Effect of Vit. C, Vit. E and yeast on sperm parameters: a friend or foe?!

Authors

Ahmed Samy Saad1,2, Sahar Afify Eissa2 Tamer M. Assar1 Aziza ali negm1

1Department of Obstetrics and Gynecology, Faculty of Medicine, Banha University,
2Hawaa Fertility Center, Banha, Egypt

drahmedsaad@live.com
Aziza.negm@fmed.bu.edu.eg
Tamer.assar2000@gmail.com
saharafify@yahoo.com

Abstract

Objective: Comparing different effects of supplementation of Ham’s F10 media with vitamin E, Baker’s Yeast (Saccharomyces cerevisiae) extraction and different concentrations of vitamin C on sperm parameters.

Patients and methods: Forty samples of ejaculated semen from infertile males were collected. Six equal fractions of each semen sample were created. A Control group (activated with Ham’s F10 only). 5 study groups were activated with Ham’s F10 plus: Group 1: Vit E (conc. 2 mg/ml Ham’s F10), groups 2,3,4 (Vit C1, Vit C2 and Vit C3) (conc.0.02, 0.04, 0.06 mg/ml Ham’s F10, respectively ) , Group 6: Yeast ( 20 mg/ml Ham’sF10).

Results: The treated groups showed considerable improvements over the control group in vitality, total motility, progressive motility, and oxidative stress. Comparable results were found between the Vit. E group (72%, 45%, 95%& 0) and the Yeast group (66%, 39%, 95%& 0), and the best result for Vit. C was with conc. (0.06 mg/ml) in group Vit. C3(65%,39%, 95%& 0).

Conclusions: Vitamin C, vitamin E, and yeast all have positive effects on semen parameters. Vit.E has the best results than Vit. C and yeast. The best concentration of Vit. C to achieve results is 0.06 mg/ml Ham’s F10.

Keywords: Reactive oxygen species (ROS); Antioxidant activities (AOA); Baker’sYeast; Vitamin C, Vitamin E; in-vitro sperm activation.

This trial: was registered on clinical trials.gov (identifier: NCT02814695).
Introduction

Worldwide, between 13 and 20% of couples experience infertility, irrespective of race or ethnicity. (1). Male infertility is estimated to account for between 25 and 50% of couple infertility (2). 30% to 80% of patients with male infertility have elevated reactive oxygen species (ROS) in their ejaculate. Oxidative stress is a condition characterized by an increase in ROS production and a decrease in the ability of biological mechanisms to quickly neutralize the reactive intermediates or restore the damage (3).

It is well-established that a minute amount of ROS is necessary for the steps involved in the crucial physiological reaction of fertilization, such as sperm maturation, hyperactivation, capacitation, sperm acrosomal reaction, and sperm-oocyte fusion. (4). Conversely, sperm oxidative stress (OS) is associated with a decline in sperm motility, decreased acrosome reaction, DNA damage and lower levels of implantation for in vitro fertilization (5).

The robust association between oxidative stress (OS) and male infertility has led researchers to suggest the term “Male Oxidative Stress Infertility (MOSI)” to express OS-associated male infertility (6).

Antioxidants serve as free radical scavengers, in order to shield sperm from ROS. Enzymatic antioxidants include superoxide dismutase (SOD), catalase, and peroxidase glutathione (GPX). Nevertheless, semen includes a mixture of non-enzymatic antioxidants such as vitamin C and E, pyruvate, glutathione, and carnitine and peroxidase glutathione (GPX), which decrease endogenous restore mechanisms and enzymatic defenses (7).

OS is managed by sperm selection techniques that remove sperm with oxidative DNA damage. These include intracytoplasmic morphologically selected sperm injection, electrophoretic separation, density gradient centrifugation (DGC), annexin-V magnetic-activated cell separation and hyaluronic acidbinding assay(8).

Numerous papers have demonstrated that a shorter time between ejaculatory abstinence lead to a poorer seminal reactive oxidative species and sperm DNA fragmentation.
index (DFI), thereby increasing sperm motility. A shorter period between ejaculatory abstinence may recover DNA integrity and sperm quality by reducing sperm exposure to excessive ROS in the epididymis (9).

Antioxidants such as lycopene, carnitine, vitamin C and E, zinc, selenium and coenzyme Q10 have been found valuable in balancing ROS production and scavenging activities. Oral antioxidants such as vitamins C and E eliminate ROS and improve sperm parameters and pregnancy outcomes in patients with OS and SDF. (10).

