

# Effect of vitamin E administered to men in infertile couples on sperm and assisted reproduction outcomes: a double-blind randomized study

Roberto Matorras, M.D., Ph.D.,<sup>a,b,c</sup> Jairo Pérez-Sanz, Ph.D.,<sup>a</sup> Beatriz Corcóstequi, Ph.D.,<sup>a</sup> Irantzu Pérez-Ruiz, Ph.D.,<sup>a</sup> Iker Malaina, B.S., Ph.D.,<sup>d</sup> Sara Quevedo, B.S.,<sup>a</sup> Fermín Aspichueta, Ph.D.,<sup>a</sup> Lorena Crisol, Ph.D.,<sup>a</sup> Lorea Martínez-Indart, B.S.,<sup>e</sup> Begoña Prieto, M.D., PhD,<sup>a,b,c</sup> and Antonia Expósito, Ph.D.<sup>a</sup>

<sup>a</sup> Reproduction Unit, Cruces Hospital, Biocruces, Baracaldo; <sup>b</sup> Department of Obstetrics and Gynecology, University of the Basque Country, Lejona; <sup>c</sup> IVI Bilbao, Lejona; <sup>d</sup> Applied Mathematics, Statistics, and Operative Research Department, University of the Basque Country, Biocruces, Lejona; and <sup>e</sup> Clinic Epidemiological Unit, Cruces Hospital, Biocruces, Baracaldo, Spain

**Objective:** To evaluate the influence on sperm parameters and in vitro fertilization (IVF) outcomes of the administration of 400 mg/day of vitamin E for 3 months to men from infertile couples who are undergoing IVF.

**Design:** Double-blind, placebo-controlled, randomized study.

**Setting:** Human reproduction unit of a university hospital.

**Patient(s):** A total of 101 couples, 50 in the vitamin E group and 51 in the placebo group, undergoing IVF, among whom 64.4% of cases had an abnormal spermogram according to World Health Organization (WHO) criteria.

**Intervention(s):** Vitamin E ( $\alpha$ -tocopherol), 400 mg daily by mouth for 3 months, with sperm analysis performed immediately before starting the treatment and 3 months later on the day of IVF.

**Main Outcome Measure(s):** WHO sperm parameters and IVF outcomes.

**Result(s):** Although there was a statistically significant increase in progressive motility in the vitamin E group compared with before-treatment values, a similar increase occurred in the placebo group. Normal morphology was even better in the placebo group. Regarding IVF outcomes, better fertilization rates were observed in the placebo group, but the live-birth rate per transfer was statistically significantly higher in the vitamin E group: 17 (41.46%) of 41 versus 9 (20.46%) of 44 in the placebo group. Although the clinical pregnancy rates (both per transfer and per cycle started) and the implantation rate were somewhat higher in the vitamin E group (43.9% and 25%; 36.0% and 22.0%; and 24.7% and 14.1%, respectively), the increase was not statistically significant.

**Conclusion(s):** The effect of vitamin E on classic sperm parameters was not an improvement over placebo. Nonetheless, vitamin E administration was associated with a statistically significantly higher live-birth rate, and there was a trend toward better results in other IVF parameters.

**Clinical Trial Registration Number:** Eudra CT 2007-000960-25. (Fertil Steril Rep® 2020;1:219-26. ©2020 by American Society for Reproductive Medicine.)

**Key Words:** Fertilization rate, pregnancy rate, ROS, sperm parameters, vitamin E

**Discuss:** You can discuss this article with its authors and other readers at <https://www.fertstertdialog.com/posts/xfre-d-20-00117>

**M**ale factor infertility affects nearly 50% of infertile couples (1). Although basic semen analysis according to World Health Organization (WHO) criteria is

the cornerstone of the evaluation of male infertility, it has limitations as a predictor of fertility status (2). Several other tests, such as DNA fragmentation and reactive oxygen species (ROS) mea-

surement, yield abnormal results even in sperm considered normal by WHO criteria (3-5).

In 30% to 80% of cases of male subfertility, oxidative stress (OS) may

Received June 10, 2020; revised August 25, 2020; accepted September 13, 2020.

R.M. has nothing to disclose. J.P.-S. has nothing to disclose. B.C. has nothing to disclose. I.P.-R. has nothing to disclose. I.M. has nothing to disclose. S.Q. has nothing to disclose. F.A. has nothing to disclose. L.C. has nothing to disclose. L.M.-I. has nothing to disclose. B.P. has nothing to disclose. A.E. has nothing to disclose.

Partially supported by a grant from the Department of Health of the Basque Government (code URH 01-07, 2008).

Reprint requests: Irantzu Pérez-Ruiz, Ph.D., Plaza Santa Ana N2 4A, Getxo, 48930 Vizcaya, Spain (E-mail: [irantzuperez0@gmail.com](mailto:irantzuperez0@gmail.com)).

Fertil Steril Rep® Vol. 1, No. 3, December 2020 2666-3341

© 2020 The Authors. Published by Elsevier Inc. on behalf of American Society for Reproductive Medicine. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

<https://doi.org/10.1016/j.xfre.2020.09.006>

be the cause of decline in sperm quality (6). Human spermatozoa are redox active cells capable of generating the small amount of ROS that are needed for sperm maturation, capacitation, hyperactivation, and acrosome reaction as well as sperm-oocyte fusion (7). However, excessive production of ROS results in DNA damage, reduces motility, negatively affects membrane integrity, and reduces sperm-oocyte fusion ability (7, 8).

