

Beneficial effects of oral antioxidant supplementation on semen quality parameters, reproductive hormones, and sperm DNA integrity in men with idiopathic oligoasthenoteratozoospermia

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Objective: Recently, oral antioxidants in combined forms have been used to treat men with idiopathic infertility. This study aimed to evaluate the effects of treatment with vitamin C, vitamin E, selenium, zinc, arginine, L-carnitine, and coenzyme Q10 on sperm quality parameters, DNA integrity, reproductive hormones, and pregnancy rates in men with infertility and idiopathic oligoasthenoteratozoospermia (OAT).

Methods: A prospective study was conducted on 420 men with infertility and idiopathic OAT who took an oral supplement of antioxidant SP-Power tablets twice daily for 6 months. Semen quality, reproductive hormones, and the DNA fragmentation index (DFI) were evaluated at baseline and at 3 and 6 months after supplementation, using the World Health Organization 2021 guidelines.

Results: No significant difference was observed in volume or the percentage of typical morphology during treatment. A significant improvement in sperm concentration was observed after supplementation (8.67 ± 1.41 , 12.17 ± 1.91 , and 19.01 ± 0.86 at baseline, 3, and 6 months respectively, $p < 0.01$). The total motility, progressive motility, and total motile sperm count also increased significantly ($p < 0.01$), whereas the DFI decreased after 6 months. There was an increase in normal FSH levels and testosterone levels after 6 months of supplementation of antioxidant SP-Power but these differences were not statistically significant ($p = \text{not significant}$ and $p = 0.06$, respectively).

Conclusion: Supplementation with SP-Power tablets improved sperm quality parameters, sperm DFI, some reproductive hormones, and pregnancy rates in men with infertility and idiopathic OAT, which could be attributed to the supplement's synergistic antioxidant action. Further studies are needed to determine the effects of supplementation on oxidative stress markers.

Keywords: DNA fragmentation; Idiopathic male infertility; Oligoasthenoteratozoospermia; Pregnancy rate; Semen quality; SP-Power

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Introduction

According to reports, there are between 10% and 15% of infertile couples worldwide, which represents more than 48.5 million couples. 34 million of the 40 million couples who are actively looking for infertility treatment. It is recognized that half of all cases are caused by male-factor infertility [1]. Infertility is defined as the inability of couples to have children despite 1 year of regularly engaging in unprotected sexual intercourse. Its medical, socio-cultural, psychologi-

cal, and financial impacts constitute an important public health problem [2]. However, despite the existence of many congenital and acquired causes (varicocele, cryptorchidism, hypogonadism, and genetic factors), the etiological analysis of male infertility shows a predominance of idiopathic origin, affecting 31% of infertile men [3].

Idiopathic male infertility with oligoasthenoteratozoospermia (OAT) is a condition in which the sperm concentration, percentage of progressively motile sperm, and typical morphology are below the World Health Organization (WHO) reference values [4]. Many environmental, genetic, and physiological factors, including oxidative stress (OS) [5], have been implicated in idiopathic male infertility. OS is an imbalance between the production of reactive oxygen species (ROS) and antioxidative mechanisms, resulting in cellular damage. It has been shown that low levels of ROS are essential for several sperm functions. A high ROS level and OS have been implicated in the etiology of male infertility [6]. Moreover, ROS have been linked to sperm DNA damage, altered sperm motility, pregnancy rate, and embryo development [7]. In fact, the human body has a highly organized antioxidant defense system that protects against ROS. These defense systems involve a synergy among diverse endogenous and exogenous components of scavenger-free radicals [8]. The antioxidants are divided into enzymatic antioxidants and nonenzymatic antioxidants, which can eliminate free radicals produced during normal cellular metabolism [9,10].