Reviewing the Cochrane database, 61 randomized controlled trials comparing the effects of antioxidants and placebo in a population of 6,264 infertile men were conducted. The findings showed that antioxidants have the potential to increase clinical pregnancy and live birth rates (11).

Vitamin C alone (400-1000 mg per day) (12), vitamin E alone (300-600 mg per day) (13), or a combination of vitamin C and E are examples of classic antioxidants taken orally. Vitamins C and E interact synergistically, and a number of studies have documented the beneficial effect of complex antioxidants on lowering SDF and increasing clinical pregnancy rates (14).

Zinc is required for spermatogenesis and the synthesis of sperm DNA. It also prevents lipid peroxidation (LPO) and acts as a component of antioxidant enzyme superoxide dismutase (15). Selenium is also a crucial component of the glutathione peroxidase GPX selenoproteins (16). Meta-analysis and other studies showed that both L-Carnitine and coenzyme Q10 improved conventional sperm parameters (17).

Saccharomyces cerevisiae (Baker's yeast) include numerous enzymatic antioxidants such as: SOD, peroxidase, catalase, glutathione s-transferase (18) as well as, non-enzymatic antioxidants like apiquinone, glutathione, mineral ions and sulphydryl amino acid (19). The carboxymethylated (1-3) p-glucan (CMG) in the cell wall of Sacch. cerevisiae can inhibit lipid peroxidation in liposomes caused by hydroxyl radicals (20). S. cerevisiae is a probiotic and has been used habitually as an antidiabetic, neuroprotective, antioxidant, anti-inflammatory, immune booster, antimalarial, and antitumoral (21).
A positive effect of vitamin E on sperm viability and motility has been demonstrated at various dosages and incubation times using Camel spermatozoa (22). Vitamin E is an extremely efficient free radical scavenger. Vitamin E is localized in the cell membrane; therefore, it cannot defend the cytosol from free radicals; its counterpart "selenium" is responsible for cytosol protection. Vitamin E boosts intracellular ATP levels and reduces cell permeability and enzyme inactivation (23).

Sperm preparation process for ART, may induce OS. Vitamin E supplementation in vitro may prevent spermatozoa from the harmful effects of oxidative stress during sperm processing by maintaining normal antioxidant processes (24).

Vitamin C is one of the most important antioxidants in seminal fluid. The concentration of vitamin C in seminal plasma is more than 10 times of its concentration in blood plasma. This gives a clue about its role in semen (25).

**Aim of the work:** Is to look for the best concentration of vitamin C which would improve semen parameters in infertile asthinozoospermic men versus vitamin E and Baker’s yeast.

**Statistical methods:**
Using SPS version 25, data administration and statistical analysis were performed (IBM, Armonk, New York, United States). Using the Shapiro-Wilk test and direct data visualization methods, quantitative data is analyzed for normality. In accordance with the principle of normality, numerical data were summarized as standard deviations, means, or medians and ranges. Using repeated-measures ANOVA or Friedman's test, quantitative data for various preparations were compared. Bonferroni's method was applied to post hoc analyses. All statistical analyses had two sided. P values below 0.05 were considered statistically significant.
RESULTS

Semen characteristics according to different vitamin C concentrations:

Sperm motility showed an overall significant difference between different preparations (P < 0.001). Post hoc analyses revealed that it was significantly higher in vitamin C3 samples (65%) than in control (59%), vitamin C1 (59%) and vitamin C2 (62%) samples. In contrast, sperm motility in control and vitamin C1 samples were significantly lower than in vitamin C2 samples (62%) (Table 1).

Progressive motility showed an overall significant difference between different preparations (P < 0.001). Post hoc analyses revealed that it was significantly higher in vitamin C3 samples (39%) than in control (30%), vitamin C1 (31%) and vitamin C2 (35%) samples. In contrast, progressive motility in control samples was significantly lower (30%) than in vitamin C1 (31%) and C2 (35%) samples. Also, progressive motility in vitamin C1 samples was significantly lower (31%) than in vitamin C2 samples (35%) (Table 1).

Vitality showed an overall significant difference between different preparations (P < 0.001). Post hoc analyses revealed that it was significantly lower in control samples (94%) than in vitamins C1, C2, and C3 samples (95% for each). Also, vitamin C1 samples showed a lower progressive motility (31%) than in vitamin C2 (35%) and C3 (39%) samples. In addition, lower progressive motility was reported in vitamin C2 samples (35.0%) than in vitamin C3 samples (39%) (Table 1).

