site review.(12) Written informed consent was obtained from all male and female participants.

Study Treatment

Men received a placebo or an antioxidant formulation containing 500 mg of Vitamin C (ascorbic acid), 400 mg of Vitamin E (d-alpha tocopheryl), 0.20 mg of selenium (L-selenomethionine), 1000 mg of L-carnitine, 20 mg of zinc, 1000 mcg of folic acid, 10 mg of lycopene, and 2000 IU of Vitamin D daily (IND #125753) for at least 3 months and up to 6 months. The antioxidant and placebo were purchased from and packaged by a commercial manufacturer for the study. This formulation was selected as it was commercially available

and each component at comparable doses had been previously studied in a randomized controlled trial and found to positively impact sperm structure or function and/or pregnancy rates following ART. (13) Study medications were assigned in a double-blind fashion. The randomization scheme was generated using a computer generated random number sequence in randomly varying blocks of 4 and 6 stratified by site and female age (<35 years and ≥35 years of age) with allocation 1:1 by the data coordinating center through a web-based, secured randomization service. Pill counts were conducted at each study visit to monitor compliance.

Male participants provided a semen sample on day of randomization and following 90 days of treatment. Semen analysis included standard measurements such as volume, pH, count, and motility. Semen smears were prepared from each sample and shipped to the University of Utah School of Medicine Andrology and IVF Laboratory for centralized assessment of sperm morphology using WHO 5.0 criteria. In addition, 1ml of semen was stored at −80C and subsequently shipped frozen to the Utah Andrology Laboratory for DNA fragmentation assessment using the Sperm Chromatin Structure Analysis (SCSA) test, (14) when 10 million sperm were present. A blood sample was obtained at randomization and after 3 months of treatment. The samples were shipped to ARUP laboratories, where they were analyzed for selenium, vitamin E-α Tocopherol, Vitamin E-γ Tocopherol, and Zinc.

Couples were provided with free ovulation predictor tests and instructed on timing intercourse during the first 3 months of treatment (phase 1). Couples that had not conceived after 3 months of timed intercourse received up to 3 cycles of ovarian stimulation with clomiphene citrate with intrauterine insemination (phase 2). Women who conceived were followed through pregnancy and delivery.

Outcomes

The primary outcome for the trial was live birth, defined as a delivery of a live infant after 20-weeks gestation. Secondary outcomes included pregnancy, defined as a positive home pregnancy test, within 6 months of treatment. A pre-specified, internal pilot was created to examine the effect of the antioxidant formulation on male semen parameters and DNA fragmentation at 3 months of treatment compared to controls. The protocol was designed such that if the pilot failed to reject the null hypothesis that motility and DNA fragmentation did not differ between the two treatment groups (antioxidant and placebo) after 3 months of treatment, the MOXI trial would stop enrollment.

Statistical analysis

The primary outcome was a live birth resulting from a pregnancy occurring within the 6 months of treatment. For the power analysis, a live birth rate of 35% in the antioxidant group and 25% in the placebo group with a 17% dropout was assumed. A sample size of 395 in each group would yield 80% power using a two sided chi square test with at α=0.05. For the internal pilot, we assumed 50% of the males would have low motility (<40%) at baseline. For sample size calculations we assumed that after 3 months of treatment sperm motility would differ by 13% (95% CI:3.45–23.49%)(15) between the antioxidant and placebo groups and DNA fragmentation would be 9.1±7.2% in the antioxidant group and 22.1±7.7%

placebo group.(11) Assuming a 20% dropout rate, a sample size of 60 in each group would yield ≥80% power at an α=0.05 for both outcomes.

Intention to treat analyses were performed to compare the two groups. Categorical data are reported as frequencies and percentages, and analysis conducted using chi-square analysis and Fisher's exact test where appropriate. Nonparametric data are expressed as median (interquartile range (IQR)), and bivariate analyses completed using Wilcoxon's rank-sum test. Parametric data are expressed as mean ± standard deviation; student t-tests were used for analyses. Analyses were performed with SAS, version 9.4 (SAS Institute). Statistical significance was defined as a two-sided p-value less than 0.05.

