Effect of the addition of vitamin E to sperm freezing medium on cryosurvival rate of sperm motility in asthenozoospermic patients

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Abstract- Aim: The current study was aimed to assess the effect of the addition of vitamin E to sperm freezing medium on cryosurvival rate of sperm motility. Study Design: A case control study.

Patients and Methods: 25 semen samples of asthenozoospermic patients were included in this study. They were enrolled in the fertility center of al-sadder medical teaching city in Al-Najaf. Each semen sample was divided into equal four parts: first part (before activation), second part (after activation), third part (cryopreservation with sperm freezing medium for one month), and fourth part (cryopreservation with sperm freeze medium plus vitamin E for one month). Sperm concentration, progressive motile sperm, and normal sperm morphology were counted in each part. Cryosurvival rate was counted in third and fourth parts.

Results: After sperm activation by swim up technique, the results showed significant increase (P < 0.05) of progressive motile sperm and normal sperm morphology but it was observed a significant decrease (P < 0.05) of sperm concentration. Progressive motile sperm, cryosurvival rate and normal sperm morphology were showed a significant decrease (p < 0.05) after freezing and thawing processes while the results were revealed significant improvement in progressive motile sperm and cryosurvival rate after the addition of vitamin E to sperm freezing medium.

Conclusion: Addition of vitamin E to sperm freeze medium improves the cryosurvival rate of sperm during cryopreservation processes.

I. INTRODUCTION

The developing of assisted reproductive techniques led to found the cryopreservation processes of tissues and semen samples (Mokvotsevet et al., 2010). Cryopreservation process means the keep of the biological materials with temperature less than zero such as -80 or – 196 and this process cause the stop in cellular diffusion and decrease in temperature energy of the chemical reactions. It is aimed to the maintenance on viability and functional activity in cells within certain period (Dohleet et al., 2007).

In some cases for patients, the cryopreservation process is very important such as chemotherapy, radiotherapy, bladder neck surgery, azoospermia, vasectomy, and testicular biopsy (Saito et al., 2005). Cryopreservation process include addition of cryoprotectants, immersion of mixture in liquid nitrogen with temperature - 196 C, thawing process by water bath, centrifugation, and the addition of activation medium (Luvoni, 2006). Each step of cryopreservation influence negatively on the structure of plasma membrane of sperm, sperm motility, fertilization, and premature nuclear decondensation (Maxwell and Watson, 1996; Cormier et al., 1997). Cryopreservation process lead to incidence a significant decrease of enzymatic and nonenzymatic antioxidants and increase of reactive oxygen species levels inside the medium (Kumar et al., 2011). Oxidative stress is increase of reactive oxygen species levels and decrease of antioxidants levels (Raghureer et al., 2010). The increase of reactive oxygen species levels and the decrease of antioxidant levels lead to occurrence apoptosis process in sperm cells (Wang et al., 2003). Vitamin E is a slushy antioxidant in lipids and organic solutions but it is not slushy in water and it has yellow color at room temperature (Stahl and Sies, 1997; Zheng, 2003). Vitamin E is one of antioxidants in seminal fluid and has nearly 0.32-0.52 μmol/l in seminal fluid. This vitamin has great role in the preservation of sperm motility within the normal levels that due to it the role of fixation of the plasma membrane series of sperm cells and neutralizing the reactive oxygen species such as hydrogen peroxide (H2O2), superoxide anion and hydroxyl peroxide (OH) (Bollet et al., 2002; Agarwal ET AL., 2004), and inhibit the activity of lipid peroxidation (Zheng, 2003). The present study aimed to show the negative effect for freezing and thawing process and effect of vitamin E on cryosurvival rate of sperm after freezing and thawing processes in asthenozoospermic patients.

II. MATERIALS AND METHODS

Semen collection: This study was carried out in the laboratory of sperm freezing to the fertility center – medical sadder city-Al-Najaf province – Iraq from 1/2/2015 to 3/6/2015. 25 Asthenozoospermic patients were selected for this study who enrolled in fertility center for treatment or entry the IVF program. They had 30-35 years old age. The semen samples have less than 2 ml were excluded. Each semen sample was divided into equal four parts: first part (before activation, raw semen), second part (after activation by swim up from pellet), third part (cryopreservation with sperm freeze medium for one month), and fourth part (cryopreservation with sperm freeze medium plus vitamin E (40 μmol/l) for one month). Sperm concentration, progressive motile sperm, normal sperm morphology, and cryo-survival rate were counted in third and fourth parts. Rate survival was assessed according to the following formula:
Percentage of cryosurvival = Post thaw sperm motility /Pre freeze sperm motility x 100. (Petyim and Choavaratana,2006 ;Julavijitphonget al ; 1996)

**Seminal fluid analysis:**

Fresh semen samples were collected after 3-5 days of abstinence by masturbation directly into container made of disposable glass or plastic, in a private and quite room adjacent to the semen analysis laboratory under oral instruction(WHO, 2010). The specimens were placed in an incubator(Binder – Germany) at 37°C for 30 minutes up to 60 minutes for liquefaction. Sperm concentration, sperm motility, and sperm morphology were assessed according to WHO guidelines (1999).

**Sperm preparation by swim up from pellet:**

0.5ml of semen specimen was transferred from a plastic cup to a sterile 15 ml conical. The specimen was mixed with 1IVF medium(fertiprobelgium)(1-2ml) by using a sterile pasture pipette. The tubes were centrifuged at 3000 rpm for 10 minutes. Carefully discard the supernatant and resuspend the pellet in 0.5 ml of IVF medium. Incubate the tubes at 45° angle for one hour for sperm swim-up in vertical rack in a 37°C incubator 5% CO2. (Esteves, 2012).

