



Superoxide Dismutase-Rich *Tetraselmis chuii* Supplementation Improves Semen Quality in Idiopathic Infertile Males: A Double-Blind Randomized Placebo-Controlled Trial

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Purpose: Male infertility is a growing but poorly understood condition, largely evaluated through sperm parameters. Oxidative stress plays a crucial role in idiopathic cases, with 30%–80% of patients showing elevated reactive oxygen species levels in seminal plasma. Antioxidants are increasingly used in reproductive medicine, but their effectiveness may be limited by the lack of prior assessment in patients. This study aimed to evaluate the efficacy of a *Tetraselmis chuii*-derived ingredient, with a high superoxide dismutase (SOD) activity (TSOD), in improving sperm quality parameters, including sperm DNA fragmentation (SDF), and static oxidation-reduction potential (sORP).

Materials and Methods: In this randomized, double-blind, placebo-controlled clinical study, a total of 80 males with idiopathic infertility were recruited and randomly assigned to receive either TSOD (n=40) or placebo (n=40) at 250 mg/d for three months. Blood and semen samples were collected at baseline and post-treatment to assess hormonal profiles, semen parameters, SDF, and sORP. Additionally, socio-demographic data and reproductive outcomes were recorded.

Results: After three months of supplementation, the TSOD group exhibited a significant increase in serum levels of luteinizing and follicle-stimulating hormone, resulting in improved sperm concentration and progressive motility. A significant reduction in both total and double-strand SDF was also observed exclusively in the TSOD group, particularly among males with initially pathological sORP values. Following a 3-month wash-out period, seminal parameters tended to return toward baseline values, while serum hormonal levels and SDF remained relatively stable. Notably, the improvements in semen quality were

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translated into superior reproductive outcomes, as the TSOD group achieved significantly higher live birth rates compared to the control group.

Conclusions: TSOD supplementation demonstrates clinical efficacy in improving sperm quality parameters and reproductive outcomes in males with oxidative stress-related idiopathic infertility. However, its use should be preceded by redox status assessment to identify patients most likely to benefit.

Keywords: Antioxidants; DNA fragmentation; Gonadal hormones; Infertility, male; Oxidative stress; Semen analysis

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INTRODUCTION

Infertility is an increasingly prevalent condition with complex, multifactorial, and poorly understood causes [1], linked to rising utilization of assisted reproductive technologies (ART). Around 3 million ART cycles are performed globally each year, driven by delayed parenthood and increasing infertility rates [1]. Numerous studies have also reported a global decline in sperm concentration and total count over recent decades, suggesting potential environmental influences [2]. Male infertility accounts for half of infertility cases; however, around 60% of these remain diagnosed as idiopathic or of unknown cause [3]. Conventional semen analysis, based on World Health Organization (WHO) guidelines [4] overlooks molecular factors with potential crucial roles in ART outcomes, with oxidative stress (OS) emerging as a key contributor to idiopathic male infertility.

Redox balance refers to the equilibrium between oxidizing (such as reactive oxygen species [ROS] or oxidized thiols) and antioxidant agents (such as free thiols and β -carotenes) [5,6]. ROS, including superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2), are naturally produced in male germ line during metabolic processes in organelles like peroxisomes and mitochondria [7]. In the seminiferous epithelium, Sertoli cells produce antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase and catalase, that protect germ cells from ROS. Furthermore, non-enzymatic antioxidants such as vitamins C and E also help balance ROS production under healthy conditions [7,8]. Once sperm leave to seminiferous tubules, they lose antioxidant protection and become vulnerable to oxidative attack [7,8].

At physiological levels, ROS are essential for the

fertilization capacity of sperm, as they represent key signalling molecules in processes such as capacitation and hyperactivation [7,9,10]. However, insufficient ROS levels impair spermatogenesis and sperm quality [9,11,12], while excessive ROS or insufficient reducing agents lead to an imbalance known as OS [4]. Between 30% and 80% of males with unexplained infertility exhibit elevated ROS levels in seminal plasma, which can damage cellular structures, including the plasma membrane, mitochondria, and chromatin, compromising sperm function [3]. Sperm cells are particularly vulnerable to oxidative damage due to their low antioxidant enzyme content, their polyunsaturated fatty acid-rich membrane and their high mitochondrial content, which generate ROS as a by-product, accumulating in the epididymis during maturation [7,10]. As the nucleus of the mature spermatozoa is mainly transcriptionally and translationally inactive [13], they are unable to synthesize *de novo* antioxidant proteins in response to oxidative insults. Furthermore, ROS excess in seminal plasma can impair sperm capacitation and fertilizing potential [10]. Numerous potential sources of OS, both intrinsic and extrinsic, are known, including diet, toxic habits, and diseases [7].

Due to the increasing number of patients with altered semen parameters, fertility clinics have increasingly turned to antioxidant supplements [14,15]. *In-vitro* studies reported the dose-dependent efficacy of vitamins C and E in protecting sperm motility and morphology by suppressing lipid peroxidation during cryopreservation. Glutathione, N-acetyl-L-cysteine, co-enzyme Q10, and albumin have been used to protect sperm motility and vitality during sperm preparation for ART. Other studies support the benefit of catalase and SOD in preserving sperm motility by mitigating ROS. Nonetheless, high doses of antioxidants in the

capacitation medium have been related to negative effects, inducing peroxidative damage to sperm [16]. Parallely, *in-vivo* studies reported improvements in semen parameters, sperm DNA fragmentation (SDF), and pregnancy rates after oral administration of vitamins C and E, either alone or with glutathione or selenium. Similarly, carnitines or pentoxifylline have also shown dose-dependent efficacy in improving sperm motility. Other studies failed to demonstrate the benefits, probably due to insufficient doses or too short treatment duration [16]. Despite this evidence, debates persist about the efficacy of antioxidants for the treatment of idiopathic infertility in male, as many studies lack placebo controls, double-blind design, or correlation of improved semen parameters with ART outcomes [14,16].