RESULTS

We prescreened 822 couples. Of the 264 couples who provided written informed consent and completed the screening, 171 were eligible and were randomly assigned to a treatment group (see Supplemental Figure 1); 144 of those couples completed the study. The frequency of dropouts did not differ significantly among study groups (21% in the antioxidant group, 11% in the placebo group, p=0.055). Adherence, defined as intake of 80% or more of study drug during phase 1, was 88% among antioxidant users and 82% among placebo users (p=0.26). Baseline characteristics are presented in Table 1 for male participants and Supplemental Table 1 for female participants. Mean (standard deviation) selenium levels at randomization were 160.3 (19.8) ug/L, mean vitamin E-α tocopherol levels 9.6 (2.7) mg/L, and mean Zinc levels 89.3 (12.1) ug/dL. Baseline characteristics were no different between the two groups, except males in the placebo group were more likely to have fathered a pregnancy in the past. Baseline semen characteristics (Table 2) were similar in the two groups, except males in the antioxidant group had a lower percentage of morphologically normal sperm.

Changes in semen parameters between baseline and month 3 of treatment are presented in Table 3. Change in sperm concentration, total sperm count, and total motile sperm count differed significantly between the two groups, with an increase in the placebo group and a decline in the antioxidant group. Change in morphology, motility, and DNA fragmentation did not differ between the two groups. Selenium, vitamin E-α tocopherol, and Zinc levels increased after 3 months of treatment in the antioxidant group but did not change in the placebo group. Vitamin E-γ tocopherol levels did not change in either group (Supplemental Table 2). (The antioxidant formulation contained vitamin E-α tocopherol and did not contain Vitamin E-γ tocopherol.)

Changes in semen parameters between baseline and month 3 of treatment between treatment groups among subgroups of men with oligospermia, asthenospermia, teratospermia, and high DNA fragmentation are presented in Table 4. Among the 66 men with oligospermia, there were no statistically significant differences in change in sperm concentration between the two groups. Of the 48 men with teratospermia, there were no significant differences in change in normal sperm morphology between the two groups. Among the 75 men with asthenospermia, there were no significant differences in change in sperm motility between the two treatment groups. There were no significant differences in change in DNA

fragmentation between the treatment groups, among the 44 men with high DNA fragmentation at baseline.

As we failed to reject the null hypothesis for the internal pilot, further enrollment in the trial was stopped based on the recommendation of the Data Safety and Monitoring Board (DSMB); all enrolled couples completed the study protocol. Fifteen percent (13/85) of the couples whose male partner received antioxidants had a live birth, compared to 24% (21/86) of those randomized to the placebo (p=0.14). Pregnancy rates in the antioxidant group and in the placebo group did not differ in phase 1 (9% versus 9%, p=0.98), when couples received no additional treatment, or in phase 2 (12% versus 21%, p=0.11), when women received ovarian stimulation with clomiphene citrate and timed intrauterine insemination. First trimester pregnancy loss did not differ between groups (22% versus 19%, p=1.0). Similar results were observed when the male participants were stratified based on baseline sperm morphology or prior pregnancy history (Supplemental Table 3). Serious adverse events were not observed among any male participants. The percentage of males who had at least one adverse event did not differ between groups (41% in antioxidant group, 40% in placebo group, p=0.83) (Supplemental Table 4). Pregnancy and live birth rates also did not differ between groups in a per protocol analysis (Supplemental Table 5).

DISCUSSION

In this randomized controlled trial of couples with male factor infertility, the use of an antioxidant combination in the male partner did not result in a significant improvement in semen parameters after 3 months of therapy, compared to placebo. Furthermore, men with asthenospermia or high DNA fragmentation did not exhibit an improvement in motility or a decrease in DNA fragmentation, as had been hypothesized. While the internal pilot was not powered to examine differences in pregnancy rates, couples whose male partner received an antioxidant were not more likely to conceive during natural cycles or with intrauterine insemination.