Previous research has found that the seminal fluid of men with infertility has a lower antioxidant capacity than that of fertile men [11]. To increase the chances of success in applying assisted reproductive technology [12], oral antioxidant supplementation is a therapeutic option used increasingly for men with idiopathic infertility. Recently, research has been conducted on the relationship between oral antioxidants and male infertility. In addition, several studies have shown that antioxidants and micronutrients can improve sperm quality parameters in men with infertility [13]. Multi-antioxidant supplementation is considered more effective than the use of a single antioxidant for male fertility parameters owing to the ingredients' synergetic effects [14]. One study investigating the effect of treatment with a combination therapy of antioxidants (vitamin C, vitamin E, selenium, zinc, and coenzyme Q10) on seminal fluid parameters in men with infertility and idiopathic OAT reported an improvement in sperm quality parameters [15]. Another study has shown that supplementation with coenzyme Q10 improved sperm quality parameters and relieved sperm DNA damage and OS markers in men with infertility and idiopathic oligoasthenospermia [16]. Oral antioxidant supplementation therapy is widely used to improve the fertility of men, especially those with idiopathic infertility [17]. However, to the best of our knowledge, this is the first study that investigated the treatment of men with infertility and idiopathic OAT with a nutraceutical con-

taining seven micronutrients (vitamin C, vitamin B9, selenium, zinc, arginine, L-carnitine, and coenzyme Q10) and compared the effects on sperm quality parameters, sperm DNA fragmentation, reproductive hormones, and the pregnancy rate at baseline, after 3 months, and after 6 months of treatment.

Methods

1. Ethics statement

This study was approved by the Research Ethical Review Committee of Faculty of Medicine and Pharmacy of Oujda, Morocco (approval code: 02/2023). The participants were informed of the scientific nature of our study.

2. Recruitment of subjects

A prospective study was conducted on 420 men (ages 26 to 59 years, mean age 38.5 ± 1.2 years) with infertility and idiopathic OAT who met the inclusion criteria. The patients were recruited at our Fertility Center, Mohammed VI University Hospital Center in Oujda, Morocco, and the study was conducted from November 2022 to May 2023. The inclusion criteria were a history of infertility of at least 12 months despite regular unprotected intercourse and a seminal fluid analysis showing abnormal sperm concentration (oligozoospermia) (<16 million/mL), progressive motility (asthenozoospermia) $<30\%$, total motility $<42\%$, normal morphology according to the modified David classification (teratozoospermia $<15\%$), and DNA fragmentation index (DFI) $<25\%$, as defined by the sixth edition (2021) of the WHO manual for semen analysis [1]. The exclusion criteria consisted of azoospermia, varicocele, genital tract infection, cryptorchidism, testicular trauma or scrotal surgery, and endocrine disorders.

3. Composition of antioxidant supplement

All patients received orally a daily supplement of antioxidant SP-POWER tablet (Health Innovation), taken two times daily. The main composition is presented in Table 1. We explained the aim and methods of the study to each patient. All patients stated that they understood and accepted that they would receive antioxidant supplements. Each patient signed an informed written consent form before entering the study, and they were informed that they could terminate their cooperation with us whenever they wanted without any consequences.

4. Semen sample analysis

The semen samples used in the study were classified according to the 2021 WHO guidelines for examination and processing of human semen. Samples were obtained and analyzed for sperm volume, concentration, motility, and morphology at baseline, 3, and 6

Table 1. Content of the antioxidant SP-Power (antioxidant formula)

Component	Amount (mg)
Vitamin C	40
Vitamin B9	0.1
Selenium	25
Zinc	50
Arginine	200
L-carnitine	200
Coenzyme Q10	15

months. A clean sterile plastic container confirmed to be non-toxic for spermatozoa was given to each participant to collect a semen sample by masturbation (after 3 days of sexual abstinence). To minimize temperature fluctuations and control the time between semen sample collection and analysis, samples were collected in our Fertility Center. Macroscopic analysis of the sperm was performed with the observation of liquefaction time, viscosity, semen volume, color, and pH [18]. The study of the sperm was undertaken only with all assurances that the collection was done without loss and in the good conditions required for this purpose.