There is growing interest in microalgae-based supplements due to their high content of bioactive metabolites potentially beneficial to human health [15,16]. In 2014, the European Union approved the microalga *Tetraselmis chuii* for human consumption. A freeze-dried and natural ingredient derived from this microalgae exhibits significantly high SOD activity, over 30,000 U/g (TSOD). TSOD also contains polyunsaturated fatty acids, vitamins, carotenoids, polyphenols, and phytosterols, all of them exhibiting potential bioactivity [15,17]. *In-vitro* studies have shown that TSOD promotes antioxidant and metal-chelating responses, inhibits acetylcholinesterase, and may upregulate genes involved

in mitochondrial biogenesis and ATP synthesis [15,17]. Additionally, preliminary *in-vivo* studies reported immunostimulant and anti-inflammatory effects after 3 weeks of supplementation [15].

Despite all, there is a lack of proper screening to identify which patients would truly benefit from antioxidant supplementation, as diagnostic techniques to assess seminal OS are not yet established [4,16]. The MiOXSYS[®] system has recently emerged as a fast and simple method suitable for andrology labs [4,18]. However, its diagnostic relevance remains uncertain due to a lack of standardized protocols and clinical trials correlating this variable to improvements in sperm quality and ART outcomes [4,19,20].

The aim of this study was to evaluate: (1) the efficacy of dietary supplementation with TSOD in improving sperm quality, including SDF and static oxidation-reduction potential (sORP), and (2) the reproductive outcomes during a 6-month post-treatment follow-up period.

MATERIALS AND METHODS

1. Study design

A monocentric, prospective, randomized, double-blind, placebo-controlled clinical study was conducted with two experimental groups: a group supplemented with TSOD (n=40), and a control group supplemented with

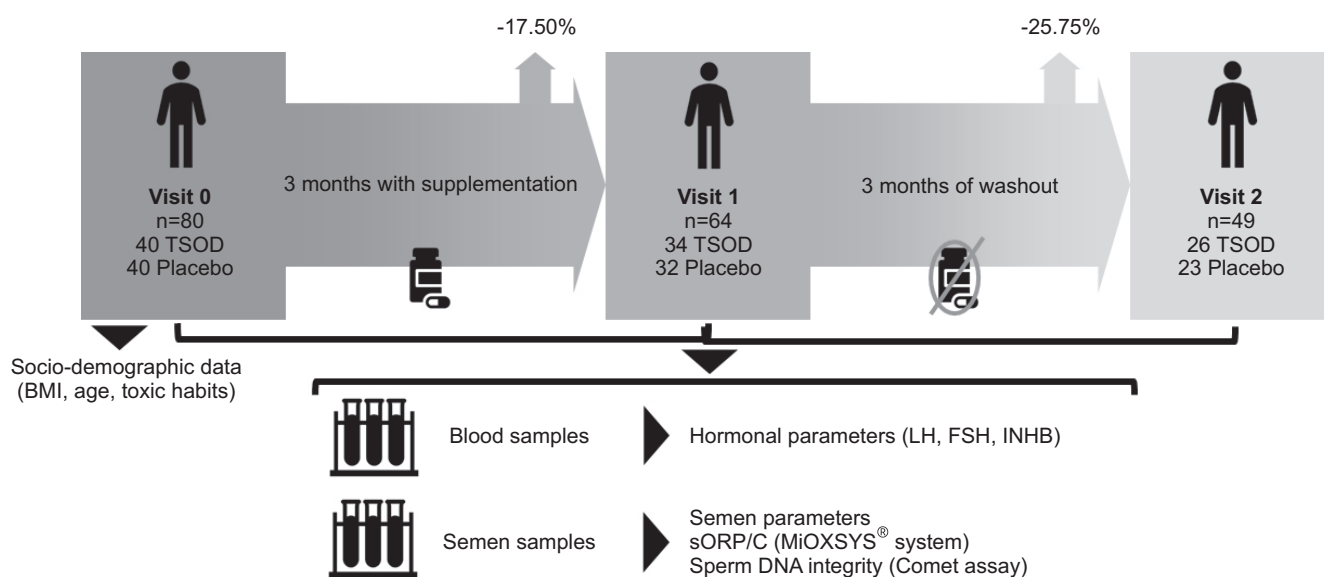


Fig. 1. Study design of present monocentric, prospective, randomized, double-blind, and placebo-controlled clinical study. TSOD: superoxide dismutase-rich *Tetraselmis chuii*, BMI: body mass index, LH: luteinizing hormone, FSH: follicle-stimulating hormone, INHB: inhibin, sORP/C: static oxidation-reduction potential normalized by sperm concentration.

placebo (n=40) (Fig. 1). The TSOD group received 250 mg/d for three months, equivalent to one spermatogenesis cycle. The control group received the same daily dose of placebo for the same period. Capsules were manufactured to be identical in appearance. After supplementation, both groups underwent a 3-month wash-out period. Clinical parameters, seminal quality, OS, and SDF were assessed in samples obtained at Visit 0 (baseline), Visit 1 (post-supplementation) and Visit 2 (post-wash-out).

2. Selection and processing of biological samples

Semen samples were obtained with informed consent from adult males (aged 18–45 years) after 3–5 days of sexual abstinence. Participants had a body mass index (BMI) under 30 kg/m² and were diagnosed with idiopathic infertility. Exclusion criteria included azoospermia, severe oligozoospermia (<5×10⁶ spermatozoa/mL), urinary or testicular disorders, hormonal or chromosomal abnormalities, oncological treatment, and recent (past 3 months) use of drugs or antioxidant supplements. Sociodemographic data (age, height, weight, and lifestyle habits, including physical activity and smoking) were recorded for all participants. Both blood and semen samples were collected at Visit 0, Visit 1, and Visit 2 to assess hormonal profiles and semen parameters. Seminograms were performed post-liquefaction following WHO guidelines [4] evaluating ejaculate volume, sperm concentration, motility, morphology, and vitality as routinely. A portion of each semen sample was used for sORP and the remainder was cryopreserved in liquid nitrogen using cryoprotectant CryoSpermTM (ORIGIO[®]; CooperSurgical[®]), following the manufacturer's instructions. Blood levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), and inhibin were routinely measured by enzyme-linked immunoassay.

3. Measurement of semen sORP using the MiOXSYS[®] system

The MiOXSYS[®] galvanostatic system (Caerus Biotechnologies) was used to measure the balance between total oxidant and reductant activity in seminal plasma. For each measurement, 30 µL of fresh ejaculate was deposited on a single-use sample holder chip. The seminal sORP was then displayed in mV on the device screen and subsequently normalized to sperm concen-

tration (sORP/C). Samples with sORP/C values >1.34 mV/10⁶ spermatozoa/mL were classified as exhibiting OS, with an upper limit of 9.00 mV/10⁶ spermatozoa/mL [6,18].