Treatment with an antioxidant formulation did not increase motility among the entire cohort nor in the subgroup with asthenospermia at baseline. Benefits of Vitamin C (16), selenium (9), N-acetylcysteine (10), L-carnitine (17), and Zinc (18) on sperm motility have been seen after 3 months of treatment. However, most of these studies have been small and heterogeneous. While most studies included only infertile men, some included those with normal baseline semen parameters and some with abnormal baseline semen parameters. A recent Cochrane meta-analysis by Smits et al., which included only with men with abnormal sperm, found that only N-acetylcysteine, Selenium, and Vitamin E alone improved sperm motility. (7) Given the degree of heterogeneity, pooling of all antioxidant results was not possible. However, the Cochrane meta-analysis did find a 12% absolute increase in motility in men treated with combination antioxidants for 3 months compared to controls. (7) A trial by Raigani et al of 84 men with oligoasthenoteratospermia using a combination of folic acid and zinc for 14 weeks found no improvement in sperm motility after 14 weeks of therapy, (19) similar to our findings.

Treatment with an antioxidant formulation did not decrease DNA fragmentation as measured by the SCSA among the entire cohort or among men with high DNA fragmentation at baseline. Only a few clinical trials have compared sperm DNA fragmentation between those treated with and without antioxidants. Greco et. al enrolled 64 men with DNA fragmentation levels >15%, as measured using Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay. (11) After two months of treatment with vitamin E and Vitamin C, the DNA fragmentation index decreased from 22 to 9%, with no change noted in the placebo group. Although the DNA fragmentation levels were no different in our cohort at baseline, we did not observe a similar reduction over 3 months, despite using a combination that included both vitamin E and C. Our cohort was over twice the size, and we used SCSA, not TUNEL, to quantify DNA fragmentation. As we noted in men with high DNA fragmentation (>25%), Stenqvist et al also found no improvement in DNA fragmentation as measured by SCSA after 6 months of therapy with an antioxidant combination. (20) The recent Cochrane meta-analysis, which included 5 trials with a variety of antioxidants with a total of 254 subjects, found that men treated with antioxidants had on average 5% lower DNA fragmentation, but the confidence interval was broad and crossed 0. (7) Taken together with previous findings, our results indicate that while antioxidants may reduce ROS, this does not appear to translate into reduced sperm DNA fragmentation.

Couples in which the male partner was treated with antioxidants were not more likely to have a pregnancy resulting in a live birth in the first 3 months of treatment with timed intercourse, nor in the second 3 months of treatment with ovarian stimulation with intrauterine insemination. Antioxidants did not improve pregnancy or live birth rates. Given that the trial also found no improvement in semen parameters, the DSMB concluded that continuing the trial in light of the lack of response was not justifiable. The trial stopping rule had the strong underlying hypothesis that the effect of the intervention on live births is (at least partially) mediated through improvements in sperm motility and a reduction in sperm DNA fragmentation. The internal pilot study was designed to provide further evidence that antioxidants could improve semen quality, in order to justify an investment in a trial of sufficient magnitude to study the outcome of live birth. However, conventional semen quality parameters and even sperm DNA fragmentation are, at best, modest predictors of a couple’s fertility when trying with or without medical assistance.

The recent Cochrane Review found that antioxidant use increased the odds of pregnancy by 2.97 fold and the odd of live birth by 1.8 fold. (7) The meta-analysis included nine studies of 6 antioxidant or antioxidant combinations for a total of 750 participants in the live birth analysis. Two of the trials, which strongly favored antioxidants, were in couples undergoing IVF. (21, 22) Follow up in the natural conception trials was not systematic. (23, 24) In the Omu trial, the couples were followed for 6 months *after* cessation of antioxidant therapy. (24) Similar to the MOXI trial, the high quality trials included in the Cochrane review did not find a benefit to antioxidants on live birth. (25, 26)

Our negative findings contradict the overall conclusions from the Cochrane Review and meta-analysis. This could be due to many factors. Henkel et al. suggest that excessive use of antioxidants may upset the balance between oxidation and reduction, leading to reductive stress. (27) Although a theoretical concern, the antioxidant formulation used in this study did

not include excessive amounts of any given antioxidant. Doses aligned with those in prior trials.

