Yeast Cells as Antioxidant-Producing Probiotics: Can they emulate Vitamin E on in-vitro protection of human spermatozoa against oxidative stress?

Sahar-Eissa A., Saad A. S., Gamal Enan, El-Dougoud K. A

Hawaa Fertility Center, Banha City, Egypt.
Obstetrics and gynecology Department, Faculty of Medicine, Banha University, Egypt.
Microbiology Department, Faculty of Science, Zagazig University, Egypt.
Microbiology Department, Faculty of Agriculture, Ain shams University, Egypt.

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ABSTRACT

Oxidative stress- associated defective sperm function is one of the commonest causes of male infertility, and until recently, it is difficult to evaluate and treat. Overcoming of such oxidative stress status (OSS) by suitable antioxidants sure may help those infertile patients. It is well established that Vitamin E is one of the major membrane protectant against reactive oxygen species (ROS). This study aimed to determine the effect of supplementation of Ham’s F10 media with Vitamin E and comparing the results with Baker’s Yeast (Saccharomyces cerevisiae) extraction, which according to EFSA (The European Food Safety Authority) has a QPS (Qualified Presumption of Safety) status, so it was selected to be our probiotic model. Statistically significant positive influence towards sperm reproductive potential has been revealed in both supplementations of Vitamin E and Baker’s Yeast extraction. The sperm vitality, motility percent, and grade of motility were significantly increased (P<0.05) in both treated groups and control groups as compared to pre-activation groups using Ham’s F10 medium by direct mixing technique, the results showed that all YE concentrations significantly improved Motility and progressive motility (P<0.05) whiles’ Vit E significantly improved motility and PR-motility only with conc. (0.2 mg/ml). In conclusion, Yeast Extraction improved sperm motility, vitality and decreased OS level in comparable manner to Vitamin E.

KEYWORDS: Antioxidant activities (AOA); Reactive Oxygen specimens (ROS); baker’s Yeast; Vitamin E; in-vitro sperm activation.

INTRODUCTION

As known, it’s difficult to evaluate and treat oxidative stress which affect human sperm function and so influence male infertility. Antioxidants supplementation may help those infertile patients to overcome such OSS. It was reported that male factor infertility accounts for up to half of the cases who suffered from infertility [1]. Evidence now suggests that oxidative stress (OS)-mediated damage to sperm is a significant contributing pathology in 30–80% of cases [2]. Oxidative stress occurs when the production of reactive oxygen species (ROS) overcome the power of the bodies antioxidant defense mechanisms and lead to cellular damage [3].

Many non-controlled trials reported significant improvements in sperm count, motility and morphology of infertile patients’ semen samples while subjected to antioxidant therapy [4]. The catching point in antioxidant therapy is its ability to guard against oxidative stress, when they were added to sperm preparation media. The addition of different antioxidants as for example; catalase/SOD [5], Vitamin C [6], Vitamin E [7], EDTA [8], glutathione/hypotaurine [9], albumin [10] and N-acetyl-cysteine [11] to sperm preparation media have all been shown to protect sperm from prolonged oxidative attack.
Until now, commercial sperm preparation media does not contain any antioxidants aside from albumin and amino acids, therefore optimized sperm culture media containing protective antioxidants needs intensive research as soon as possible. There are many reasons to consider probiotics in the management of infertility, as for oxidative stress status (OSS) antioxidant producing probiotics maybe helpful.

Saccharomyces cerevisiae (Baker’s yeast) contain several endogenous substances act as antioxidant, There are enzymatic components such as superoxide dismutase (SOD), catalase, peroxidase, glutathione s-transferase [12,13] as well as, non enzymatic compounds like glutathione, apiquinone, Sulhydryl amino acid and mineral ions [14,15]. The carboxymethylated (1-3) β-glucan (CMG) which prepared from the cell wall of baker’s yeast Sacch. cerevisiae has the ability to inhibit lipid peroxidation in liposomes induced by hydroxyle radicals, the protective efficiency of glucan can imply its possible application as antioxidant [16].