First, a Pasteur pipette (Health Innovation) was used to homogenize the semen samples, and an evaluation of the concentration and motility of spermatozoa parameters was conducted using the Sperm Class Analyzer (Computer Aided Semen Analysis system; Microptic S.L.). For each measurement, a 2.5 μ L aliquot of sperm was loaded into a standard four-chamber slide (Leja Products BV). The spermatozoa with fast and slow progressive motility (A and B) were counted first, followed by the non-progressive motile (C) and non-motile spermatozoa (D). The sperm concentration count and sperm motility were determined using $\times 10$ magnification. Sperm vitality was assessed using the eosin-nigrosine test.

The evaluation of spermatozoon morphology was based on modified David criteria and was performed using a Diff-Quik kit (Dade Behring AG) containing one stain fixative and two stains (A and B). Morphological assessment was performed with a Nikon microscope using an oil immersion (Nikon Company) [18]. A minimum of 100 sperm cells were counted per sample. Strict scoring criteria were used to classify each participant's samples as showing normal or abnormal morphology according to the modified David classification.

Participants were required to undergo an extensive physical examination. A urologist used a "test-size" Prader orchidometer to measure each participant's testicular volume.

5. Analysis of the total motile sperm count

Using a two-layered density gradient centrifugation technique, sperm samples were prepared (50% and 90% Isolate; Irvine Scientif-

ic). Male-factor infertility was not strictly defined but rather was assessed by analyzing the number of motile sperm in the ejaculate [18]. The total motile sperm count (TMSC) in the ejaculate was calculated using the formula: $TMSC = \text{semen volume (mL)} \times \text{sperm concentration (millions/mL)} \times \text{percentage motility divided by 100 (\%)}$.

The participants were divided into four groups according to their TMSC results, based on the WHO guideline, as less than 0.5×10^6 , $0.5 - 1 \times 10^6$, $1 - 2 \times 10^6$, and greater than 2×10^6 .

6. Hormonal evaluation

Quantitative analyses of luteinizing hormone (LH), testosterone, and follicle-stimulating hormone (FSH) were carried out for each participant to determine their hormonal profile. After 10 mL of peripheral blood was collected from each participant in a dry tube and centrifuged for 1 hour using an Eppendorf 5810R centrifuge, the plasma was used for determining LH, testosterone, and FSH levels in our biochemistry department with an Architect ci8200 [18].

7. DNA fragmentation assessment

DNA fragmentation was assessed by the sperm chromatin dispersion (SCD) test. In the absence of massive sperm DNA breakage, and following acid denaturation and removal of nuclear proteins, dispersed DNA loops produce a characteristic halo [19]. Sperm with fragmented DNA does not develop a halo, or the halo is small. DNA fragmentation testing was performed using the SCD method with Spermfunc DNA kits at BRED Life Science Technology Inc. The agarose was placed at a temperature of 90 to 100 $^{\circ}$ C for 20 minutes and heated at 37 $^{\circ}$ C for 5 minutes. The semen was then added to the agarose and mixed well. The suspension was poured on agarose-coated slides and covered with a 20 \times 20 mm cover glass. The slides were cooled at 4 $^{\circ}$ C for 5 minutes, then were slowly opened. They were then incubated in a denaturing solution at 22 $^{\circ}$ C for 7 minutes and with lysis solution at room temperature for 25 minutes. After being washed with H₂O for 5 minutes, they were dehydrated with graded ethanol at 70%, 90%, and 100% for 2 minutes per concentration. The slides were dried and stained with Wright's solution for 25 minutes. This was followed by observation via a light microscope based on various halo images, namely large, medium, small, no halo, and degraded sperm. A minimum of 500 sperm were counted on each sample under $\times 100$ magnification. Based on the observations of two researchers, sperm with large and medium halos were classified as having unfragmented DNA and the rest as having fragmented DNA.

The rate of sperm DNA fragmentation was calculated as follows: $DFI (\%) = 100 \times (\text{number of spermatozoa with fragmented DNA} / \text{total number of spermatozoa})$. A DFI <25% was considered normal.