To counteract OS, seminal plasma and spermatozoa themselves have a number of antioxidant systems and substances, among them vitamin E (9–11). It has been suggested that antioxidants be used to treat male infertility. Nonetheless, there could be some concern because an excess of antioxidants might not be beneficial because an adequate balance between oxidative and antioxidative systems is needed for optimal performance of biological functions (12, 13).

Previous studies on antioxidant treatment of male factor infertility are very heterogeneous in terms of population characteristics (normal population, infertile men, men who are participating in in vitro fertilization [IVF]), the antioxidant(s) employed, and the concomitant therapies (IVF, intrauterine insemination, ovarian stimulation, natural intercourse). In a meta-analysis performed in 2019, which included data from the work we report here (which was in process at the time), 61 randomized studies were included (6,264 subfertile males). It was concluded that antioxidant supplementation in subfertile males may improve live-birth rates for couples attending fertility clinics (14).

One of the best-known antioxidants is vitamin E. This vitamin has eight isoforms. Of them,  $\alpha$ -tocopherol has the highest in vivo bioactivity and is the only one that is essential in humans. It is considered the most important lipophilic antioxidant in vivo—in humans in particular—metabolizing peroxyl radicals (15, 16). Given these characteristics, it has been employed for treating and/or preventing various conditions (16).

Several points should be highlighted first. [1] The assessment of OS is complex and many different ROS and antioxidant systems could be analyzed (17, 18). [2] There is currently no consensus concerning the best method to measure OS in clinical settings (19). [3] In men with normal semen characteristics who are part of couples experiencing unexplained infertility, the role of OS is not well defined (19). [4] There is controversy concerning the relationship of some OS markers with sperm parameters in assisted reproduction technology (ART) (20). [5] Oxidative stress may be circumvented by ART (20). In this context, our study with couples undergoing IVF and intracytoplasmic sperm injection (ICSI) assessed whether vitamin E treatment of men—regardless of their diagnosis—could increase pregnancy rates.

## MATERIALS AND METHODS

### Population

For a 2-year period (from March 2012 to June 2014), couples presenting at the reproduction unit of our university hospital to undergo IVF-ICSI treatment were invited to participate in our study. The inclusion criteria were [1] woman's age of

18–40 years; [2] infertility duration >2 years; [3] fewer than two previous IVF-ICSI cycles; [4] antral follicle count of >6 and/or antimüllerian hormone level of >0.4 ng/mL; [5] no contraindications to IVF or pregnancy; [6] no use of donor sperm; [7] use of own fresh oocytes; [8] woman's body mass index (BMI) <36 kg/m<sup>2</sup> because this is the general criterion for inclusion in our public health system; [9] absence of uterine abnormalities or hydrosalpinges; [10] man's age of 18–50 years; and [11] man's BMI <45 kg/m<sup>2</sup>. The exclusion criteria were [1] certain conditions in the man (endocrinologic or cardiovascular conditions, oncologic infective condition, or psychiatric disorders), [2] illicit drug use, [3] azoospermia, [4] abnormal karyotype, [5] preimplantational genetic diagnosis cycles, [6] donor oocyte cycles, and [7] testicular biopsy cycles.

### Study design

Once the indication for IVF-ICSI had been established based on sperm analysis, medical records, and ancillary tests, couples were invited to participate in the study. If the couples agreed and gave written informed consent, they were randomized into one to two groups: [1] a vitamin E group or [2] a placebo group. The groups began to take either vitamin E ( $\alpha$ -tocopherol) at 400 mg/day or identical placebo capsules from 3 months before the expected date of oocyte pickup (OPU). The vitamin treatment was started 90 days before the scheduled day of OPU because the spermatogenesis cycle takes approximately 72 days (21).

Randomization was performed using a sealed opaque envelope system. The study was double-blinded (for couples and the team of clinicians and biologists involved in the research). The blind was not broken until the completion of the study. This study was approved by Cruces Hospital's institutional review board (CEIC-07/17) and was registered in Eudra CT (ref. 2007–000960–25). It was partially funded by a public grant from the Health Department of the Basque Country and received no funding from pharmaceutical companies.

### Sample size calculation

The main objective was to ascertain an increase in the sperm concentration. Accepting an alpha risk of 0.05 and a beta risk of 0.2 in a two-sided test, 102 couples (51 in each group) were required to detect a difference greater than or equal to 10 million spermatozoa/mL as statistically significant. The common standard deviation was assumed to be 18 million spermatozoa/mL (based on our previous data) and the dropout rate 0. The secondary outcomes were the remaining WHO sperm parameters as well as IVF outcomes (fertilization, implantation, and pregnancy rates), total pregnancy rates (including natural cycle conceptions), and adverse effects.

### Patient selection

Among the 160 couples assessed for eligibility, 113 met selection criteria for enrollment in this study. Of these, 55 were randomized to vitamin E and 58 to placebo. The main demographic characteristics of the population included are summarized in Table 1. The most common indications for IVF were

TABLE 1

Population characteristics before starting treatment (vitamin E or placebo) in patients eventually undergoing in vitro fertilization.

