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

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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: 40 ejaculated semen samples of infertile men were obtained. Each semen sample was divided into 6 equal fractions. 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's F10).

Results: Sperm total motility, progressive motility, vitality and oxidative stress were significantly increased in treated groups than the control group. The results showed that Vit. E has the best results (72%, 45%, 95%& 0) with comparable results with 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), respectively.

Conclusions: Vit. C, E and yeast, all have beneficial 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: Antioxidant activities (AOA); Reactive oxygen species (ROS); Baker's Yeast; Vitamin E; Vitamin C; *in-vitro* sperm activation.

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

Introduction

Infertility affects 13–20% of couples worldwide, regardless of race or ethnicity **(1).**The male factor of couple infertility is anticipated to be around 25-50% **(2).** Between 30% and 80% of patients with male infertility produce excessive reactive oxygen species (ROS) in their ejaculate. Oxidative stress is a condition characterized by an elevated generation of ROS and a reduced response of biological mechanisms to promptly neutralize the reactive intermediates or to repair the damage **(3).**

It is well established that a tiny amount of ROS is crucial for the steps involved in the critical physiological reaction of fertilization—sperm maturation, hyperactivation, capacitation, acrosomal reaction of sperm, 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).**

Sperm selection techniques manage OS by removing sperms with oxidative DNA damage. These include density gradient centrifugation (DGC), electrophoretic separation, intracytoplasmic morphologically selected sperm injection, hyaluronic acid binding assay, and annexin-V magnetic-activated cell separation**(8)**.

Numerous papers have shown that a shorter time between ejaculatory abstinence lead to a poorer seminal reactive oxidative species (ROS) 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 antioxidant therapies such as vitamins C and E eliminate ROS and improve sperm parameters and pregnancy outcomes in patients with OS and sperm DNA fragmentation (SDF) (**10).**

According to the Cochrane database, 61 randomized controlled trials comparing the effect of antioxidants and a placebo in a population of 6,264 infertile men were reviewed. The results confirmed that antioxidants could enhance clinical pregnancy and live birth rates. **(11).**

Classic oral antioxidants are vitamin C alone (400-1000 mg/day)(**12),** vitamin E alone (300-600 mg/day)**(13),** or a combination of vitamin C and E. Vitamin C and E act synergistically, and several studies have reported the valuable effect of complex antioxidants on decreasing SDF and raising clinical pregnancy rates (**14)**.

Zinc is a vital component for spermatogenesis and sperm DNA synthesis. It also prevents lipid peroxidation (LPO) and acts as a component of antioxidant enzyme superoxide dismutase (**15).** Selenium is also an essential 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, catalase, peroxidase, glutathione s-transferase (**18)** as well as, non-enzymatic antioxidants like apiquinone, glutathione, mineral ions and sulfhydryl amino acid (**19**). The carboxymethylated (1-3) p-glucan (CMG) in the cell wall of Sacch. cerevisiae has the capacity to restrain lipid peroxidation in liposomes induced 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 Vit E on sperm motility and viability have been proven at different doses and different incubation time on spermatozoa of Camel**(22).**Vitamin E is a very efficient scavenger of free radicals. Vitamin E is localized in cell membrane, therefore it cannot protect the cytosol from free radicals, its counterpart "selenium" present in cytosol is responsible for protection in cytosol. Vitamin E increases intracellular ATP and decreases cell permeability and enzyme inactivation **(23).**

Sperm preparation process for ART, may induce OS. In vitro Vit. E supplementation may guard spermatozoa from the undesirable effect of oxidative stress during sperm processing via preserving antioxidant processes in normal form (**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:

Data management and statistical analysis were done using SPS version 25. (IBM, Armonk, New York, United States). Quantitative data assessed for normality using the Shapiro-Wilk test and direct data visualization methods. According to normality, numerical data were summarized as means and standard deviations or medians and ranges. Quantitative data were compared in different preparations using repeated measures ANOVA or Friedman's test. Post hoc analyses were done using Bonferroni's method. All statistical tests were two-sided. P values less than 0.05 were considered 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 C2samples(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 vitaminC1 (31%) and C2 (35%) samples. Also, progressive motility in vitamin C1 samples was significantly lower (31)than in vitamin C2samples (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 C1samples 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). Posthoc 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 C1and C2 samples (1for each) (Table 1).