8. Statistical analysis

GraphPad Prism ver. 6.01 (GraphPad Software) for Windows was used for statistical analysis. Results were expressed as mean±standard deviation (SD). The Kolmogorov-Smirnov test was used to assess data normality. Nominal variables were expressed as proportions, whereas continuous variables were presented as mean and SD. The paired Student *t*-test was used to compare sperm quality parameters before (baseline) and after supplementation (3 and 6 months). A *p*<0.05 was considered to indicate statistical significance.

Results

1. The effect of SP-Power on sperm quality and DNA fragmentation

Baseline patient characteristics are shown in Table 2. The participants were aged 26 to 59 years with a mean of 38.5±1.2 years. Primary infertility affected 85% of the participants, whereas 15% had secondary infertility; the mean duration of infertility was 5.4±1.2 years.

Table 3 shows the mean±SD of sperm parameters at three time points: before treatment, after 3 months, and after 6 months of the antioxidant treatment used in our study. No significant change in semen volume occurred after treatment (2.7±0.22, 2.73±0.15, and 2.37±0.3 mL at 0, 3, and 6 months respectively). Significant improvements in sperm motility parameters and the DFI after treatment. The DFI decreased, with mean±SD values of 26.13%±1.75% before treatment and 23.51%±0.86% and 21.85%±0.54% at 3 and 6 months, respectively (*p*<0.01).

2. The effects of SP-Power on hormone levels

Table 4 shows the mean±SD values of serum hormone concentrations among participants treated with SP-Power for 3 and 6 months. At the 6-month mark, the mean±SD value of LH for patients treated with SP-Power (11.4±2.2 IU/L) was significantly higher than the

pre-treatment value (13.2±2.1 IU/L) (*p*<0.01). The final mean±SD value of FSH in participants (17.1±2.5 IU/L) was higher than the baseline mean±SD value (16.4±1.3 IU/L), but this difference did not reach statistical significance. Similarly, the mean±SD value of testosterone for participants treated with SP-Power (18.4±2.7 nmol/L) was higher than the baseline value (17.1±3.3 nmol/L), but the effect did not reach statistical significance (*p*=0.06). The final mean±SD value for inhibin B (162.3±7.2 pg/mL) was higher than the baseline value (158.1±12.2 pg/mL), and the difference was statistically significant (*p*<0.01).

3. The effect of SP-Power on pregnancy rate

A total of 56 (13.33%) pregnancies were achieved, including 40 (9.52%) spontaneous pregnancies as follows: 12 pregnancies after 3 months, 18 pregnancies after 6 months, and 10 pregnancies after 9 months of treatment. Overall, 16 pregnancies were achieved using

Table 2. Baseline patient demographics and serum hormone levels of 420 infertile men

Characteristic	Range	Mean ± SD
Age (yr)	26–59	38.5 ± 1.2
Age of female partner age (yr)	21–39	31 ± 2.7
Serum hormones		
LH (IU/L)	10–14.02	13.2 ± 2.1
FSH (IU/L)	13–20.3	16.4 ± 1.3
Testosterone (mmol/L)	14.6–21.04	17.1 ± 3.3
Inhibin B (pg/mL)	148.1–164.8	158.1 ± 12.2
Testicular volume (mL)		
Right	6–27	17 ± 2.1
Left	2–30	14.6 ± 5.4

SD, standard deviation; LH, luteinizing hormone; FSH, follicle-stimulating hormone.

Table 3. Sperm quality parameter before and after 3 and 6 months of treatment of 420 infertile men