4. Evaluation of SDF with the alkaline and neutral comet assay

The comet assay, performed under both alkaline and neutral conditions, has been established as the most robust and sensitive method for detecting SDF, with the neutral assay specifically identifying double-strand DNA breaks [21,22]. Briefly, approximately 10,000 sperm from cryopreserved samples were embedded in 1% low-melting-point agarose gel and subjected to lysis. Slides were immersed in either neutral or alkaline electrophoresis buffer for 30 min, followed by electrophoresis at 22 V for 10–15 min, maintaining current below 280 mA to prevent overheating. DNA was then stained with SYBRTM Gold (InvitrogenTM, ThermoFisher scientific[®]) and visualized by fluorescence microscopy. DNA fragments migrated further than intact chromatin, forming a characteristic comet tail. The percentage of fragmented sperm was quantified by analyzing 60–120 cells per sample.

5. Determination of the success of ART

For each couple undergoing ART, reproductive outcomes during a 6-month post-treatment follow-up period were recorded and classified as spontaneous pregnancy, pregnancy achieved through *in-vitro* fertilization (IVF), pregnancy loss (miscarriage), or unsuccessful IVF attempt (Supplement Table 1). Two variables were then compared by chi² test between the TSOD and placebo groups: (1) Embryo proportion (encompassed live births from both spontaneous gestation and ART and miscarriages), and (2) Live births proportion (included live births from spontaneous gestation or ART). p-values<0.05 were considered statistically significant.

6. Statistical analysis

All statistical analyses were performed using RStudio version 4.3.2 [23]. Because not all data followed a normal distribution (determined *via* Shapiro test), Wilcoxon test was applied for pairwise comparisons. p-values<0.05 were considered statistically significant. Baseline homogeneity between the TSOD and placebo groups was evaluated for sociodemographic, hormon-

al, seminal, and molecular parameters. Intra-group analyses assessed changes from Visit 0 to Visit 1, and inter-group comparisons were conducted to identify supplementation-related effects. Patients were stratified based on baseline sORP/C into pathological and non-pathological OS groups. Finally, the dynamics of significantly altered parameters were examined after the wash-out period.

7. Ethics statement

The study protocol was approved by the Institutional Review Board of Clinical Research Ethics Committee of Clínic Barcelona Hospital (Reg. No. HCB/2020/1147) and research project was registered in a primary registry (ClinicalTrials.gov ID: NCT04864314). Written informed consents were obtained from all participants. All procedures were conducted in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments.

RESULTS

1. Baseline characteristics of the study population

Sociodemographic parameters (BMI, age, and toxic habits), hormonal levels (LH, FSH, and inhibin blood levels) and semen parameters (ejaculate volume, sperm concentration, sperm motility, morphology, and vitality) were evaluated alongside molecular parameters not typically included in routine semen analysis, such as sORP/C and SDF, in the 80 enrolled patients. A descriptive analysis at baseline showed no significant differences between the TSOD and placebo groups, confirming the homogeneity of the study population and supporting the validity of subsequent comparisons (Table 1).

Patient retention was high: between Visit 0 and Visit 1 (after three months of supplementation), the dropout rate was below 20%, with a higher loss (above 25%) to follow-up during the wash-out period (Fig. 1). Additionally, four patients were excluded from the analysis because their baseline sperm concentration and progressive motility values exceeded the WHO minimum reference thresholds [4], ensuring a homogeneous co-

Table 1. Baseline parameters in individual groups (Visit 0)

Variable	Thresholds	Placebo (n=40)	TSOD (n=40)	p-value
Age (y)	-	37.13±4.39	37.02±5.71	ns
Body mass index (kg/m ²)	-	26.35±3.81	26.49±4.36	ns
Smokers (%)	-	27.50	34.20	ns
Hormonal parameters				
Luteinizing hormone (U/L)	1.50–7.50 ^a	4.02±1.49	3.98±1.98	ns
Follicle-stimulating hormone (U/L)	1.70–8.00 ^a	5.60± 3.18	5.51±2.26	ns
Inhibin (ng/L)	120.00–400.00 ^a	188.86±70.95	166.82±70.97	ns
Seminal parameters				
Seminal volume (mL)	1.50–2.00 ^a	2.83±1.62	2.53±1.36	ns
Sperm concentration (10 ⁶ /mL)	>16.00 ^a	37.48±48.29	38.70±55.00	ns
Progressive motility (A+B) (%)	>30.00 ^a	17.43±10.89	20.30±13.63	ns
Normal morphology (%)	>4.00 ^a	5.15±3.15	5.12±3.63	ns
Vitality (%)	>58.00 ^a	63.83±12.80	58.70±14.28	ns
Molecular parameters				
sORP/C (mV/10 ⁶ /mL)	<1.34 ^b	3.26±2.95	3.10±2.90	ns
Single-strand DNA fragmentation (%)	<36.09 ^c	33.74±11.68	33.71±11.13	ns
Double-strand DNA fragmentation (%)	<27.72 ^c	28.85±13.77	29.24±12.84	ns

Values are presented as mean±standard deviation.

p-values<0.05 were considered statistically significant.

TSOD: superoxide dismutase-rich *Tetraselmis chuii*, 10⁶: millions of spermatozoa, sORP/C: static oxidation-reduction potential normalized by sperm concentration, mV: millivolts, ns: not significant.

Threshold values were established according to ^aWHO 2021 indications, ^bscientific literature, or ^cown data.