The antioxidant formulation was selected based on input from the steering committee, advisory board, and data safety monitoring board. While one or two individual antioxidants could have been selected for study, a combination formulation was selected as 1) there are multiple antioxidants, 2) antioxidant formulations are being marketed and prescribed, and 3) there was no single “superior” antioxidant. A commercially available antioxidant formulation was selected to reduce the potential for opposing effects of antioxidants, reductive stress due to excessive antioxidants, or poor or impure product selection. Unfortunately, the design of the MOXI trial does not allow the differentiation of effects of individual nutrients and inherently assumes namely that there are no interacting effects between the different antioxidants in the formulation. Since this assumption may not be true, future randomized controlled trials could study individual components through a factorial design.

Another theoretical concern is that we selected patients who would be unlikely to benefit from antioxidants. For example, only men with elevated ROS should have been included. However, this is not how antioxidants are currently marketed and prescribed. Our inclusion criteria were similar if not more selective compared to prior trials. We also evaluated subgroups who were more likely to have ROS damage, those with asthenospermia and high DNA fragmentation, and did not see any evidence of benefit.

This multi-site, randomized, double-blind, placebo-controlled trial was designed with adequate power to determine the extent to which antioxidants improve semen parameters and DNA fragmentation. Prior trials have been small and of low or very low quality. (13) All males enrolled in the MOXI trial had male factor infertility, with at least one abnormal semen parameter and a partner with normal fertility testing. Plasma vitamin levels confirm that men in the antioxidant group complied with the regimen, and men randomized to placebo did not crossover. The trial was powered to examine changes in semen parameters in the entire cohort and in subgroups with specific sperm abnormalities. While not powered to determine group differences in live birth, it is the largest, appropriately designed trial to date to examine the impact of antioxidant treatment in the male partner on subsequent non-ART outcomes, showing no increase in live birth either with timed intercourse or with intrauterine insemination. Future studies may seek to determine if there are sub-populations (e.g. men with low vitamin levels, men with high levels of reactive oxygen species in their semen) for which antioxidants may improve semen parameters. Larger trials are needed to examine live birth as an outcome.

CONCLUSIONS

Antioxidant treatment does not improve semen parameters or DNA integrity in infertile males. Although limited by sample size, this study suggests that combination antioxidant treatment of the male partner does not improve in vivo pregnancy or live birth rates in couples with male factor infertility, but larger trials are needed to confirm this finding.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Wang A, Fanning L, Anderson DJ, Loughlin KR. Generation of reactive oxygen species by leukocytes and sperm following exposure to urogenital tract infection. Arch Androl 1997;39:11–7. [PubMed: 9202828]
2. Agarwal A, Prabakaran S, Allamaneni SS. Relationship between oxidative stress, varicocele and infertility: a meta-analysis. Reprod Biomed Online 2006;12:630–3.
3. Saleh RA, Agarwal A, Sharma RK, Nelson DR, Thomas AJ Jr. Effect of cigarette smoking on levels of seminal oxidative stress in infertile men: a prospective study. Fertil Steril 2002;78:491–9. [PubMed: 12215323]
4. Aitken RJ. Generation of reactive oxygen species, lipid peroxidation, and human sperm function. Biology of reproduction 1989;41:183–97. [PubMed: 2553141]
5. Gharagozloo P, Aitken RJ. The role of sperm oxidative stress in male infertility and the significance of oral antioxidant therapy. Human Reproduction;26:1628–40.
6. Song GJ, Norkus EP, Lewis V. Relationship between seminal ascorbic acid and sperm DNA integrity in infertile men. Int J Androl 2006;29:569–75. [PubMed: 17121654]
7. Smits RM, Mackenzie-Proctor R, Yazdani A, Stankiewicz MT, Jordan V, Showell MG. Antioxidants for male subfertility. Cochrane Database Syst Rev 2019;3:CD007411. [PubMed: 30866036]
8. Ener K, Aldemir M, Isik E, Okulu E, Ozcan MF, Ugurlu M et al. The impact of vitamin E supplementation on semen parameters and pregnancy rates after varicocelectomy: a randomised controlled study. Andrologia 2016;48:829–34. [PubMed: 26780969]
9. Scott R, MacPherson A, Yates RW, Hussain B, Dixon J. The effect of oral selenium supplementation on human sperm motility. Br J Urol 1998;82:76–80. [PubMed: 9698665]