The median oxidative stress level showed an overall significant difference between different preparations (P < 0.001). Post hoc analyses revealed that it was significantly lower in vitamin C3 samples (0) than in control (2), vitamin C1 (1), and vitamin C2 (2) samples. In contrast, it was significantly higher in control samples (2) than in vitamins C1 and C2 samples (1 for each) (Table 1).

So, we decided to use C3 as a representative of the vitamin C groups.
**Patients and methods**

From August 2020 to November 2021, the study's fieldwork was conducted in a private fertility center in Banha, Qalyubiya, Egypt. The study included 40 infertile male caes. The inclusion criteria were as follows: (i) Unable to conceive their wives after 1 year of marriage; (ii) Must not have received an antibiotic in the past four weeks; (iii) Aged between 22 and 35 years; (iv) Asthinozoospermic cases. Exclusion criteria: severe oligospermic cases and necrozoospermia.

**Seminological analysis**

Semen and sperm characteristics were analyzed using a plastic container with a large opening and the masturbation method from 40 infertile patients who agreed to participate in the study and had abstained from sexual activity for 3-7 days [17]. Samples were allowed to liquefy for 20 minutes before being analyzed for viscosity, volume, spermatozoa count using a Neubauer hemocytometer, total sperm motility, progressive and non-progressive motility, oxidative stress level (measured using Oxisperm; Modern Bio-Systems; Spain), and sperm vitality using an eosin-negrosin stain (fertipro; Belgium).

**In-vitro antioxidant activation**

The semen specimens were divided into six equal fractions:

- **a. Control** (1st fraction) 0.1ml of liquefied semen was mixed with 0.1ml Ham’s F10 medium and incubated at 37°C for 30 minutes.

- **b. Vitamin E** (2nd fraction)

  A powder of Vitamin E (α-tocopherol) (Sigma, Germany) is dissolved in Ham’s F10 to reach a conc. of (2 mg/ml). 0.1ml liquefied semen was mixed with 0.1ml Ham’s F10 medium supplemented with (2 mg/ml) and incubated at 37°C for 30 minutes.

- **a. Vitamin C** (3rd, 4th, 5th fraction)

  A powder of Vitamin C (L-ascorbic acid) (Sigma, Germany) is dissolved in Ham’s F10 to have 3 aliquots with 3 conc. (0.02, 0.04, 0.06 mg/ml). Three fractions of 0.1ml liquefied semen, each was mixed with 0.1ml Ham’s F10 medium supplemented with Vit. C, as Vit C1,2,3 (0.02, 0.04, 0.06 mg/ml), respectively and incubated at 37°C for 30 minutes.
C. Antioxidant producing Yeast: (6th fraction)

This study utilized the yeast strain Sacch. Cerevisiae ATCC 58523 from the Egypt Microbiology Culture Collection (Cairo MIRCEN, Fac. of Agric., Ain Shams Univ., Cairo, Egypt).

Growth of Yeast and preparation of cell extract:

The yeast was inoculated into malt extract broth medium and incubated at 25°C for four days, after which the cells were harvested and resuspended in lyses buffer (50 mM K-phosphate "pH 7.0", 1 mM PMSF, 0.5 mM EDTA). 15 cycles of 1 minute of vortexing with 1 volume of glass beads (0.5 mm) followed by 1 minute of cooling on ice were used to disrupt cells. Cell debris was removed by centrifugation at 6,000 rpm for 10 minutes, and the precipitate was rinsed with saline puffer (40 g/100 ml dist. water) before being acidified with HCl and cultured at a pH of 4.5. The resulting ppt (Lysed cells) and cell suspension (yeast extraction (YE)) were stored overnight in the refrigerator after a one-hour water bath at 65 °C.

A mixture of 0.1 ml of liquefied semen and 0.1 ml of Ham's F10 medium with 20 mg/ml of yeast extract was incubated at 37°C for 30 minutes.

Total motility, vitality, progressive motility, and amount of oxidative stress (OS) were evaluated 30 minutes after treating semen specimens across all fractions.