Sperm variable	Mean ± SD	Mean ± SD; <i>p</i> -value (95% CI)		WHO criteria (2021)
	Baseline	3 months	6 months	
Semen volume (mL)	2.7 ± 0.22	2.73 ± 0.15; 0.1 (–0.41 to –0.34)	2.37 ± 0.3; 0.1 (0.29 to 0.36)	≥ 1.4 mL
Sperm concentration (10 ⁶ /mL)	8.67 ± 1.41	12.17 ± 1.91; < 0.01 (–3.72 to –3.28)	19.01 ± 0.86; < 0.01 (–8.56 to –8.12)	≥ 16 million/mL
Sperm progressive motility (%; grade a+b)	19.90 ± 1.04	23.78 ± 2.30; < 0.01 (–4.20 to –3.54)	28.78 ± 2.71; < 0.01 (–9.20 to –8.55)	≥ 30%
Sperm total motility (%)	28.46 ± 1.00	32.32 ± 0.95; 0.03 (–4.00 to –3.71)	35.6 ± 0.92; < 0.05 (–7.28 to –6.99)	≥ 42%
Viability (%)	56.05 ± 1.11	56.07 ± 1.3; < 0.01 (–0.22 to 0.17)	57.02 ± 1.38; < 0.01 (–1.17 to –0.78)	≥ 54%
Normal sperm morphology (%)	10.42 ± 0.73	11.45 ± 0.29; < 0.3 (–1.15 to –0.89)	11.37 ± 1.26; < 0.2 (–1.08 to –0.82)	≥ 15%
Total motile sperm count	1.05 ± 0.13	1.35 ± 0.21; < 0.01 (–0.32 to –0.28)	1.10 ± 0.11; < 0.01 (–0.08 to –0.03)	-
DFI (%)	26.13 ± 1.75	23.51 ± 0.86; < 0.01 (–4.29 to –3.96)	21.85 ± 0.54; < 0.01 (–9.63 to –9.30)	≥ 25%

Values are presented as mean±SD, of seminal fluid parameters before and at 3 and 6 months of antioxidant combination treatment used in our study. Paired Student's *t*-test was used to compare seminal fluid parameters before (baselines) and after supplement (3 and 6 months), *p*<0.05 was considered statistical significant.

SD, standard deviation; CI, confidence interval; WHO, World Health Organization; DFI, DNA fragmentation index.

Table 4. Reproductive hormones before and after 3 and 6 months of treatment with SP-Power of 420 infertile men

Reproductive hormone	Mean ± SD	Mean ± SD; <i>p</i> -value		Normal value
	Baseline	3 months	6 months	
LH (IU/L)	13.2 ± 2.1	13.9 ± 1.7 0.02	14.8 ± 3.6 < 0.01	5–15
FSH (IU/L)	16.4 ± 1.3	16.0 ± 0.2 0.08	17.1 ± 2.5 NS	5–16
Testosterone (nmol/L)	17.1 ± 3.3	17.7 ± 0.9 < 0.01	18.4 ± 2.7 0.06	8.2–34.6
Inhibin B (pg/mL)	158.1 ± 12.2	160.4 ± 9.4 NS	162.3 ± 7.2 < 0.01	100–164

This table shows mean ± SD, of reproductive hormones before and at 3 and 6 months of antioxidant combination treatment used in our study. Paired Student's *t*-test was used to compare seminal fluid parameters before (baselines) and after supplement (3 and 6 months), *p* < 0.05 was considered statistically significant.

SD, standard deviation; LH, luteinizing hormone; FSH, follicle-stimulating hormone; NS, not significant.

assisted reproductive technology, and 16 couples used artificial insemination by the husband.

Oral administration of an antioxidant treatment containing a combination of 40 mg of vitamin C, 0.1 mg of vitamin E, 25 mg of selenium, 50 mg of zinc, 200 mg of arginine, 200 mg of L-carnitine, and 15 mg of coenzyme Q10 (SP-Power), was generally tolerated, and no undesirable or negative laboratory results were observed.

Discussion

OS has been linked to several factors such as smoking, poor diet, alcohol abuse, pollution and environmental toxins, obesity, and psychological stress. Various approaches have been suggested to manage infertility resulting from OS [20]. The present study demonstrated that antioxidant supplementation improved sperm concentration, total motility, progressive motility, normal morphology, TMSC, and DFI in participants with infertility and idiopathic OAT after 3 and 6 months.

Various antioxidant treatments used alone or in combined form have been tested for treating idiopathic male infertility, yet with no significant difference in sperm volume after treatment. This finding is consistent with results from a study that showed no difference in sperm volume in men with idiopathic OAT after antioxidant treatment with vitamin C, vitamin E, and coenzyme Q10 [21,22]. Another study showed that semen volume increased after supplementation with a combined antioxidant treatment for 3 months. Sperm concentration, progressive motility, and normal morphology improved significantly after 3 months of a combined antioxidant treatment [23].