Characteristic	Study group		P value
	Placebo (n = 51)	Vitamin E (n = 50)	
Man's age (y)	37.2 ± 3.8	37.8 ± 3.3	.449
Woman's age (y)	35.9 ± 2.2	35.2 ± 2.7	.310
Woman >35 y (%)	56.9 (29/51)	52 (26/50)	.6914
Man smoking (%)	23.5 (12/51)	28.0 (14/50)	.692
Woman smoking (%)	41.2 (21/51)	40.0 (20/50)	.9042
First IVF cycle (%)	50.98 (26/51)	48.0 (24/50)	.8431
Main IVF indications (%) <sup>a</sup>			
Male infertility	60.8 (31/51)	68 (34/50)	.449
Idiopathic infertility	23.5 (12/51)	20 (10/50)	.6675
Tubal factor	11.8 (6/51)	8 (4/50)	.5265
Endometriosis	9.8 (5/51)	8 (4/50)	.7504
Main male diagnosis (%)			
Normozoospermia	39.2 (20/51)	32 (16/50)	.449
Oligozoospermia	9.8 (5/51)	24 (12/50)	.05659
Asthenozoospermia	15.7 (8/51)	8 (4/50)	.2326
Teratozoospermia	35.3 (18/51)	36 (18/50)	.941
Days of abstinence	3.6 ± 1.3	3.6 ± 1.7	.452
Median (min–max)	3 (1–6)	3 (1–7)	
Semen volume (mL)	3.3 ± 1.6	3.2 ± 1.4	.750
Median (min–max)	2 (1–8)	3 (1–8)	
Total sperm count (millions)	186.3 ± 168.3	185.8 ± 167.5	.7988
Median (min–max)	135 (5–600)	150 (1–570)	
Concentration (10 <sup>6</sup> /mL)	60 ± 37.3	59.5 ± 42.3	.7274
Median (min–max)	50 (2–120)	60 (1–120)	
Motility grade A+B (%)	49.7 ± 14.5	52.5 ± 14.8	.4090
Median (min–max)	55 (15–70)	56 (20–75)	
Normal morphology (%)	3.8 ± 2.3	3.7 ± 2.6	.7033
Median (min–max)	4 (0–10)	3 (0–10)	

Note: Values are mean ± standard deviation unless otherwise indicated. IVF = in vitro fertilization.

<sup>a</sup> Totalling >100%.

Matorras. Vitamin E administration and ART outcome. *Fertil Steril Rep* 2020.

male factor infertility (65.5%), tubal factor infertility (13.5%), endometriosis (8.8%), and idiopathic infertility (22.1%). The most common sperm diagnoses were normozoospermia (34.5%), oligozoospermia (15.9%), asthenozoospermia (12.4%), and teratozoospermia (33.6%).

Semen analysis was performed after 4 days of sexual abstinence. The patients collected a semen sample by masturbation into sterile containers at the hospital, and the samples were analyzed within 1 hour of ejaculation. After liquefaction of the semen at room temperature (22°C) for 30 minutes, ejaculate analysis was performed in accordance with the 2010 WHO criteria (22), which included the assessment of semen volume, and sperm concentration, and morphology.

Sperm concentration was measured using a hemocytometer (Improved Neubauer; Hauser Scientific). Briefly, 10 µL of well-mixed semen was placed on a clean glass slide that had been stored at 37°C and covered with a 22 × 22 mm coverslip. The preparation was placed on the heating stage of a microscope at 37°C and was immediately examined at ×400 magnification. The same specialized biologist (P-S, J) performed all the semen analyses.

The patients were categorized according to the 2010 WHO criteria into the following categories: normozoospermia, oligozoospermia, asthenozoospermia, or teratozoospermia (22). In cases with more than one abnormal sperm parameter, the

most severe was considered for classification purposes. For this study, sperm analysis was performed on two occasions: [1] immediately before starting treatment (vitamin or placebo); and [2] 3 months later, on the same day as IVF, with an aliquot of the sample used for IVF-ICSI.

### IVF methodology

Our IVF management has been described elsewhere (23, 24). Briefly, it consists of down-regulation with the gonadotropin-releasing hormone analogue triptorelin acetate (Decapeptyl; Ipsen) on a long protocol, ovarian stimulation; [1] women ≤35 years received only recombinant follicle-stimulating hormone (FSH) (Gonal F, Merck Serono), and [2] women >35 years received recombinant FSH, and highly purified urinary menopausal gonadotropins (Menopur, Ferring) or recombinant FSH and recombinant luteinizing hormone (Pergoveris; Merck Serono). Ovulation was triggered with 250 µg of Ovitrelle (Merck). We scheduled OPU 36 hours after the human chorionic gonadotropin injection, and the luteal phase was supplemented with micronized progesterone (Utrogestan; Laboratorios Seid), 200 mg vaginally/12 hours. During the study period, no ovarian triggering was performed with gonadotropin-releasing hormone agonists, and cycles with hyperstimulation risk were canceled.

Implantation rate was defined as the number of embryos transferred that developed to the stage of a gestational sac on ultrasound divided by the total number of embryos transferred. Clinical pregnancy was defined as an intrauterine gestational sac on ultrasound with fetal heart activity at 7–8 weeks of gestation. Live birth was defined as the birth of a viable infant born after 24 weeks of gestation, and twins delivered by one mother were counted as one live birth. Global live-birth rate was defined as the sum of live births resulting from IVF transfers and those resulting from natural intercourse during the treatment period (with placebo or vitamin E) divided by the total number of couples in each treatment group.

### Statistical evaluation

All variables were checked for normal distribution by applying the Kolmogorov-Smirnov one-sample test for goodness-of-fit. Factorial analysis of variance (ANOVA) for repeated measurements was performed for testing statistically significant differences between the study groups. Two-sided  $P < .05$  was considered statistically significant. Confidence intervals for differences in sperm motility before and during treatment were calculated from the binomial distribution. Values determined before and after treatment were compared with the paired  $t$ -test. Qualitative parameters were studied with the chi-square test as well as odds ratio (OR) and 95% confidence interval (CI). Computations were performed using SPSS (version 22; IBM).