Table(1) Semen characteristics according to different vitamin C concentrations

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Vitamin C1</th>
<th>Vitamin C2</th>
<th>Vitamin C3</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Sperm motility</td>
<td>59±11^3,4</td>
<td>59±10^3,4</td>
<td>62±9^1,2,4</td>
<td>65±9^1,2,3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Progressive motility</td>
<td>30±7^2,3,4</td>
<td>31±6^1,3,4</td>
<td>35±6^1,2,4</td>
<td>39±7^1,2,3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Non-progressive motility</td>
<td>29±9^2,3,4</td>
<td>28±8^1,4</td>
<td>27±7^1,4</td>
<td>25±7^1,2,3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vitality (%)</td>
<td>94±1.8^2,3,4</td>
<td>95±1.6^1</td>
<td>95±1.8^1</td>
<td>95±1.9^1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Oxidative stress level</td>
<td>2(0-4)^1,2,4</td>
<td>1(0-3)^1,4</td>
<td>1(0-3)^1,4</td>
<td>0(0-2)^1,2,4</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data was presented as mean and SD except for oxidative stress level which was presented as median and range. Repeated measures ANOVA was used. Friedman's test was used for oxidative stress level. Post-hoc analyses were done using Bonferroni's method. 1: significantly different from control, 2: significantly different from vitamin C1, 3: significantly different from vitamin C2; 4: significantly different from vitamin C3.
Semen characteristics in the rest of preparations versus vitamin C3 group

Sperm motility showed an overall significant difference between different preparations (P <0.001). Post hoc analyses revealed that it was significantly higher in vitamin E samples (72%) than in control, vitamin C3, and yeast samples (59%, 65%, 66%, respectively). In contrast, sperm motility in control samples was significantly lower (59%) than in vitamin C3 (65%) and yeast (66%) samples (Table 2).

Progressive motility showed an overall significant difference between different preparations (P<0.001). Post hoc analyses revealed that it was significantly higher in vitamin E samples (45%) than in control, vitamin C3, and yeast samples (30%, 39%, and 39%, respectively). In contrast, sperm motility in control samples was significantly lower (30%) than in vitamin C3 and yeast samples (39 for each%) (Table 2).

Vitality showed an overall significant difference between different preparations (P <0.001). Post hoc analyses revealed that it was significantly lower in control samples (94%) than in vitamin C3, vitamin E, and yeast samples (95% for each) (Table 2).

The median oxidative stress level showed an overall significant difference between different preparations (P < 0.001). Post hoc analyses revealed that it was significantly higher in control samples (2) than in vitamin C3, vitamin E, and yeast samples (0 for each) (Table 2).
Table (2) Semen characteristics in different preparations

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>vitaminC3</th>
<th>VitaminE</th>
<th>Yeast</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Sperm motility</td>
<td>59±112,3,4</td>
<td>65±91,3</td>
<td>72±81,2,4</td>
<td>66±71,3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Progressive motility(%)</td>
<td>30±72,3,4</td>
<td>39±71,3</td>
<td>45±61,2,4</td>
<td>39±51,3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Non-progressive motility(%)</td>
<td>29±92,3</td>
<td>25±71</td>
<td>26±51</td>
<td>27±5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vitality(%)</td>
<td>94±22,3,4</td>
<td>95±21</td>
<td>95±21</td>
<td>95±21</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Oxidative stress level</td>
<td>2(0-4)2,3,4</td>
<td>0(0-2)1</td>
<td>0(0-1)1</td>
<td>0(0-2)1</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data was presented as mean and SD except for oxidative stress level which was presented as median and range. Repeated measures ANOVA was used. Friedman’s test was used for oxidative stress. Post-hoc analysis was done using Bonferroni’s method. 1: significantly different from after 1h, 2: significantly different from vitamin C3, 3: significantly different from vitamin E, 4: significantly different from yeast.
Discussion

Human spermatozoon has low antioxidant enzymes capacity and is unable to effectively deal with oxidative stress; consequently, oxygen-free radicals produced by spermatozoa and leucocytes can contribute to a decrease in intracellular ATP levels, which negatively impacts sperm motility. In addition, the sperm membrane contains polyunsaturated fatty acids (PUFA) that may serve as a catalyst for lipid peroxidation (26).

Compounds containing reactive oxygen species (ROS) can be released from the mitochondria and middle region of spermatozoa and gain access to the chromatin structure in the sperm head. Oxygen-free radicals, such as hydrogen peroxide, can damage the sperm nucleus due to their ability to penetrate the membrane surrounding the nucleus (27).

Kobayashi et al. also showed a relation between a decline in the number of live & active spermatozoa and increased ROS levels (28). Increasing the Malondialdehyde (MDA) enzyme causes disorder in cell membranes, which disrupts the transfer of ionic and chemical mediators as well as the ionic gradient on both sides of the membrane. (29, 30).