Aim of the study: the present in-vitro study aimed at finding out the efficacy of Baker’s yeast extractions, probiotic antioxidant, in scavenging the OSS mediated damage on sperm motility, progressive motility and vitality as well as comparing this potency to that of Vitamin E in infertile men attending Hawaa fertility center in Benha City, Qaliubiya, Egypt.

**MATERIALS AND METHODS**

**Selection of respondents:**

The practical part of the study took place from August 2013 to May 2015 to collect and test 40 cases. The study population was made up of infertile males attending Hawaa fertility center, Benha city, Qaliubiya, Egypt. The inclusion criteria were as follows: (i) must have been married for 1 year before inclusion into the study and were unable to achieve pregnancy; (ii) must have not received an antibiotic treatment for the last 4 weeks prior to sampling; (iii) aged 22-35 years old; (iv) showed oxidative stress-mediated asthinozoospermia in their semen.

**Sampling technique:**

The study samples were collected from patients who indicated willingness to participate in the study and have had 3 to 7 days of sexual abstinence, using the masturbation method and ejaculated in wide-mouthed plastic container as described by WHO [17].

**Seminological analysis:**

Fourty ejaculated semen samples from infertile males were first analyzed for semen and sperm characteristics as diminished by WHO [17]. Samples were allowed to liquefy for 20 minutes and were examined for volume, viscosity, spermatozoa count by Neubauer haemocytometer, oxidative stress level measurement by Oxisperm; Modern Bio-systems; Spain, percentage of progressive motility and non-progressive motility and sperm vitality using eosin-negrosin stain; fertipro; Belgium.

**In-vitro antioxidant activation:**

a. Vitamin E:

Vitamin E was obtained from (α tocopherol, Sigma). The semen specimens were divided into seven equal fractions. First fraction was control, 0.1ml of liquefied semen mixed with 0.1ml Ham’s F10 medium and incubated at 37°C for 30 minutes. 2nd, 3rd, 4th fractions (Vit. E 1,2,3), 0.1ml liquefied semen was mixed with 0.1ml Ham’s F10 medium supplemented with (1, 2, 5 mg/ml) Vitamin E respectively and incubated at 37°C for 30 minutes.

b. Antioxidant producing Yeast:

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 buffer (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)).

5th, 6th, 7th parts fractions (YE 1,2,3), 0.1ml liquefied semen was mixed with 0.1ml Ham’s F10 medium supplemented with (10, 20, 50 mg/ml) Yeast extraction and incubated at 37°C for 30 minutes.
After semen specimen treated in all fractions, they were examined and assessed for microscopical changes, total motility, progressive motility, vitality, level of oxidative stress (OS) were detected in all fractions 30 minutes later after addition of the antioxidants.

**Statistical analysis:**
The collected data were tabulated and analyzed using IBM SPSS version 19 software. Categorical data were presented as frequency and percentages while quantitative data were expressed as mean ± standard deviation.
The collected data were represented graphically using Microsoft Excel 2010 software. Categorical data were represented by Pie chart, while quantitative data were expressed by Bar chart.
The accepted level of significance in this work was stated at 0.05 (P < 0.05 was considered significant, P < 0.01 was highly significant and P value > 0.05 was insignificant).

**Results:**

1. **Abiotic in-vitro activation:**
   Results of this study showed that there was decrease, but not significant, in vital spermiogram characters in comparison to fresh sample levels (after 30 minutes), the sperm motility, progressive motility and vitality were significantly improved (P < 0.05) in both control and treated groups with (Ham’s F10 and Ham’s F10 supplemented with Vit. E) Compared to preactivation groups (Table 1), while there were no significant differences in the assessment values among different concentrations of vitamin E (Table 1).
   However the best in vitro results showed in the sperm motility percent, grade of motility and vitality were observed with ham’s F 10 media supplemented with Vitamin E in a concentration of 2 mg/ml (Table 1 and Fig 1,2 and 3).