## RESULTS

### Study flow chart

A total of 160 couples were contacted, and of these, 113 (89.4%) were enrolled in the study. Three natural cycle pregnancies were achieved during the study period, at 45, 50, and 70 days of treatment. After breaking the study blinding, we found that two were in the placebo group and the other in the vitamin E group. For various reasons, nine other couples eventually did not perform IVF in this study: five in the placebo group (three cycles canceled due to low response and two due to hyperresponse) and four in the vitamin E group (two for low response and two for hyperresponse). Therefore, in the end 101 couples were included in the study: 50 men received vitamin E, and 51 received placebo (Fig. 1). No after-treatment sperm analysis was performed in cases of pregnancy or canceled cycles. The main demographic and clinical characteristics of the population who eventually underwent IVF are summarized in Supplemental Table 1 (available online).

### Baseline sperm characteristics in vitamin E and placebo groups

As expected in a randomized trial, the main demographic characteristics and sperm parameters were similar in the two groups (Table 1), as were the main IVF indications.

### Sperm parameters before and after 3 months of treatment (with vitamin E or placebo) in the entire population

Sperm concentration ( $74.45$  million/mL  $\pm$  40.06) statistically significantly increased from the day immediately before starting treatment to the day of IVF ( $59.75 \pm 39.85$ ,  $P = .01$ ). Similarly, progressive motility statistically significantly increased ( $70.6\% \pm 33.9$  vs.  $51.1 \pm 14.65$ ,  $P < .001$ ) (Table 2). Total motile sperm count almost doubled ( $83.4 \pm 87.2$  million vs.  $156.4 \pm 141.4$  million,  $P = .001$ ). There were no statistically significant changes in total spermatozoa or normal morphology.

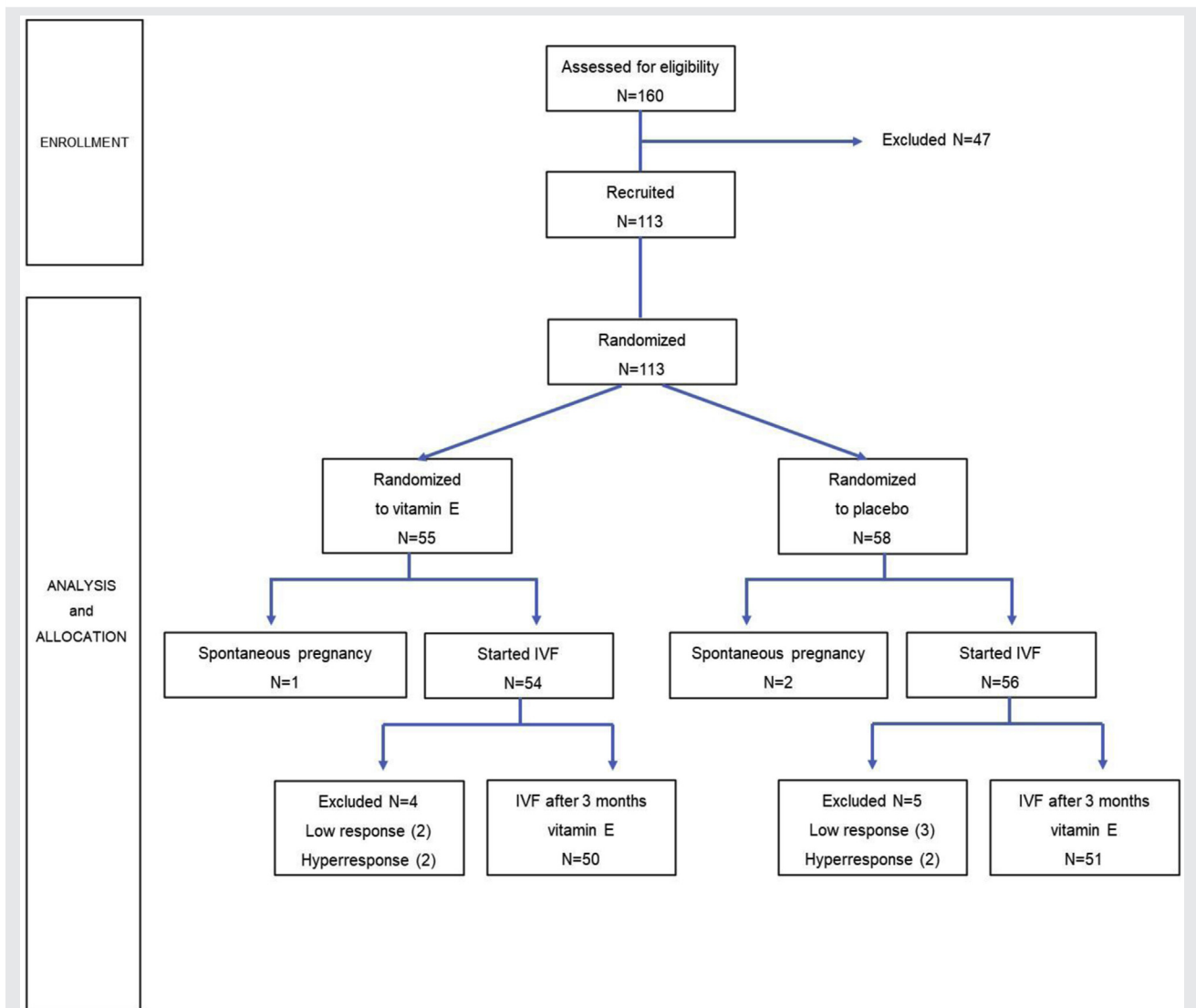
### Sperm parameters before and after 3 months treatment considered separately in the vitamin E and in the placebo group

In the vitamin E group, the progressive motility statistically significantly increased ( $52.5\% \pm 14.8$  vs.  $72.3 \pm 41.6$ ) ( $P = .001$ ) (Table 2). The total motile sperm count almost doubled ( $86.9 \pm 90.1$  million vs.  $160.7 \pm 155.6$  million,  $P = .025$ ). The total sperm count and sperm concentration, although somewhat higher, did not statistically significantly change. There were no changes in the percentage of normal morphology.