10. Ciftci H, Verit A, Savas M, Yeni E, Erel O. Effects of N-acetylcysteine on semen parameters and oxidative/antioxidant status. Urology 2009;74:73–6. [PubMed: 19428083]
11. Greco E Reduction of the incidence of sperm DNA fragmentation by oral antioxidant treatment. Journal of andrology 2005;26:349–53. [PubMed: 15867002]
12. Diamond MP, Eisenberg E, Huang H, Coutifaris C, Legro RS, Hansen KR et al. The efficiency of single institutional review board review in National Institute of Child Health and Human Development Cooperative Reproductive Medicine Network-initiated clinical trials. Clin Trials 2019;16:3–10. [PubMed: 30354458]
13. Showell MG, Mackenzie-Proctor R, Brown J, Yazdani A, Stankiewicz MT, Hart RJ. Antioxidants for male subfertility. Cochrane Database Syst Rev 2014:CD007411. [PubMed: 25504418]
14. Evenson DP, Larson KL, Jost LK. Sperm chromatin structure assay: its clinical use for detecting sperm DNA fragmentation in male infertility and comparisons with other techniques. J Androl 2002;23:25–43. [PubMed: 11780920]
15. Showell MG, Brown J, Yazdani A, Stankiewicz MT, Hart RJ. Antioxidants for male subfertility. Cochrane Database Syst Rev:CD007411.
16. Dawson EB, Harris WA, Powell LC. Relationship between ascorbic acid and male fertility. World Rev Nutr Diet 1990;62:1–26. [PubMed: 2180213]
17. Peivandi S, Abasali K, Narges M. Effects of L-carnitine on infertile men's spermogram; a randomised clinical trial. Journal of Reproduction and Infertility 2010; 10:331.
18. Omu AE, Al-Azemi MK, Kehinde EO, Anim JT, Oriowo MA, Mathew TC. Indications of the mechanisms involved in improved sperm parameters by zinc therapy. Med Princ Pract 2008;17:108–16. [PubMed: 18287793]
19. Raigani M, Yaghmaei B, Amirjannti N, Lakpour N, Akhondi MM, Zeraati H et al. The micronutrient supplements, zinc sulphate and folic acid, did not ameliorate sperm functional parameters in oligoasthenoteratozoospermic men. Andrologia.
20. Stenqvist A, Oleszczuk K, Leijonhufvud I, Giwercman A. Impact of antioxidant treatment on DNA fragmentation index: a double-blind placebo-controlled randomized trial. Andrology 2018;6:811–6. [PubMed: 30298673]
21. Kessopoulou E, Powers HJ, Sharma KK, Pearson MJ, Russell JM, Cooke ID et al. A double-blind randomized placebo cross-over controlled trial using the antioxidant vitamin E to treat reactive oxygen species associated male infertility. Fertil Steril 1995;64:825–31. [PubMed: 7672157]
22. Tremellen K, Miari G, Froiland D, Thompson J. A randomised control trial examining the effect of an antioxidant (Menevit) on pregnancy outcome during IVF-ICSI treatment. Aust N Z J Obstet Gynaecol 2007;47:216–21. [PubMed: 17550489]
23. Suleiman SA, Ali ME, Zaki ZM, el-Malik EM, Nasr MA. Lipid peroxidation and human sperm motility: protective role of vitamin E. J Androl 1996;17:530–7. [PubMed: 8957697]
24. Omu AE, Dashti H, Al-Othman S. Treatment of asthenozoospermia with zinc sulphate: andrological, immunological and obstetric outcome. Eur J Obstet Gynecol Reprod Biol 1998;79:179–84. [PubMed: 9720838]
25. Balercia G Placebo-controlled double-blind randomized trial on the use of L-carnitine, L-acetylcarnitine, or combined L-carnitine and L-acetylcarnitine in men with idiopathic asthenozoospermia. Fertility and sterility 2005;84:662–71. [PubMed: 16169400]
26. Blomberg Jensen M, Lawaetz JG, Petersen JH, Juul A, Jorgensen N. Effects of Vitamin D Supplementation on Semen Quality, Reproductive Hormones, and Live Birth Rate: A Randomized Clinical Trial. J Clin Endocrinol Metab 2018;103:870–81. [PubMed: 29126319]
27. Henkel R, Sandhu IS, Agarwal A. The excessive use of antioxidant therapy: A possible cause of male infertility? Andrologia 2019;51:e13162. [PubMed: 30259539]