2. **Probiotic in-vitro activation:**
   Results of this section showed that the sperm motility, progressive motility and vitality were significantly improved (P < 0.05) in both control and treated groups with (yeast extraction, YE) compared to preactivation groups (Table 1 and fig 4,5 and 6), while there were no significant differences in the assessment values among YE (Table 1).
   However, the best in vitro results showed in the sperm motility percent, grade of motility and vitality were observed with ham’s F 10 media supplemented with yeast extraction in a concentration of 20 µM/ml (Table 1).

**Table 1:** Effect of different concentrations of vitamin E and Yeast Extraction on spermiogram of asthinozoospermic infertile patients following in vitro activation technique.

<table>
<thead>
<tr>
<th>Spermiogram</th>
<th>Before activation</th>
<th>Without activation</th>
<th>After Activation control</th>
<th>Vit. E1</th>
<th>Vit. E2</th>
<th>Vit. E3</th>
<th>YE 1</th>
<th>YE 2</th>
<th>YE 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm Motility</td>
<td>57±11</td>
<td>34±11</td>
<td>60±10*</td>
<td>64±9*</td>
<td>72±8**</td>
<td>67±7*</td>
<td>69±9**</td>
<td>67±7*</td>
<td>65±11*</td>
</tr>
<tr>
<td>PR-Motility</td>
<td>25±6</td>
<td>9±7*</td>
<td>30±6</td>
<td>35±6*</td>
<td>46±5**</td>
<td>32±7</td>
<td>42±6**</td>
<td>40±5*</td>
<td>36±4*</td>
</tr>
<tr>
<td>Non PR Motility</td>
<td>32±10</td>
<td>34±8</td>
<td>30±9</td>
<td>29±6</td>
<td>27±5</td>
<td>27±4</td>
<td>27±5</td>
<td>27±6</td>
<td>28±5</td>
</tr>
<tr>
<td>Vitality</td>
<td>97±2</td>
<td>89±4</td>
<td>94±2</td>
<td>95±2</td>
<td>97±2*</td>
<td>95±3</td>
<td>96±3*</td>
<td>97±2*</td>
<td>97±1*</td>
</tr>
<tr>
<td>Oxidative stress Level</td>
<td>L2±2</td>
<td>L4±1</td>
<td>L2±2</td>
<td>L1±1*</td>
<td>L0±1**</td>
<td>L1±1*</td>
<td>L0±1**</td>
<td>L0±1**</td>
<td>L1±1*</td>
</tr>
</tbody>
</table>
Fig. 1: The mean grade of motility and sperm motility index in response to different concentrations of Vitamin E.

Fig. 2: The mean sperm vitality index in response to different concentrations of Vitamin E.
Fig. 3: The mean Value of seminal plasma oxidative stress level in response to different concentrations of Vitamin E.

Fig. 4: The mean grade of motility and sperm motility index in response to different concentrations of Yeast extraction (YE).
Fig. 5: The mean sperm vitality index in response to different concentrations of Yeast extraction (YE).

Fig. 6: The mean Value of seminal plasma oxidative stress level in response to different concentrations of Yeast extraction (YE).

Discussion:

Assisted reproductive techniques (ART) recently became the most popular and suitable treatment technique in many cases of male and female infertility and one of the factors determining successful assisted reproduction is the quality of semen samples, which were subjected to many techniques to select motile spermatozoa [18].

In the present study, we aimed to evaluate wheather antioxidant supplementation has a benefit in direct mixing technique for sperm preparation from asthenozoospermic patients in an attempt to prevent damage by centrifugation and generation of reactive oxygen species (ROS). Agarwal et al. [19] reported that increased formation of ROS has been correlated with reduction of sperm motility. The selection of sperm preparation methods depends on the quality of the ejaculates, and ejaculates with high ROS production such as that of asthenozoospermic patients should not be separated by centrifugation method due to severely spermatozoa damage [20].
The results of this study showed that among the three doses of vitamin E that have been used, there was a significant increase at P<0.05 in the sperm motility, progressive motility and vitality that assessed post-activation in vitro in both treated groups and control groups as compared to pre-activation groups using Ham’s F10 medium by direct mixing technique, but there were no significant differences in the assessed value among different concentrations of vitamin E. However, best in vitro sperm activation results regarding the motility percent and grade of sperm motility achieved with vitamin E (2 mg/ml).