In the placebo group, there were statistically significant increases in sperm concentration ( $60 \pm 37.3$  million/mL vs.  $76.7 \pm 39.6$  million/mL), progressive motility ( $49.7\% \pm 14.5\%$  vs.  $70.9\% \pm 22.6\%$ ) and also normal morphology ( $3.8\% \pm 2.3\%$  vs.  $5.1\% \pm 3.5\%$ ). The total motile sperm count almost doubled ( $79.9 \pm 84.9$  vs.  $152.1 \pm 127.5$ ,  $P = .001$ ). There were no differences concerning total sperm count. In comparing the sperm parameters between the groups after 3 months of treatment, the results were found to be similar except the percentage of normal forms, which was statistically significantly higher in the placebo group than in the vitamin E group ( $5.1\% \pm 3.5\%$  vs.  $3.7\% \pm 2.4\%$ ,  $P = .04$ ).

Exploring the different sperm diagnosis categories, similar patterns were observed. In the normozoospermia group, the sperm concentration increased from  $60 \pm 31.3$  million/mL to  $77.9 \pm 36.9$  million/mL in the placebo group ( $P = .055$ ) and from  $69.3 \pm 30.5$  million/mL to  $92.2 \pm 35.1$  million/mL in the vitamin E group ( $P = .07$ ). In the group where the main diagnosis was teratozoospermia, the percentage of normal forms increased from  $2.1\% \pm 0.7\%$  to  $4.7\% \pm 1.1\%$  in the placebo group ( $P = .001$ ) and from  $1.8\% \pm 0.8\%$  to  $3.0\% \pm 1.6\%$  in the vitamin E group ( $P = .03$ ). When the main diagnosis was asthenozoospermia, the percentage of spermatozoa with progressive motility grew from  $24.3\% \pm 6.2\%$  to  $35.8\% \pm 11.5\%$  in the placebo group ( $P = .15$ ) and from  $26.2\% \pm 6.3\%$  to  $37.9\% \pm 12.4\%$  in the vitamin E group ( $P = .10$ ). Finally, in the oligozoospermia group the sperm concentration increased from  $4.8 \pm 4.6$  million/mL to  $6.8 \pm 5.6$  million/mL in the placebo group ( $P = .55$ ) and from  $6.3 \pm 3.9$  million/mL to  $8.4 \pm 6.1$  million/mL in the vitamin E group ( $P = .49$ ) ( $n = 12$ ).

FIGURE 1



Flow chart of participants from recruitment to analysis of effect of antioxidant therapy.

Matorras. Vitamin E administration and ART outcome. *Fertil Steril Rep* 2020.

## IVF results

The mean age of the woman, the number of oocytes obtained, and the percentage of cycles undergoing ICSI were very similar in the two groups (Table 3). The fertilization rate in the vitamin E group was  $54.80\% \pm 28.6\%$ , statistically significantly lower than the rate of  $66.5\% \pm 29.1\%$  in the placebo group ( $P=.022$ ). When the fertilization rate was considered separately for couples receiving ICSI or IVF, the results were somewhat higher in placebo group, but statistical significance was not reached ( $67.2\% \pm 30.3\%$  vs.  $55.6\% \pm 32.7\%$  in ICSI,  $P=.052$  and  $64.1\% \pm 27.4\%$  vs.  $52.4\% \pm 25.5\%$  in IVF,  $P=.069$ ). Nonetheless, the mean number of oocytes fertilized was similar in the two groups ( $5.0 \pm 4.3$  vitamin E group vs.  $5.4 \pm 4.1$  placebo group). The percentage of couples with at least one high-

quality embryo and the number of embryos transferred were also similar in the two groups.

In the vitamin E group, there was a trend toward a higher clinical pregnancy rate per transfer (43.9% vs. 25.0%), pregnancy rate per cycle started (36% vs. 22%), and implantation rate (24.7% vs. 14.1%), but the differences did not reach statistical significance. The corresponding ORs were 2.34 (95% CI, 0.94–5.89), 2.05 (95% CI, 0.85–4.94), and 1.94 (95% CI, 0.92–4.06), respectively.

The live-birth rate per transfer was statistically significantly higher in the vitamin E group (41.46% vs. 20.46% in the placebo group,  $P=.04$ ; OR 2.75; 95% CI, 1.05–7.19). The live-birth rate per cycle started and global live-birth rate were somewhat higher in the vitamin E group, though the differences did not reach statistical significance.