Table 2. Differences between selected variables after three months of TSOD/placebo supplementation (Visit 1) compared with baseline parameters (Visit 0)

Variable	Placebo (n=31)		p-value	TSOD (n=31)		p-value
	Visit 0	Visit 1		Visit 0	Visit 1	
Hormonal parameters						
Luteinizing hormone (U/L)	3.89±1.49	3.96±1.65	ns	3.86±2.07	4.53±2.00	0.014
Follicle-stimulating hormone (U/L)	5.38±3.12	5.25±3.03	ns	5.39±2.14	5.82±2.30	ns
Inhibin (ng/L)	190.79±71.73	174.38±65.39	0.045	171.95±75.94	165.80±61.44	ns
Seminal parameters						
Seminal volume (mL)	2.84±1.59	2.86±1.47	ns	2.44±1.25	2.54±1.24	ns
Sperm concentration (10 ⁶ /mL)	41.34±52.13	38.70±53.72	ns	36.45±52.62	37.79±49.19	ns
Progressive motility (A+B) (%)	15.74±9.00	19.62±14.59	ns	18.80±12.08	25.11±17.03	0.041
Normal morphology (%)	5.39±3.16	5.00±3.09	ns	4.93±3.74	4.32±3.61	ns
Vitality (%)	61.68±12.91	59.58±13.09	ns	59.47±16.11	61.92±10.79	ns
Molecular parameters						
sORP/C (mV/10 ⁶ /mL)	3.01±2.86	2.59±2.67	ns	3.07±3.03	3.17±3.25	ns
Single-strand DNA fragmentation (%)	35.40±11.53	31.29±10.25	ns	34.21±11.97	30.31±12.52	ns
Double-strand DNA fragmentation (%)	30.63±14.35	31.41±15.31	ns	30.34±13.58	26.60±11.28	ns

Values are presented as mean±standard deviation.

p-values<0.05 were considered statistically significant.

TSOD: superoxide dismutase-rich *Tetraselmis chuii*, 10⁶: millions of spermatozoa, sORP/C: static oxidation-reduction potential normalized by sperm concentration, mV: millivolts, ns: not significant.

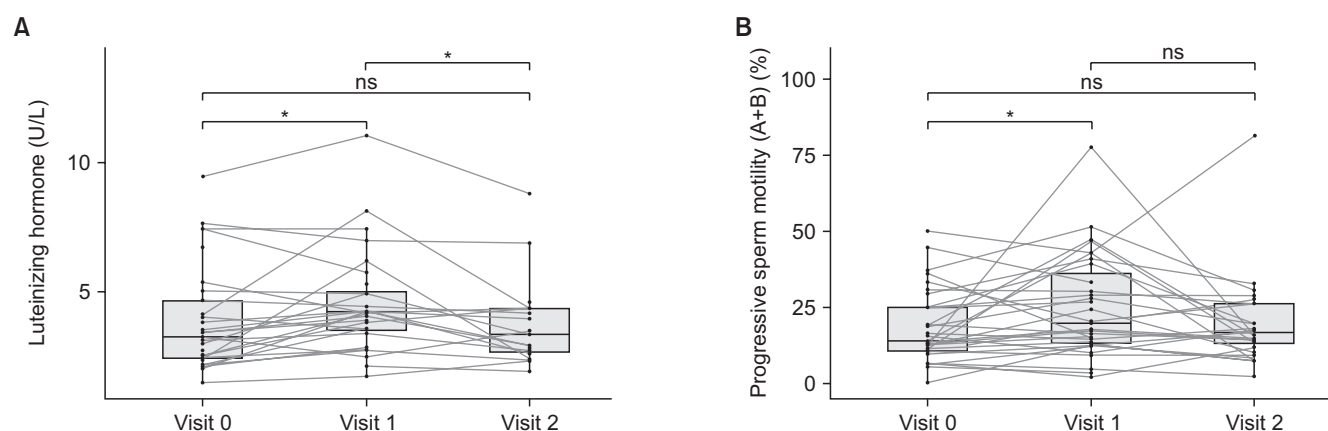


Fig. 2. Differences in blood luteinizing hormone levels (A) and progressive sperm motility (B) between baseline values (Visit 0), after three months of supplementation (Visit 1), and after three months of wash-out (Visit 2) in the TSOD group. p-values<0.05 were considered significant. TSOD: superoxide dismutase-rich *Tetraselmis chuii*, ns: not significant. *p<0.05.

hort of oligozoospermic, asthenozoospermic, or oligoasthenozoospermic patients. No adverse effects of TSOD supplementation were reported by any participant in the study.

2. Effects of TSOD supplementation on hormonal, seminal and molecular parameters

Patients who received TSOD for three months showed a significant increase in LH levels (+17%;

from 3.86±2.07 to 4.53±2.00 U/L, p<0.05) and progressive sperm motility (+33%; from 18.80%±12.08% to 25.11%±17.03%, p<0.05). These changes were not observed in the placebo group (Table 2). The placebo group exhibited a slight decrease in inhibin levels (from 190.79±71.73 to 174.38±65.39 ng/L, p<0.05) (Table 2). After the wash-out period, the improved parameters in the TSOD group regressed, returning to values not significantly different from baseline at Visit 0 (Fig. 2).

Table 3. Variables with significant improvements after three months of TSOD supplementation (Visit 1), in two cohorts according to static oxidation-reduction potential

Cohort 1: Patients with a pathologic sORP (sORP/C > 1.34 mV/10 ⁶ /mL)						
Variable	Placebo (n=20)		p-value	TSOD (n=19)		p-value
	Visit 0	Visit 1		Visit 0	Visit 1	
Luteinizing hormone (U/L)	3.77±1.36	4.01±1.08	ns	4.02±2.28	4.88±2.17	0.029
Follicle-stimulating hormone (U/L)	5.76±3.29	5.62±3.03	ns	5.39±2.01	5.92±2.16	0.024
Sperm concentration (10 ⁶ /mL)	14.32±10.53	18.33±15.51	ns	12.93±13.89	26.68±32.32	0.045
Overall DNA fragmentation (%)	34.78±12.39	32.83±11.15	ns	36.19±13.30	29.17±13.41	0.048
Double-strand DNA fragmentation (%)	31.35±15.18	34.49±17.01	ns	33.35±14.71	25.06±11.04	0.001
Cohort 2: Patients with a non-pathologic sORP (sORP/C < 1.34 mV/10 ⁶ /mL)						
Variable	Placebo (n=11)		p-value	TSOD (n=12)		p-value
	Visit 0	Visit 1		Visit 0	Visit 1	
Progressive motility (A+B) (%)	14.68±6.94	23.20±17.98	ns	16.18±10.47	21.50±14.26	0.027

Values are presented as mean±standard deviation.

p-values < 0.05 were considered statistically significant.