Table 1.
Characteristics at screening for all enrolled male subjects.

Data are presented as the number (%) or median (interquartile range).

	Antioxidants (n=85)	Placebo (n=86)
Age (years)	34.0 (30.0, 38.0)	34.0 (30.0, 37.0)
Body mass index (kg/m^2)	27.8 (24.2, 31.7), n=82	27.6 (24.4, 31.0)
Ethnicity		
Hispanic or Latino	7 (8.2)	5 (5.8)
Non-Hispanic	72 (84.7)	78 (90.7)
Unknown	6 (7.1)	3 (3.5)
Race		
White	63 (74.1)	69 (80.2)
Black	6 (7.1)	7 (8.1)
Asian	7 (8.2)	2 (2.3)
American Indian or Alaska Native	1 (12)	1 (12)
Unknown	8 (9.4)	5 (5.8)
Mixed Race	0 (0)	2 (2.3)
Abnormal semen parameters		
Single abnormal parameter		
Sperm concentration ≤15 Million/ml	4 (4.7)	5 (5.8)
Total motility ≤40%	9 (10.6)	10 (11.6)
Normal morphology[#] ≤4%	33 (38.8)	29 (33.7)
>1 abnormal parameters	39 (45.9)	42 (48.8)
Fathered a prior pregnancy[^]		
Yes	25 (29.4)	38 (44.2)
No	60 (70.6)	48 (55.8)
Prior infertility treatment and/or surgery		
Yes	25 (29.4)	24 (27.9)
No	60 (70.6)	62 (72.1)
Duration of infertility (months)	24.0 (18.0, 48.0), n=81	24.0 (15.0, 36.0), n=83
History of smoking		
Never	54 (63.5)	47 (54.7)
Current	8 (9.4)	11 (12.8)
Former	23 (27.1)	28 (32.6)
History of alcohol use		
Never	6 (7.1)	4 (4.7)
Current (in the past year)	72 (84.7)	81 (94.2)
Former (not in the past year)	7 (8.2)	1 (12)

[#]WHO 5th criteria.

[^]$p<0.05$, Wilcoxon's rank-sum test was used for the continuous variables, and Chi-square or Fisher's exact test was used for categorical variables. Wilcoxon's rank-sum test was used to test the distributional difference, instead of mean or median of the two groups

Table 2.

Semen parameters at randomization.

Data are presented as median (interquartile range) or mean ± standard deviation or number (%).