**TABLE 2**

**Comparison of the sperm parameters in vitamin E and placebo groups before and after treatment.**

Parameter	Placebo or vitamin E (n = 101)			Placebo (n = 51)			Vitamin E (n = 50)			Placebo versus vitamin E		
	Before	After	P value	Before	After	P value	Before	After	P value	Before	After	P value
Days of abstinence	3.5 ± 1.5	3.5 ± 1.1	1.00	3.48 ± 1.3	3.6 ± 1.0	.3011	3.5 ± 1.7	3.5 ± 1.1	.9780	3.5 ± 1.1	3.5 ± 1.1	.4552
Median (min-max)	3 (1-7)	3 (1-7)		3 (1-6)	3 (1-7)		3 (1-7)	3 (1-6)		3 (1-6)	3 (1-6)	
Total sperm count (millions)	186.05 ± 167.9	237.3 ± 180.4	.1072	186.3 ± 168.3	231 ± 175.7	.201	185.8 ± 167.5	243.6 ± 185.1	.111	185.8 ± 167.5	243.6 ± 185.1	.6975
Median (min-max)	145 (3-585)	185 (4-710)		135 (5-600)	175 (3-720)		150 (1-570)	190 (4-700)		150 (1-570)	190 (4-700)	
Concentration (10 <sup>6</sup> /ml)	59.75 ± 39.85	74.45 ± 40.6	.0123	60 ± 37.3	76.7 ± 39.6	.012	59.5 ± 42.3	72.2 ± 41.6	.138	59.5 ± 42.3	72.2 ± 41.6	.4575
Median (min-max)	55 (2-120)	70 (3-120)		50 (2-120)	80 (5-120)		60 (1-120)	80 (1-120)		60 (1-120)	80 (1-120)	
Motility grade A+B (%)	51.1 ± 14.65	70.6 ± 33.39	.0001	49.7 ± 14.5	70.9 ± 22.6	.001	52.5 ± 14.8	72.3 ± 41.6	.001	52.5 ± 14.8	72.3 ± 41.6	.9553
Median (min-max)	56 (18-73)	80 (1-105)		55 (15-70)	80 (0-90)		56 (20-75)	80 (1-120)		56 (20-75)	80 (1-120)	
Morphology (%)	3.75 ± 2.45	4.4 ± 3.01	.1765	3.8 ± 2.3	5.1 ± 3.5	.049	3.7 ± 2.6	3.7 ± 2.4	.866	3.7 ± 2.6	3.7 ± 2.4	.041
Median (min-max)	3.5 (0-10)	4 (1-16)		4 (0-10)	4 (1-20)		3 (0-10)	3.5 (1-12)		3 (0-10)	3.5 (1-12)	
Total motile sperm count (millions)	83.4 ± 87.2	156.4 ± 141.4	.0001	79.9 ± 84.9	152.1 ± 127.5	.001	86.9 ± 90.1	160.7 ± 155.6	.025	86.9 ± 90.1	160.7 ± 155.6	.6779
Median (min-max)	72 (1-380)	138 (3-462)		69 (3-350)	130 (3-451)		74 (1-380)	150 (4-462)		74 (1-380)	150 (4-462)	

Note: Values are mean ± standard deviation unless otherwise indicated.

Matarras. Vitamin E administration and ART outcome. *Fertil Steril Rep* 2020.

**Adverse effects and costs**

Oral administration of vitamin E was well tolerated, and no adverse effects or laboratory abnormalities were observed. The cost of the complete vitamin E treatment per patient, based on Spanish market prices, would be 10,53 euro (3.51 euro per month for 30 capsules of 400 mg of vitamin E multiplied by 3 for the 3-month treatment).

**DISCUSSION**

Oxidative stress is recognized to be a major cause of male infertility (6). Nonetheless, there is no agreement concerning the best method to assess it or the cutoff points of normality. Many different antioxidants have been proposed, alone or in combination, in different clinical settings. The results, although controversial, have suggested a beneficial effect of antioxidants (14). On the other hand, the beneficial effect, if any, of antioxidants on normal sperm is unknown. Therefore, we directed our study to our general IVF population, in which the rate of abnormal sperm is nearly 65% following WHO criteria.

We selected vitamin E as the antioxidant based on previous data in the literature on male fertility (25-27), as well as its safety profile (16, 28) and price. Vitamin E is a collective term that describes eight naturally occurring homologues with potent antioxidant properties. All eight homologues are differentially distributed within food sources (29), but α-tocopherol is the only form that is recognized to meet human requirements (16). The recommended dietary allowance for α-tocopherol is currently 15 mg/day in adults with a recommended upper intake level of 1,000 mg/day for supplemental vitamin E (28).

One interesting finding in our study is that after 3 months of treatment there were notable improvements in many sperm parameters, regardless of whether the man had received vitamin E or placebo. In our opinion, the explanation could be the well-known Hawthorne effect. This effect was first described when it was observed in a population of workers that their work productivity increased regardless of the changes made due to the psychological stimulus of being singled out and made to feel important (30). It could be speculated that the men in our study adopted healthier habits (concerning exercise, diet, smoking, and alcohol consumption or other drugs) and that these changes led to improvements in sperm characteristics. This could also be responsible for the non-negligible natural cycle pregnancy rate (nearly 1% per month). This underlines the great importance of double-blind protocols in studies focusing on male fertility.

The results of previous studies on the effect of vitamin E on sperm parameters have been controversial (10, 25, 31). As for the composition of the vitamin E group there was a higher proportion of oligozoospermia, close to statistical significance. One could speculate that this might have influenced the results. In our study, it should also be highlighted that some of the changes were similar in the placebo and vitamin E groups. There was a marked, statistically significant increase in progressive motility in both groups. Sperm concentration also increased in both groups, although the change only reached statistical significance in the placebo group.