TSOD: superoxide dismutase-rich *Tetraselmis chuii*, sORP/C: static oxidation-reduction potential normalized by sperm concentration, mV: millivolts, 10⁶: millions of spermatozooids, ns: not significant.

3. Effects of TSOD supplementation in patients classified by baseline OS levels

We specially detected a direct and significant impact on semen quality in patients with an altered initial sORP/C (Table 3). Evaluating only those patients who presented a sORP/C over 1.34 mV/10⁶ spermatozoa/mL at Visit 0 (Table 3, Cohort 1), we found a significant increase not only in LH levels (from 4.02±2.28 to 4.88±2.17 U/L, p<0.05), but also in FSH levels (from 5.39±2.01 to 5.92±2.16 U/L, p<0.05) after TSOD supplementation. Reflecting this improvement in hormonal parameters, sperm concentration significantly doubled between Visit 0 and Visit 1 (from 12.93±13.89 to 26.68±32.32 10⁶ sperm/mL, p<0.05) and progressive sperm motility showed an upward trend (from 20.46%±12.99% to 27.39%±18.57%, p>0.05). Similarly, the proportion of cells with global SDF and, specifically, with double-stranded DNA breaks, was significantly reduced after TSOD supplementation (from 36.19%±13.30% to 29.17%±13.41%, p<0.05, and from 33.35%±14.71% to 25.06%±11.04%, p<0.01, respectively). These parameters did not show statistically significant differences in the placebo group (Table 3, Cohort 1). After three months of wash-out, sperm concentration rapidly returned to baseline levels. In contrast, the percentage of cells with double-strand DNA breaks remained at lower values, more similar to those observed at Visit 1. Other parameters showed less consistent recovery patterns, neither fully returning to baseline nor remaining as stable as double-strand DNA

fragmentation (Fig. 3).

Alternatively, those patients with an initial sORP/C considered non-pathological (<1.34 mV/10⁶ spermatozoa/mL spermatozoa/mL) who were supplemented with the antioxidant TSOD showed a significant increase only in progressive sperm motility (from 16.18%±10.47% to 21.50%±14.26%, p<0.05) (Table 3 Cohort 2 and Fig. 4A). No significant changes in sORP/C were detected (Fig. 4B).

4. Higher live birth rates after TSOD supplementation

Within the entire cohort, fertility outcomes were available for 49 patients: 27 in the TSOD group and 22 in the placebo group. Among patients with known fertility outcomes within the first three months post-supplementation, 63% of the TSOD group achieved spontaneous pregnancy or successful ART cycles (including miscarriages), compared to 28% of the placebo group. Similar trends were observed for live birth rates (Fig. 5A). During the 6-month post-supplementation period, comparable patterns emerged for embryo generation outcomes. However, pronounced differences in live birth rates were observed, with 66% of patients in the TSOD group achieving offspring compared to 21% in the control group (p<0.05) (Fig. 5B).