Vitamin C in different concentrations (0.02, 0.04, 0.06 mg/ml) was evaluated in the present study. Our results indicate that vitamin C in conc. (0.06 mg/ml) could significantly improve semen parameters (65%, 39%, 95%, 0) for total motility, progressive motility, vitality, level of oxidative stress (OS), respectively. In comparison to control were (59%, 30%, 94%, 2).

Vitamin C is currently used in-vitro to improve spermatozoa quality in infertility clinics, as previous research has demonstrated that it is the primary seminal anti-oxidant, accounting for approximately 65% of the seminal anti-oxidant capacity (31,32).

Alagbonsi, &Olayaki in 2020 reported in 2020 that dose-response and time-course of modulation demonstrated that vitamin C increased the percentage of spermatozoa motility in a dose-dependent but not time-dependent manner. For instance, 100 M, 1 mM, 5 mM, and 10 mM, but not 10 M, significantly enhanced the motility of spermatozoa throughout the observation period compared to the starting point. Moreover, incubation of spermatozoa in a 5 mM vitamin C solution increased their motility by 22% relative to the control. This report concludes that vitamin C enhances the kinematics of spermatozoa, thereby increasing motility (33).
These findings confirm a previous report that in-vitro treatment of caprine animal spermatozoa with vitamin C improves their motility and kinematics. In addition, they found that vitamin C increased total and progressive sperm motility (34).

The motility, viability, and lipid peroxidation (LPO) of human spermatozoa were examined in Ringer-Tyrode with varying concentrations of ascorbic acid (AA). It varied between 50 and 4000 μm. As demonstrated by an enhancement in their motility and viability, ascorbic acid concentrations below 1000 μm scavenges spermatozoa from free radical damage. Concurrently, malondialdehyde production (an end product of LPO) decreases significantly following AA treatment. Ascorbic acid concentrations of 1000 μm and above are not protective, as evidenced by an immediate reduction in sperm motility and viability and an increase in LPO (35).

The results of the present study showed that Vitamin E significantly increased total and progressive motility and viability of spermatozoa (72%, 45%, 95%) compared to the control group (59%, 30%, 94%).

Consistent with previous studies, the results of this study demonstrate a positive effect of Vitamin E on sperm motility and viability at varied concentrations and incubation times on spermatozoa from camel (22; 36), rooster (37), Ram (38), and men (26). By binding to endoperoxides, vitamin E protects sperm plasma membranes from damage and stabilizes sperm morphology and motility (39).

Vitamin E enhances sperm viability by boosting the antioxidant system and protects spermatozoa by preventing oxidative DNA damage (26). This antioxidant aids spermatozoa in overcoming oxidative damage and enhances sperm motility and viability (36). By inhibiting lipid peroxidation of the sperm plasma membrane, Vitamin E may improve sperm quality when added to semen diluent (37).
Saccharomyces cerevisiae possesses a network of anti-oxidative stress defense mechanisms. These defense mechanisms included antioxidant enzymes such as superoxide dismutase (SODs), which catalyze the conversion of O2- to H2O2 and O2. (40;41). Both SOD and catalase work in harmony to protect cellular proteins from oxidation by ROS, but they may operate in different ways due to the fact that they reduce the levels of superoxide anion and hydrogen peroxide, respectively (42). In the case of UV-irradiation, the intracellular protective activity of oxidized yeast cells may be associated with the antioxidant effect of SOD, catalase, and glutathione (43).

As for yeast extraction outcomes, the current study demonstrates the efficacy of yeast extraction in significantly protecting and enhancing sperm total motility, progressive motility, and viability (66%, 39%, 95%) compared to the control (59%, 30%, 94%). This result is consistent with a previous trial by Eissa and colleagues in 2016 which showed a positive effect on sperm motility after adding yeast extraction (44).

**Conclusions**

1- Vitamin C, vitamin E, and yeast all have beneficial influences on semen parameters.

2- Vitamin E has superior results to vitamin C and yeast.

3- The optimal vitamin C concentration for achieving outcomes is 0.06 mg/ml Ham's F10.

**4- Recommendations**

1- Both Vit. C and Vit. E should be considered for sperm preparation medium in IUI, IVF and ICSI cycles as they have beneficial effects on sperm parameters.

2- Baker’s yeast, Vit C and Vit. E should be considered for oral supplementation in cases of asthinozoospermia especially if associated with oxidative stress.

**ClinicalTrials.gov ID:** NCT02814695
Conflict of interest: -

The authors have no conflict of interest.

Author contribution:-

All authors contributed to data collection, literature review and editing report.
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