TABLE 3

## IVF outcomes in the placebo and vitamin E groups.

Outcome	Placebo treatment (n = 51)	Vitamin E treatment (n = 50)	OR (95% CI)	P value
Intention to treat	58	55		
Woman's age (y)	35.9 ± 2.2	35.2 ± 2.7		.310
Total no. of oocytes retrieved	11.0 ± 6.7	11.2 ± 7.1		.992
ICSI (%)	78.4 (40/51)	76 (38/50)		.82
Fertilization rate (%)	66.5 ± 29.1	54.8 ± 28.6		.022
No. of oocytes fertilized	5.4 ± 4.1	5.0 ± 4.3		.429
Patients with at least 1 top-quality embryo on day 3 (%)	55.0 (28/51)	46.0 (23/50)		.273
No. of embryos transferred	2.0 ± 0.9	1.9 ± 1.0		.766
CPR per transfer (%)	25.0 (11/44)	43.9 (18/41)	2.34 (0.94–5.89)	.069
CPR per cycle started (%)	22.0 (11/51)	36.0 (18/50)	2.05 (0.85–4.94)	.11
Implantation rate (%)	14.1 (14/99)	24.7 (22/91)	1.94 (0.92–4.06)	.08
LBR per transfer (%)	20.46 (9/44)	41.46 (17/41)	2.75 (1.05–7.19)	.04
LBR per cycle started (%)	17.62 (9/51)	34.0 (17/50)	2.4 (0.95–6.07)	.06
Global LBR (%)	18.9 (11/58)	32.7 (18/55)	2.08 (0.88–4.93)	.097

Note: Values are expressed as mean ± standard deviation, unless indicated otherwise. CI = confidence interval; CPR = clinical pregnancy rate; ICSI = intracytoplasmic sperm injection; LBR = live-birth rate; OR = odds ratio.

Matorras. Vitamin E administration and ART outcome. *Fertil Steril Rep* 2020.

Finally, the percentage of normal forms only statistically significantly increased in the placebo group. With the specific sperm categories, similar patterns were observed although in some categories the statistical analysis was hampered by the small sample size of the subgroups. Although the separate analysis found no statistically significant differences, it should be remembered that the study was not powered to analyze these subgroups. Our results are consistent with those of a recent randomized trial in which antioxidants did not improve sperm parameters among men with male factor infertility (32).

Regarding IVF outcomes, our results are paradoxical. On the one hand, in agreement with the aforementioned better sperm parameters in the placebo group, the fertilization rate was statistically significantly higher in the placebo group. Nonetheless, the live-birth rate per transfer was statistically significantly higher in the vitamin E group (41.46% vs. 20.46% placebo group,  $P=.04$ ; OR 2.75; 95% CI, 1.05–7.19). In all the other IVF outcome parameters considered (implantation rate and clinical pregnancy rate both per transfer and per cycle started, live-birth rate per cycle started, and global live-birth rate), the ORs were nearly 2 in favor of vitamin E, but the trends did not reach statistical significance.

It has been highlighted that vitamin E does not simply have an antioxidant effect. Specifically, the reported nonantioxidant effects of vitamin E involve the modulation of cellular responses including survival, inflammation, cellular adhesion, migration, secretion, and immunity either by modulating enzymes in signal transduction pathways (especially protein kinase) or regulating activities of specific transcription factors (16, 33–36). It could be speculated that, by some of the aforementioned mechanisms, vitamin E-treated spermatozoa have a somewhat impaired fertilization ability, but this resulted in better embryo quality. Our relatively small sample size meant that we were unable to investigate the effects on IVF in each male diagnosis subgroup. More studies are needed to confirm our results, especially in relation to

male diagnosis and OS status. Our work opens the door to different lines of research to investigate the influence of vitamin E on the spermatozoa, such as sperm DNA fragmentation and markers of OS in the seminal plasma and in the spermatozoa membranes.

## CONCLUSION

The effect of vitamin E on classic sperm parameters is not better than that of placebo. Nonetheless, we found vitamin E administration was associated with a statistically significantly higher live-birth rate, and there was a trend toward better results in other IVF parameters.

## REFERENCES

- Oseguera-López I, Ruiz-Díaz S, Ramos-Ibeas P, Pérez-Cereales S. Novel techniques of sperm selection for improving IVF and ICSI outcomes. *Front Cell Dev Biol* 2019;7:298.
- Oehninger S, Ombelet W. Limits of current male fertility testing. *Fertil Steril* 2019;111:835–41.
- Agarwal A, Bui AD. Oxidation-reduction potential as a new marker for oxidative stress: correlation to male infertility. *Investig Clin Urol* 2017;58:385–99.
- Agarwal A, Majzoub A, Esteves SC, Ko E, Ramasamy R, Zini A. Clinical utility of sperm DNA fragmentation testing: practice recommendations based on clinical scenarios. *Transl Androl Urol* 2016;5:935–50.
- Simon L, Zini A, Dyachenko A, Ciampi A, Carrell D. A systematic review and meta-analysis to determine the effect of sperm DNA damage on in vitro fertilization and intracytoplasmic sperm injection outcome. *Asian J Androl* 2017;19:80–90.
- Gharagozloo P, Aitken RJ. The role of sperm oxidative stress in male infertility and the significance of oral antioxidant therapy. *Hum Reprod* 2011;26:1628–40.
- Gagnon C, Iwasaki A, De Lamirande E, Kovalski N. Reactive oxygen species and human spermatozoa. *Ann NY Acad Sci* 1991;637:436–44.
- Agarwal A, Saleh RA, Bedaiwy MA. Role of reactive oxygen species in the pathophysiology of human reproduction. *Fertil Steril* 2003;79:829–43.
- Rolf A, Coope TG, Yeong CH, Nieschlag E. Antioxidant treatment of patients with asthenozoospermia or moderate oligoasthenozoospermia with high-