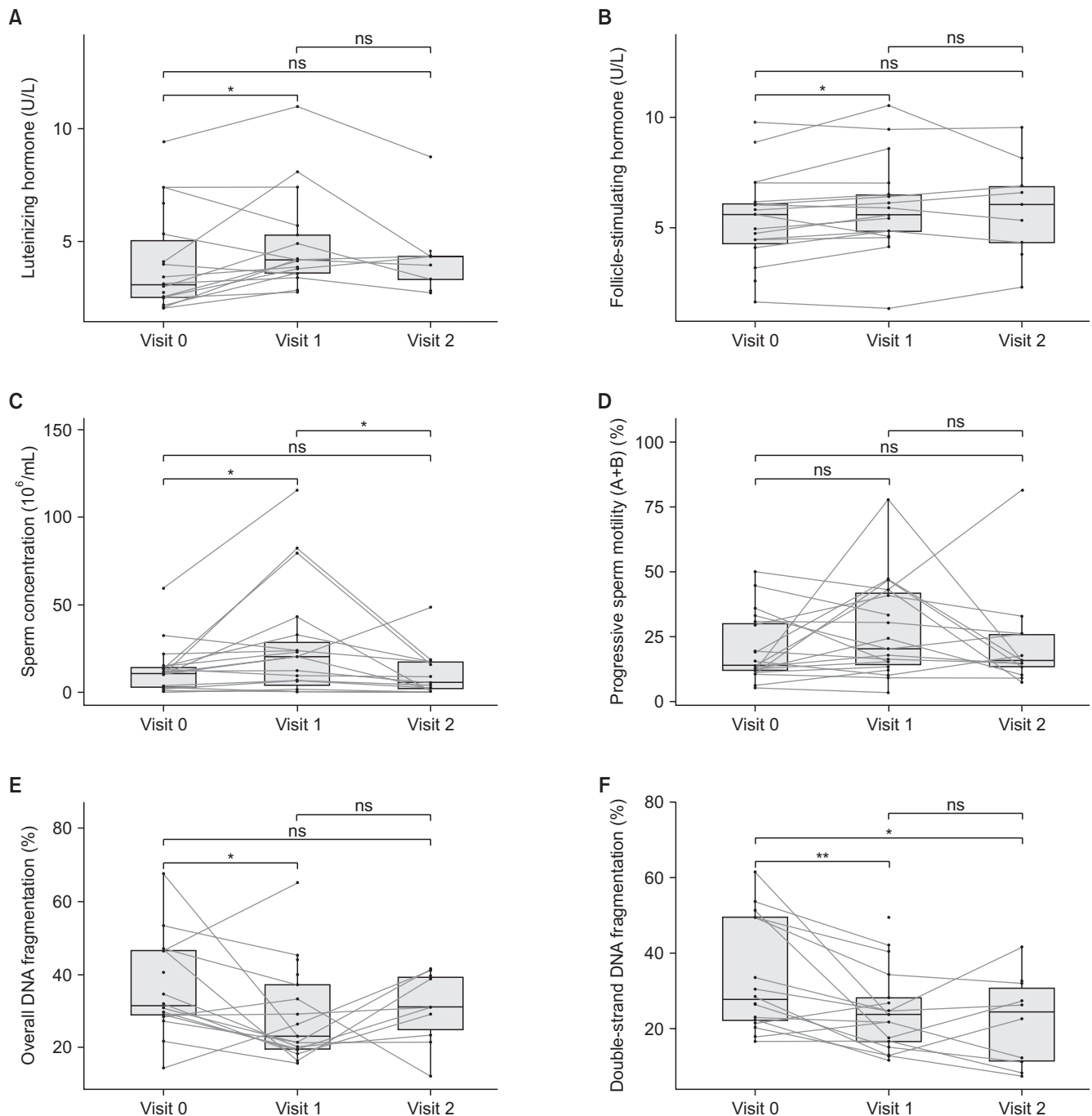


Fig. 3. Differences in blood luteinizing hormone levels (A), blood follicle-stimulating hormone level (B), sperm concentration (C), progressive sperm motility (D), overall DNA fragmentation (E), and double-strand DNA fragmentation (F) between baseline values (Visit 0), after three months of supplementation (Visit 1) and after three months of wash-out (Visit 2) in the TSOD group, selecting only those patients with a pathologic sORP (sORP/C > 1.34 mV/ $10^6/\text{mL}$). p-values < 0.05 were considered significant. TSOD: superoxide dismutase-rich *Tetraselmis chuii*, sORP/C: static oxidation-reduction potential normalized by sperm concentration, mV: millivolts, 10^6 : millions of spermatozoa, ns: not significant. *p < 0.05 and **p < 0.01.

DISCUSSION

This study confirms the clinical efficacy of TSOD, a marine microalgae-derived antioxidant supplement, in improving semen quality in patients with idiopathic

infertility [24], assessing molecular parameters such as SDF or sORP/C for the first time. We have determined and report novel correlations on antioxidant supplementation, SDF and sORP/C and reproductive outcomes. OS is widely recognized as a key contributor

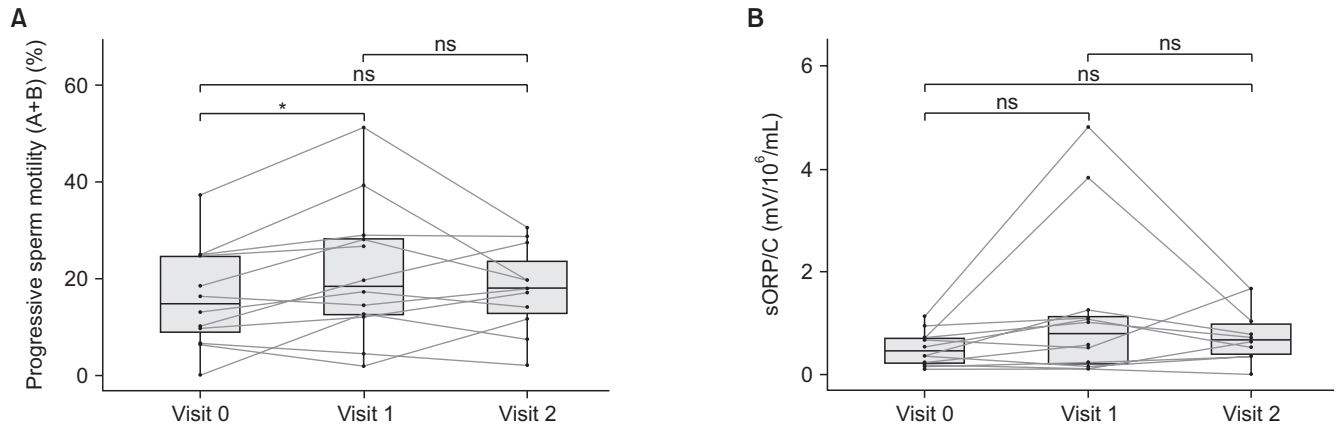


Fig. 4. Differences in progressive sperm motility (A) and static oxidation-reduction potential (sORP/C) (B) values between baseline (Visit 0), after three months of supplementation (Visit 1), and after three months of wash-out (Visit 2) in the TSOD group, selecting only those patients with a non-pathologic sORP (sORP/C < 1.34 mV/10⁶/mL). p-values < 0.05 were considered significant. TSOD: superoxide dismutase-rich *Tetraselmis chuii*, sORP/C: static oxidation-reduction potential normalized by sperm concentration, mV: millivolts, 10⁶: millions of spermatozoa, ns: not significant. *p < 0.05.

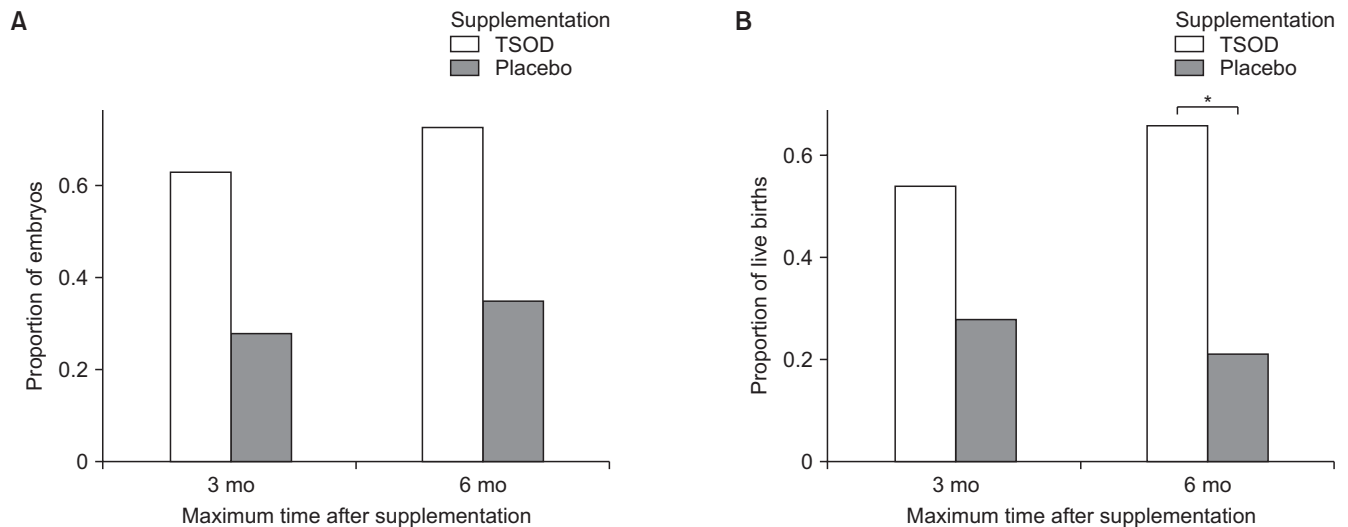


Fig. 5. Clinical outcomes by groups (TSOD and placebo) and maximum time point after supplementation. Embryo proportion (A) refers to spontaneous pregnancy or successful ART cycle, including those ending in miscarriages. Live birth rates (B) encompasses spontaneous gestation or successful ART ending with live birth. p-values < 0.05 were considered significant. TSOD: superoxide dismutase-rich *Tetraselmis chuii*, ART: assisted reproductive technologies. *p < 0.05.