Our results agreed with the results of Lewis et al. [21] who recommended adding low molecular weight antioxidants like α-tocopherol to sperm during preparation for ART, especially in asthenozoospermic patients. They also indicated that it would be of more clinical benefit to add these antioxidants directly to the sperm rather than using dietary supplements, because of that defective seminal vesicle function may be one of the causes of decreased α-tocopherol levels in these patients.

Gomez et al. [8] demonstrated that levels of ROS produced by spermatozoa were negatively correlated with the quality of sperm in the original semen. Vitamin E acts as a scavenger of a wide range of ROS, which explains its ability to counteract the effects of free radicals both in terms of induced DNA damage and ROS production. Thus, it is suggested that vitamin E may be effective in preventing the rapid loss of motility that normally occurs during incubation of spermatozoa and maintains the motility under oxidative stress conditions. This study shows that all doses of vitamin E improve the motility of human sperm, but the dose of 2 mg/ml vitamin improves better.

These results may be explained based on the fact that vitamin E protects the spermatozoa by preventing from endogenous oxidative DNA and membrane damages, thereby helping the sperm to overcome the oxidative attack. Thus, by maintaining the membrane integrity and optimum functioning of sperm, vitamin E improves the per cent sperm motility [22]. The present study shows the effectiveness of vitamin E in protecting sperm motility and viability. Similar observations have been made on humans [4]. It is concluded that all doses of vitamin E increased the percentage of sperm motility and viability. Most effective occurred the 2 mg/ml dose, though differences among the doses were non-significant (P≥0.05).

Thus, vitamin E protects spermatozoa against the damages caused by reactive oxygen species. Supplementing the samples with vitamin E could, therefore, be of clinical importance for extending the time of spermatozoa storage before artificial insemination (AI), in vitro fertilization (IVF) and intrauterine insemination (IUI).

As for yeast extraction results, the present study shows the effectiveness of yeast extraction in significantly protecting and enhancing sperm motility and viability (P<0.05). And also it is concluded that all doses of yeast extraction increased the percentage of sperm motility and viability, but most effective occurred the 20mg/ml dose, though differences among the doses were non-significant (P≥0.05), and this results was expected because as was demonstrated about Baker’s yeast; Saccharomyces cerevisiae contain several endogenous substances act as antioxidant, enzymatic components [12,13] as well as, non-enzymatic compounds [15].

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 [23,24]. Lushchak [25] reported that both SOD and catalase operate in concert to protect cellular proteins from oxidation by ROS, but they may act in different ways because they reduce the cellular levels of superoxide anion and hydrogen peroxide, respectively.

Also, zyrack et al. [26] found that 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.

The carboxymethylated (1-3) β-glucan (CMG) which prepared from the cell wall of baker’s yeast Saccharomyces cerevisiae has the ability to inhibit lipid peroxidation in liposomes induced by hydroxyl radicals, the protective efficiency of glucan a model can imply its possible application as antioxidant [16].

Moreover, the antioxidant β-glucan was much stronger than that of D-mannitol. This is due to fact that polysaccharides multiply anomic hydrogen atoms, which are primarily abstracted by the active free radicals, while monosaccharide possess only one anomic hydrogen [16]. From our results we concluded that Yeast extraction showed improvement in more high scale than Vit E, but it remains needs further investigations.

Conclusion:

In vitro and in vivo experiments in this study have demonstrated the beneficial effect of many antioxidant (Vit E) on sperm motility and vitality and lowers oxidative stress level. The in vitro antioxidant activity of lyophilized Sac. cerevisiae has been investigated in oxidative stress-associated male infertile patients and it showed positive efficacy comparing with other known antioxidants like Vit E.
REFERENCES