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

Patients and methods

The practical part of the study took place from August 2020 to November 2021in 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 have not received an antibiotic treatment for the last 4 weeks; (iii) Aged 22-35 years old; (iv)Asthinozoospermic cases. Exclusion criteria: severe oligospermic cases and necrozoospermia.

Seminological analysis

Fourty ejaculated semen samples from infertile patients who indicated willingness to participate in the study and have had 3-7 days of sexual abstinence, using the masturbation method and ejaculated in wide mouthed plastic container were analyzed for semen and sperm characteristics as described by WHO, 2010 [17].

Samples were allowed to liquefy for 20 minutes and were examined for volume, viscosity, spermatozoa count by Neubauer haemocytometer , total sperm motility, progressive and non-progressive motility, oxidative stress level (measurement by Oxisperm; Modern Bio-systems; Spain) and sperm vitality using 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 37º 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 37º 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 37º C for 30 minutes.

C. Antioxidant producing Yeast:(6^{th} fraction)

The yeast strain *Sacch. Cerevisiae* ATCC 58523 used in this study was obtained from 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 in malt extract broth medium and incubated at 25°C for 4 days, then cells were harvested and re-suspended in lyses buffer (50 mM K-phosphate "pH 7.0", 1 mM PMSF, 0.5 mM EDTA). Cells were disturbed by vortexing for 15 cycles for 1 minute with 1 volume of glass beads (0.5 mm) followed by 1 minute of cooling on ice. Cell debris were removed by centrifugation for 10 minutes at 6,000 rpm, then precipitate was washed with saline puffer (40 gm/100ml dist. Water) then acidified with HCl and culture at pH 4.5. Then was put on water path at 65°C for 1 hour and was kept in refrigerator overnight to have ppt (Lysed cells) and cell suspension (yeast extraction (YE)).

0.1ml liquefied semen was mixed with 0.1ml Ham's F10 medium supplemented with (20 mg/ml) Yeast extraction and incubated at 37° C for 30 minutes.

Thirty minutes after semen specimen treatment in all fractions, they were examined and assessed for total motility, progressive motility, vitality and level of oxidative stress (OS).

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

	Control	Vitamin C1	VitaminC2	VitaminC3	P-value
Total Sperm motility	59±11[3,4]	59±10[3,4]	62±9[1,2,4]	65±9[1,2,3]	<0.001
Progressive motility(%)	30±7[2,3,4]	31±6[1,3,4]	35±6[1,2,4]	39±7[1,2,3]	<0.001
Non-progressive motility(%)	29±9[2,3,4]	28±8[1,4]	27±7[1,4]	25±7[1,2,3]	<0.001
Vitality(%)	94±1.8[2,3,4]	95±1.6[1]	95±1.8[1]	95±1.9[1]	<0.001
Oxidative stress level	2(0-4)[2,3,4]	1(0 -3)[1,4]	1(0 -3)[1,4]	0(0-2)[1,2,4]	<0.001

Data was presented as mean and SD except for oxidative stress level which was presented as median and range. RepeatedmeasuresANOVA wasused.Friedman'stest wasusedforoxidativestresslevel.Post-hocanalysesweredoneusingBonferroni'smethod.1:significantlydifferentfromcontrol,2:significantlydifferentfromvitC1,3:significantlydifferentfromvitC2;4:significantlydifferentfrom vitC3.

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, vitaminC3, 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) (Table2)

Table (2) Semen characteristics in different preparations

	Control	vitaminC3	VitaminE	Yeast	P-value
Total Sperm motility	59±11[2,3,4]	65±9[1,3]	72±8[1,2,4]	66±7[1,3]	<0.001
Progressive motility(%)	30±7[2,3,4]	39±7[1,3]	45±6[1,2,4]	39±5[1,3]	<0.001
Non-progressive motility(%)	29±9[2,3]	25±7[1]	26±5[1]	27±5	<0.001
Vitality(%)	94±2[2,3,4]	95±2[1]	95±2[1]	95±2[1]	<0.001
Oxidative stress level	2(0-4)[2,3,4]	0(0-2)[1]	0(0-1)[1]	0(0-2)[1]	<0.001

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 doneusingBonferroni'smethod.1:significantlydifferentfromafter1h,2:significantlydifferentfromvitC3,3:significantlydifferentfrom vitE;4:significantlydifferentfromyeast.