Parameters	Antioxidants (n=85)	Placebo (n=86)
Sperm concentration (million/ml)	21.0 (11.0, 41.2)	16.7 (10.4, 42.0)
Sperm concentration ≤ 15 million/ml	31 (36.5)	39 (45.4)
Normal morphology (%)^	4.0 (2.0, 8.0), n=63	6.0 (3.0, 10.0), n=63
Normal morphology ≤ 4%^	33 (52.4)	19 (30.2)
Total motility (%)	44.9 ± 17.3	43.0 ± 15.7
Total motility ≤40%	36 (42.4)	43 (50.0)
DNA fragmentation (SCSA, DNA fragmentation index) (%)	18.7 (14.3, 28.3), n=73	21.1 (14.1, 28.6), n=74
DNA fragmentation > 25%	23 (31.5)	26 (35.1)
Total sperm count (million)	47.6 (24.7, 84.0)	53.4 (26.4, 90.0)
Total motile sperm count (million)	20.7 (7.4, 44.5)	23.4 (8.6, 46.7)

^ $p<0.05$.

Student's t test or Wilcoxon's rank-sum test was used for continuous variables; Chi-square test was used for categorical variables.

Table 3.

Change in semen parameters from baseline to month 3.

Data are presented as median (interquartile range) or mean ± standard deviation (SD).

Parameters	Antioxidants	Placebo	P value for comparison of change between Antioxidants and Placebo groups*
Sperm concentration (million/ml)	−4.0 (−12.0, 5.7)^, n=82	2.4 (−9.0, 15.5), n=82	0.029
Normal morphology (%)	0 (−2.0, 1.0), n=55	0 (−2.0, 1.0), n=55	0.470
Total motility (%)	−1.6 ± 16.0, n=82	−1.1 ± 13.7, n=82	0.822
DNA fragmentation (SCSA, DNA fragmentation index) (%)	0.8 (−3.4, 3.8), n=65	0.2 (−5.7, 6.4), n=70	0.548
Total sperm count (million)	−10.6 (−32.5, 12.6)a, n=82	1.6 (−21.8, 42.9), n=82	0.021
Total motile sperm count (million)	−4.0 (−13.2, 9.9), n=82	1.5 (−11.8, 15.4), n=82	0.043

*Student's t test or Wilcoxon's rank-sum test was used.

^Significant change ($p<0.05$) from baseline to month 3.

Table 4.

Semen parameters from baseline to month 3 by subgroup of sperm abnormality at baseline.

Data presented as median (interquartile range) or mean ± SD.

Groups (non-exclusive)	N	Value at visit 3 Antioxidants group	Value at visit 3 Placebo group	Change of parameter in Antioxidants group	Change of parameter in Placebo group	P value for comparison of change between Antioxidants and Placebo groups*
Sperm concentration ≤15 million/ml at baseline	66	8.5 (4.8, 15.0), n=31	15.0 (6.0, 24.0), n=35	3.0 (−3.0, 9.0)^, n=31	7.0 (−2.0, 14.9)^^, n=35	0.298
Normal morphology ≤4% at baseline	48	2.0 (1.0, 5.0), n=30	2.5 (2.0, 4.0), n=18	0 (−1.0, 2.0), n=30	0.3 (−1.0, 2.0)^, n=18	0.863
Total motility ≤40% at baseline	75	34.0 ± 16.3, n=35	36.4 ± 15.8, n=40	5.1 ±16.1, n=35	5.1 ± 13.9^, n=40	0.929
DNA fragmentation (SCSA, DNA fragmentation index) >25% at baseline	44	29.5 (21.6, 36.5), n=19	28.0 (20.6, 36.4), n=25	−2.0 (−6.6, 3.7), n=19	−6.5 (−12.5, 0.7)^, n=25	0.197

*Student's t test or Wilcoxon's rank-sum test was used.

^significant change ($p<0.05$) from baseline to month 3.

^^Significant change ($p<0.01$) from baseline to month 3.