to male infertility. Chronic exposure to ROS has been shown to disrupt the hypothalamic-pituitary-testicular axis, leading to a reduction in gonadotropins such as LH and FSH and ultimately impairing testosterone production and spermatogenesis [25]. Furthermore, continuous exposure to ROS damages testicular and sperm cell structures [7,10] and alters gene transcription and enzymatic function within the testes and accessory glands [26,27]. In the present manuscript, a significant increase in LH levels after three months of TSOD supplementation was observed, consistent with results from previously reported studies after antioxidant

supplementation [28,29]. Interestingly, in patients with initial redox imbalance, FSH levels also increased after supplementation. Despite a slight decrease in inhibin levels in both groups, this parameter showed high variability, making its interpretation less clear. These hormonal improvements were accompanied by enhanced sperm concentration and progressive motility in the TSOD-supplemented patients.

At the molecular level, TSOD supplementation improved sperm DNA integrity. Specifically, it reduced the proportion of sperm cells with double-strand DNA breaks in patients with an initial redox imbalance.

These findings are consistent with previous studies demonstrating that TSOD enhances the transcription and activity of endogenous antioxidant enzymes, and stimulates biosynthesis and recycling of glutathione, a key cellular antioxidant peptide that protect cells against free radicals [15,17]. Moreover, potential activation of DNA repair mechanisms by TSOD cannot be ruled out, as this antioxidant has been demonstrated to upregulate two key cellular factors, NRF2 and SIRT1, both involved in the repair of double-strand DNA breaks [15].

Seminal parameters such as motility and concentration appear to be particularly sensitive to changes in redox balance [9,11,12]. In this study, both parameters exhibited significant improvements following TSOD supplementation but tended to regress toward baseline values once supplementation was discontinued. Unlike seminal parameters, sperm DNA integrity after supplementation appeared more sustained over time, possibly due to regulation by more complex and stable physiological mechanisms.

Importantly, these molecular and functional improvements translated into enhanced reproductive outcomes. Patients supplemented with TSOD showed higher embryo generation rates (including live births from spontaneous gestation and IVF, as well as miscarriages) and a significantly greater proportion of live births within six months post-supplementation compared to the placebo group. Notably, of the six spontaneous pregnancies recorded, five occurred in males who had received TSOD. These results could reflect the reduction of sperm with DNA fragmentation, as lower fragmentation rates have been associated with improved pregnancy and live birth outcomes in previous studies [14,16].

It is worth noting that TSOD supplementation was well tolerated, even in males with normal OS levels at baseline. However, the general clinical use of antioxidants should be approached with caution, as not all males with idiopathic infertility benefit equally, underscoring the importance of individualized treatment. It should be also considered that ROS play a physiological role in sperm capacitation and hyperactivation [10-12,16], and excessive antioxidant intake could theoretically lead to reductive stress, impairing sperm function. TSOD, being a potent natural antioxidant, has been shown to induce a homeostatic response in human tissues by modulating only the cellular responses that

require enhancement [15]. Therefore, it is unlikely to interfere with redox-mediated cellular signalling and processes after supplementation. Nevertheless, a thorough clinical assessment of redox status in each patient is essential before initiating antioxidant therapy. Additionally, healthy lifestyle habits, such as regular exercise, have independently been shown to improve semen parameters and reduce OS [3,30], suggesting that antioxidant supplementation should be considered as part of a broader reproductive health strategy.

While these are promising results, sample size of this study was modest. Larger, multicentre trials are needed to confirm the durability of these effects and to evaluate the optimal duration and dosing of TSOD supplementation. Furthermore, future studies should investigate whether combining TSOD therapy with lifestyle interventions could yield additive or synergistic benefits.

CONCLUSIONS

TSOD supplementation improved hormonal profiles, semen quality, and sperm DNA integrity in males with idiopathic infertility, particularly in those with elevated OS. These molecular and seminal improvements translated into higher pregnancy and live birth rates. However, the effects on semen parameters appeared to be transient, suggesting that sustained supplementation or additional interventions may be necessary to maintain reproductive benefits.

Conflict of Interest

Lalia Mantecón, Carlos Infante, and Carlos Unamunzaga are employees of Fitoplancton Marino, S.L., El Puerto de Santa María, Cádiz, Spain, the funding company. Eva González is employee of Fertipharm S.L., Barcelona, Spain, the company to which the product belongs. Nevertheless, none of them made any influence on this work in relation with the company or its products. Other authors have no potential conflicts of interest to disclose.

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Author Contribution

Conceptualization: CI, CU, EG, RO, JMC, MJ. Data curation: ML, MJ, JC, EG. Formal analysis: ML, MJ. Funding acquisition: LM, CI, CU, EG, JMC, MJ. Investigation: MJ, EG, ML. Methodology: ML, DW, MG, RF, AD, LN. Project administration: MJ, JMC, RO, EG, LM, CI, CU. Resources: DM, JMC, MG, EG. Software: ML, MJ. Supervision: MJ, EG, RO, JMC, JC, LM, CI, CU. Validation: MJ, EG, ML, LM, CI, CU, JMC, DM. Visualization: ML, MJ, JC, DW, RO, EG, CI, CU. Writing – original draft: ML, MJ. Writing – review & editing: ML, MJ, EG, JC, RO, LM, CI, CU, DW, DM, JMC, MG, RF, AD, LN.

Supplementary Materials

Supplementary materials can be found *via* <https://doi.org/10.5534/wjmh.250385>.