Discussion

Human spermatozoon has low antioxidant enzymes capacity and is not able to fully deal with oxidative stress and thus, oxygen-free radicals produced by spermatozoa and leucocytes can lead to a decrease in intracellular ATP levels, which in turn destructively affects sperm motility. Also, the sperm membrane has polyunsaturated fatty acids (PUFA) which could be a primer of lipid peroxidation (**26**).

Reactive oxygen species (ROS) compounds can be released from the mitochondria and middle region of the spermatozoa and access the chromatin structure in the sperm head**.** Oxygen-free radicals such as hydrogen peroxide in terms of their ability to penetrate through the membrane surrounding the nucleus, can affect the sperm nucleus and damage it **(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 leads to disorder in cell membranes so that the transfer of ionic and chemical messengers and also ionic concentration gradient on both sides of the membrane are disrupted (**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).

Earlier researches have shown that vitamin C is the main seminal anti-oxidant, representing about 65% of the seminal anti-oxidant capacity, and is presently being used in-vitro to augment spermatozoa quality in infertility clinics **(31,32)**

Alagbonsi, &Olayaki in 2020 stated that dose-response and time-course of modulation showed that vitamin C increased the percentage of motility of spermatozoa in a dose-dependent but not time-dependent manner. For instance, 100 μM, 1 mM, 5 mM, and 10 mM, but not 10 μM, drastically improved the spermatozoa motility all through the

observation period when compared to the starting point. Furthermore, incubation of spermatozoa in 5 mM of vitamin C solution furthermore augmented their motility by 22% when compared to control. From this report we can conclude that vitamin C increases motility by enhancing the kinematics of spermatozoa **(33).**

These data are consistent with a previous report that an improvement in the motility and kinematics of caprine animal spermatozoa treated with vitamin C in-vitro do happen. They also observed that vitamin C increased total and progressive spermatozoa motility (34).

Human spermatozoal motility, viability and lipid peroxidation (LPO) were studied in Ringer-Tyrode with different concentrations of ascorbic acid (AA) added to it. It ranged from 50 to 4000 μm. Ascorbic acid in concentrations lower than 1000 μm scavenges spermatozoa from free radical harm as shown by an improvement in their motility and viability. Concomitantly, there is also a sure diminution of malondialdehyde generation (an end product of LPO) following AA treatment. Ascorbic acid at 1000 μm concentration and above, however, is not protective, as evidenced by immediate decrease in sperm motility and viability and associated 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%).

The results of this study are consistent with the previous studies that indicate a positive effect of Vitamin E on sperm motility and viability at different doses and different incubation time on spermatozoa of Camel (22; **36**), Rooster (**37**), Ram (**38**) and men (**26**). Vit E protects sperm plasma membranes from any harm and stabilizes sperm morphology and motility by binding to endoperoxides (**39**).

Vitamin E increases sperm viability by increasing the antioxidant system and also protects spermatozoa by preventing oxidative damage to endogenous DNA (**26**). This antioxidant helps the spermatozoa to overcome the oxidative attack and can improve sperm motility and viability (**36**). Improvement in sperm quality by adding Vitamin E to semen diluent may be achieved by inhibiting lipid peroxidation of the sperm plasma membrane (**37**).

Saccharomyces cerevisiae has a network of defense mechanisms to protect itself against oxidative stress. These defense mechanisms involved antioxidant enzymes such as superoxide dismutase (SODs), which catalyze the dismutation of O2- to H2O2 and O2 (40;41)**.** Both SOD and catalase work together in harmony to defend cellular proteins from oxidation by ROS, but they may operate in diverse ways because they lessen the cellular levels of superoxide anion and hydrogen peroxide, respectively (42). Intracellular protective activity of oxidized yeast cell in the case of UV-irradiation may be associated with the antioxidant effect of SOD, catalase and glutathione (43)

As for yeast extraction results, the present study shows the effectiveness of yeast extraction in significantly protecting and enhancing sperm total motility, progressive motility and viability (66%, 39%, 95%), respectively, compared to 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- Vit. C, E and yeast all have beneficial effects on semen parameters.
2- Vit. E has the best results than Vit. C and yeast.
3- The best concentration of Vit. C to achieve results is 0.06 mg/ml Ham's F10.

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.

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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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