This finding is similar to that of a previous study that reported an increase in sperm concentration after the participants had taken an antioxidant supplement [24]. A recent study demonstrated that the effect of combined antioxidant treatment including vitamin C, vitamin E, zinc, selenium, and coenzyme Q10 for 3 months increased sperm concentration in men with infertility. Moreover, in another study, an increase in progressive and total sperm motility in men with idiopathic OAT was observed after treatment with coenzyme Q10 (200 mg/day) for 3 months [25]. Furthermore, sperm concentration, progressive motility, and total motility increased significantly in men with infertility and OAT when compared to fertile controls, following the administration of selenium (200 µg/day) daily for 6 months [26]. Dadgar et al. [27] demonstrated the beneficial effects of co-administering pentoxifylline and zinc in men with idiopathic infertility, including significant improvements in normal morphology, DNA integrity, and reproductive hormones. The addition of 200 mg of coenzyme Q10 once daily for 3 months significantly improved sperm quality and seminal antioxidant status and significantly reduced total ROS and sperm DNA fragmentation levels [16].

Our results are in line with previous studies that have demonstrated higher sperm motility in patients with idiopathic infertility following the administration of vitamin C, vitamin E, zinc, selenium, and coenzyme Q10. A recent study showed that sperm concentration and sperm morphology were significantly improved after 3 months of antioxidant treatment with L-carnitine [28]. In contrast, in a different study, no significant difference was found in mean semen volume, sperm concentration, total sperm motility, progressive sperm motility, or normal sperm morphology in males with idiopathic infertility after supplementation with selenium (200 µg), vitamin E (400 IU), and folic acid (5 mg) [29]. Furthermore, after the administration of 400 mg/day of vitamin E for 3 months to men from infertile couples, another study reported a statistically significant increase in progressive motility, normal morphology, and fertility rates [30]. In men with idiopathic infertility, an imbalance exists between the production of ROS and seminal fluid antioxidant defense system, resulting in OS with a negative impact on sperm quality [17]. Such men have a lower dietary intake of antioxidants, and their seminal fluid shows a lower total antioxidant capacity. In the current study, the sum of the partial improvements resulting from each compound could account for the effects of the combination of micronutrients.

The antioxidant capacity of dietary zinc has been demonstrated by several studies. The use of zinc in asthenozoospermic patients reduces the OS, apoptosis, and DNA fragmentation of their sperm and may counteract excess copper, improving sperm cell concentration and motility [12]. Selenium is an essential trace element that plays an important role in sperm formation and testosterone synthesis. Selenium-fortified probiotics were found to reduce triglyceride levels

and improve sperm count, mobility, and morphology [15]. Vitamin E (α -tocopherol) has strong antioxidant properties and inhibits the lipid peroxidation caused by free hydroxyl and superoxide radicals. This vitamin protects the cell membrane of sperm cell from damage by ROS. Vitamin C also improves human semen quality [30]. The synergistic action on the male reproductive system of the seven micronutrients used in this study could show the importance of combination therapy compared to single-supplement therapy. Variability in the dosage and usage of antioxidants may explain the differences between our findings and those of other studies.

In conclusion, the combined formulation of vitamin C, vitamin E, selenium, zinc, arginine, L-carnitine, and coenzyme Q10 improved sperm concentration, total sperm motility, progressive motility, TMSC, and DFI in men with infertility and idiopathic OAT. These effects may be due to the synergistic antioxidant action of unidentified compounds or other mechanisms that affect the production of gonadal semen and the antioxidant ability of seminal fluid.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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Conceptualization: CR, MC. Data curation: LA. Formal analysis: CR, MO, IB. Methodology: CR, MC. Project administration: MC. Visualization: CR, MO, IB, SM, AM, MC. Writing-original draft: IB. Writing-review & editing: CR, MO, SM, HT, HS, AM, MC.

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