- dose vitamin C and vitamin E: a randomized, placebo-controlled, double-blind study. *Hum Reprod* 1999;14:1028–33.
10. Keskes-Ammar L, Feki-Chakroun N, Rebai T, Sahnoun Z, Ghazzi S, Hammami K, et al. Sperm oxidative stress and the effect of an oral vitamin E and selenium supplement on semen quality in infertile men. *Arch Androl* 2003;49:83–94.
  11. Greco E, Iacobelli M, Rienzi L, Ubaldi F, Ferrero S, Tesarik J. Reduction of the incidence of sperm DNA fragmentation by oral antioxidant treatment. *J Androl* 2005;26:349–53.
  12. Diamanti-Kandarakis E, Papalou O, Kandaraki EA, Kassi G. Mechanisms in endocrinology: nutrition as a mediator of oxidative stress in metabolic and reproductive disorders in women. *Eur J Endocrinol* 2017;176:79–99.
  13. Henkel R, Sandhu IS, Agarwal A. The excessive use of antioxidant therapy: a possible cause of male infertility? *Andrologia* 2019;51:e13162.
  14. Smits RM, Mackenzie-Proctor R, Yazdani A, Stankiewicz MT, Jordan V, Showell MG. Antioxidants for male subfertility. *Cochrane Database Syst Rev* 2019;3:CD007411.
  15. Institute of Medicine Panel on Dietary Antioxidants and Related Compounds. Dietary reference intakes for vitamin C, vitamin E, selenium, and carotenoids. Washington, DC: National Academies Press; 2000.
  16. Office of Dietary Supplements, National Institutes of Health. Dietary supplement fact sheet: vitamin E. Available at: <https://www.ods.od.nih.gov/factsheets/vitamined.asp>.
  17. Aitken RJ, Drevet JR. The importance of oxidative stress in determining the functionality of mammalian spermatozoa: a two-edged sword. *Antioxidants (Basel)* 2020;9:111.
  18. Nowicka-Bauer K, Nixon B. Molecular changes induced by oxidative stress that impair human sperm motility. *Antioxidants (Basel)* 2020;9:134.
  19. Agarwal A, Parekh N, Panner Selvam MK, Henkel R, Shah R, Homa ST, et al. Male oxidative stress infertility (MOSI): proposed terminology and clinical practice guidelines for management of idiopathic male infertility. *World J Mens Health* 2019;37:296–312.
  20. Crisol L, Matorras R, Aspichueta F, Expósito A, Hernández ML, Ruiz-Larrea MB, et al. Glutathione peroxidase activity in seminal plasma and its relationship to classical sperm parameters and in vitro fertilization-intracytoplasmic sperm injection outcome. *Fertil Steril* 2012;97:852–7.
  21. Muciaccia B, Boitani C, Berloco BP, Nudo F, Spadetta G, Stefanini M, et al. Novel stage classification of human spermatogenesis based on acrosome development. *Biol Reprod* 2013;89:60.
  22. World Health Organization. Laboratory manual for the examination and processing of human semen. 5th ed. Geneva: WHO; 2010.
  23. Matorras R, Urquijo E, Mendoza R, Corcostegui B, Expósito A, Rodríguez-Escudero FJ. Ultrasound-guided embryo transfer improves pregnancy rates and increases the frequency of easy transfer. *Hum Reprod* 2002;17:1762–6.
  24. Matorras R, Otero B, Mendoza R, Expósito A, De Pablo JL, Larreategui Z, et al. Quality of additional embryos transferred on pregnancy outcomes in IVF: predictions using a mathematical approach. *Reprod Biomed Online* 2014;29:200–8.
  25. Kessopoulou E, Powers HJ, Sharma KK, Pearson MJ, Russell JM, Cooke ID, Barratt CL. A double-blind randomized placebo cross-over controlled trial using the antioxidant vitamin E to treat reactive species associated male infertility. *Fertil Steril* 1995;64:825–31.
  26. Vézina D, Mauffette F, Roberts KD, Bleau G. Selenium-vitamin E supplementation in infertile men: effects on semen parameters and micronutrient levels and distribution. *Biol Trace Elem Res* 1996;53:65–83.
  27. Showell MG, Mackenzie-Proctor R, Brown J, Yazdani A, Stankiewicz MT, Hart RJ. Antioxidants for male subfertility. *Cochrane Database Syst Rev* 2014;12:CD007411.
  28. Boccardi V, Baroni M, Mangialasche F, Mecocci P. Vitamin E family: role in the pathogenesis and treatment of Alzheimer's disease. *Alzheimers Dement (NY)* 2016;2:182–91.
  29. Bieri JG. Sources and consumption of antioxidants in the diet. *J Am Oil Chem Soc* 1984;61:1917–8.
  30. Franke RH, Kaul JD. The Hawthorne experiments: first statistical interpretation. *Am Sociol Rev* 1978;43:623–43.
  31. 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.
  32. Steiner AZ, Hansen KR, Barnhart KT, Cedars MI, Legro RS, Diamond MP, et al. Reproductive Medicine Network. The effect of antioxidants on male factor infertility: the Males, Antioxidants, and Infertility (MOXI) randomized clinical trial. *Fertil Steril* 2020;113:552–60.
  33. Rizvi S, Raza ST, Ahmed F, Ahmad A, Abbas S, Mahdi F. The role of vitamin E in human health and some diseases. *Sultan Qaboos Univ Med J* 2014;14:157–65.
  34. Zingg JM. Vitamin E: a role in signal transduction. *Annu Rev Nutr* 2015;35:135–73.
  35. Azzi A. Many tocopherols, one vitamin E. *Mol Aspects Med* 2018;61:92–103.
  36. Sozen E, Demirel T, Ozer NK. Vitamin E: regulatory role in the cardiovascular system. *IUBMB Life* 2019;71:507–15.