REFERENCES

- European IVF Monitoring Consortium (EIM) for the European Society of Human Reproduction and Embryology (ESHRE); Smeenk J, Wyns C, De Geyter C, Kupka M, Bergh C, Cuevas Saiz I, et al. ART in Europe, 2019: results generated from European registries by ESHRE. *Hum Reprod* 2023;38:2321-38.
- Levine H, Jørgensen N, Martino-Andrade A, Mendiola J, Weksler-Derri D, Mindlis I, et al. Temporal trends in sperm count: a systematic review and meta-regression analysis. *Hum Reprod Update* 2017;23:646-59.
- 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.
- World Health Organization (WHO). WHO laboratory manual for the examination and processing of human semen. 6th ed. WHO; 2021.
- Nikolaidis MG, Margaritelis NV. Free radicals and antioxidants: appealing to magic. *Trends Endocrinol Metab* 2023;34:503-4.
- Agarwal A, Roychoudhury S, Bjugstad KB, Cho CL. Oxidation-reduction potential of semen: what is its role in the treatment of male infertility? *Ther Adv Urol* 2016;8:302-18.
- Fatima S. Role of reactive oxygen species in male reproduction. In: Atukeren P, editor. *Novel prospects in oxidative and nitrosative stress*. InTech; 2018;71-91.
- Bauché F, Fouchard MH, Jégou B. Antioxidant system in rat testicular cells. *FEBS Lett* 1994;349:392-6.
- Lee D, Moawad AR, Morielli T, Fernandez MC, O'Flaherty C. Peroxiredoxins prevent oxidative stress during human sperm capacitation. *Mol Hum Reprod* 2017;23:106-15.
- 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.
- Moustakli E, Zikopoulos A, Skentou C, Katopodis P, Domali E, Potiris A, et al. Impact of reductive stress on human infertility: underlying mechanisms and perspectives. *Int J Mol Sci* 2024;25:11802.
- Sadeghi N, Boissonneault G, Tavalae M, Nasr-Esfahani MH. Oxidative versus reductive stress: a delicate balance for sperm integrity. *Syst Biol Reprod Med* 2023;69:20-31.
- Castillo J, Jodar M, Oliva R. The contribution of human sperm proteins to the development and epigenome of the pre-implantation embryo. *Hum Reprod Update* 2018;24:535-55.
- Mayorga-Torres BJM, Jodar M, Leiva M, Manau D, Asencio AA, Oliva R, et al. [Current perspective on the clinical use of antioxidants for the treatment of male infertility]. In: Parra GC, Calonge RN, Vazquez-Levin MH, editors. *[Male reproductive health]*. Yao Díaz; 2023;388-402. Spanish.
- Cocksedge SP, Mantecón L, Castaño E, Infante C, Bailey SJ. The potential of superoxide dismutase-rich tetraselmis chuii as a promoter of cellular health. *Int J Mol Sci* 2025;26:1693.
- Agarwal A, Nallella KP, Allamaneni SS, Said TM. Role of antioxidants in treatment of male infertility: an overview of the literature. *Reprod Biomed Online* 2004;8:616-27.
- Ramírez P, Torres S, Lama C, Mantecón L, Unamunzaga C, Infante C. TetraSOD[®] activates the antioxidant response pathway in human cells: an in vitro approach. *Afr J Biotechnol*

- nol 2020;19:367-73.
18. Agarwal A, Panner Selvam MK, Arafa M, Okada H, Homa S, Killeen A, et al. Multi-center evaluation of oxidation-reduction potential by the MiOXSYS in males with abnormal semen. *Asian J Androl* 2019;21:565-9.
19. Homa ST, Vassiliou AM, Stone J, Killeen AP, Dawkins A, Xie J, et al. A comparison between two assays for measuring seminal oxidative stress and their relationship with sperm DNA fragmentation and semen parameters. *Genes (Basel)* 2019;10:236.
20. Henkel R, Morris A, Vogiatzi P, Saleh R, Sallam H, Boitrelle F, et al. Predictive value of seminal oxidation-reduction potential analysis for reproductive outcomes of ICSI. *Reprod Biomed Online* 2022;45:1007-20.
21. Ribas-Maynou J, García-Peiró A, Abad C, Amengual MJ, Navarro J, Benet J. Alkaline and neutral Comet assay profiles of sperm DNA damage in clinical groups. *Hum Reprod* 2012;27:652-8.
22. Simon L, Carrell DT. Sperm DNA damage measured by comet assay. *Methods Mol Biol* 2013;927:137-46.
23. RStudio Team. RStudio. Version 4.3.2. [Internet]. Posit USA; c2020 [cited 2025 May 5]. Available from: <https://posit.co/downloads/>
24. Corral JM, Jodar M, Matheu R, González E, Castaño E, Unamunzaga C, et al. Improvement of semen quality in idiopathic infertile men by dietary supplementation with TetraSOD®: a pilot clinical trial. *Biomed J Sci & Tech Res* 2023;50:41971-5.
25. Lei T, Yang Y, Yang WX. Luteinizing hormone regulates testosterone production, leydig cell proliferation, differentiation, and circadian rhythm during spermatogenesis. *Int J Mol Sci* 2025;26:3548.
26. Agarwal A, Durairajanayagam D, Halabi J, Peng J, Vazquez-Levin M. Proteomics, oxidative stress and male infertility. *Reprod Biomed Online* 2014;29:32-58.
27. Sharma R, Agarwal A, Mohanty G, Hamada AJ, Gopalan B, Willard B, et al. Proteomic analysis of human spermatozoa proteins with oxidative stress. *Reprod Biol Endocrinol* 2013;11:48.
28. Saylam B, Çayan S. Do antioxidants improve serum sex hormones and total motile sperm count in idiopathic infertile men? *Turk J Urol* 2020;46:442-8.
29. Slowikowska-Hilczek J, Walczak-Jedrzejowska R, Dobronski P. The influence of a combination of lifestyle modification and a new formula supplement with antioxidative and antioestrogenic activity on mild idiopathic abnormalities of semen parameters-a pilot study. *Andrologia* 2022;54:e14279.
30. Hajizadeh Maleki B, Tartibian B. Combined aerobic and resistance exercise training for improving reproductive function in infertile men: a randomized controlled trial. *Appl Physiol Nutr Metab* 2017;42:1293-306.