**Vitamin E preparation:**

Vitamin E (α tocopheral) was prepared with the concentration 40 umol/l depending on Mohammad et al., 2012. Sperm freeze medium was contain 50 microliter of vitamin E.

**The freezing of specimens:**

0.25 microliter of prepared specimen (by swim up from pellet) was transferred to 1.8 ml cryovial (Thermo scientific, Denmark) then sperm freeze medium (containing 50 microliter from vitamin E or not), was added to prepared specimen (0.7:1) slowly with mixing. The mixture was exposure to liquid nitrogen vapor with 20 cm of height upon the level of liquid nitrogen inside the freezing tank for 10 minutes. The cryovial which contain the mixture was plunged into liquid nitrogen (-196°C) for one month.

**The thawing of specimens:**

The cryovials were removed from liquid nitrogen and placing them in tap water for 5 minutes, then the whole mixture was diluted with 1-2 ml of IVF medium and transferred into a test tube. The mixture was centrifuged for 10 min. at 3000 rpm. The pellet was mixed with 0.25 ml of ivf medium (Wood et al., 2004).

**III. STATISTICAL ANALYSIS**

Statistical analysis was done in our study by SPSS (Statistical Package for Social Science; Version 17) program. Independent t-test and paired test were used to assess the differences between two groups. Results were reported as (mean ±SD) unless otherwise indicated. P<0.05, was considered statistically significant (Daniel, 1999).

**IV. THE RESULTS**

The table (1) was showed the role of activation processes in improvement of sperm motility and normal morphology for sperm. It was observed a significant increase (p < 0.05) of progressive motile sperm and sperm normal morphology after activation by swim up from pellet and IVF medium compared with before activation while the results were revealed insignificant decrease (p < 0.05) in sperm concentration after activation by swim up from pellet and ivf medium compared with before activation.

**Table 1-sperm activation of asthenozoospermic patients by swim up from pellet**

<table>
<thead>
<tr>
<th>Sperm parameters</th>
<th>Before activation</th>
<th>After activation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm concentration Million/ml</td>
<td>53.54 ±12.835 (a)</td>
<td>8.20 ±2.986 (b)</td>
</tr>
<tr>
<td>Progresive motile sperm %</td>
<td>36.80 ±5.842 (a)</td>
<td>60.76 ±6.10 (b)</td>
</tr>
<tr>
<td>Normal morphology percent %</td>
<td>33.00±5.400 (a)</td>
<td>50.70 ±5.18 (b)</td>
</tr>
</tbody>
</table>

Values were expressed as mean± standard deviation.

* Significant (P < 0.05). a b Within each category, numbers with different letter superscripts are significantly different from each other (p<0.05), numbers with the same letter superscript are not significantly different.

The present study was showed the negative effect of freezing and thawing on sperm parameters. The results were showed a significant decrease (p < 0.5) in progressive motile sperm, and sperm normal morphology after the freezing and thawing process compared to that in before freezing and thawing (sperm activation by swim up). (Table 2).

**Table 2-effect of cryopreservation process of asthenozoospermic samples on sperm parameters for one month**

<table>
<thead>
<tr>
<th>Sperm parameters</th>
<th>Before freezing and thawing n=25</th>
<th>After freezing and thawing n=25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm concentration Million/ml</td>
<td>8.20 ±2.98 (a)</td>
<td>7.2400 ±2.81 (a)</td>
</tr>
<tr>
<td>Progresive motile sperm %</td>
<td>60.76 ±6.10 (a)</td>
<td>22.79 ±6.47 (b)</td>
</tr>
<tr>
<td>Normal morphology percent %</td>
<td>50.70 ±5.18 (a)</td>
<td>49.50 ±4.78 (a)</td>
</tr>
</tbody>
</table>

Values were expressed as mean± standard deviation.

* Significant (P < 0.05). a b Within each category, numbers with different letter superscripts are significantly different from each other (p<0.05), numbers with the same letter superscript are not significantly different (NS).
After the addition of vitamin E as antioxidant, the current study showed the positive role of vitamin E in improvement of sperm motility and cryosurvival rate. Progressive motile sperm and cryosurvival rate were significantly increased (p < 0.05) while sperm concentration and sperm normal morphology were not significantly different (p > 0.05) after the addition of vitamin E compared to that in the freezing without vitamin E. (Table 3).

Table 3 - Effect of cryopreservation process of asthenozoospermic samples for one month with vitamin E on sperm parameters and cryosurvival rate.

<table>
<thead>
<tr>
<th>Sperm parameters</th>
<th>Cryopreservation with vitamin E</th>
<th>Cryopreservation without vitamin E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm concentration Million/ml</td>
<td>7.24 ± 2.81 (a)</td>
<td>7.40 ± 3.11 (a)</td>
</tr>
<tr>
<td>Progressive motile</td>
<td>22.79 ± 6.47 (a)</td>
<td>41.34 ± 6.05 (b)</td>
</tr>
<tr>
<td>Normal morphology percent</td>
<td>49.50 ± 4.78 (a)</td>
<td>48.50 ± 5.10 (a)</td>
</tr>
<tr>
<td>Cryosurvival rate</td>
<td>36.35 ± 9.33 (a)</td>
<td>67.42 ± 6.87 (b)</td>
</tr>
</tbody>
</table>

Values were expressed as mean± standard deviation. * Significant (P < 0.05). a,b Within each category, numbers with different letter superscripts are significantly different from each other (p<0.05), numbers with the same letter superscript are not significantly different (NS).

Figure 1: This figure showed the results of the effect of cryopreservation process on cryosurvival rate. The results were revealed a significant increase (p < 0.05) of cryosurvival rate after the addition of vitamin E compared to without vitamin E during the cryopreservation process.

V. DISCUSSION

Sperm preparation techniques in vitro have great importance in overcoming many of the problems of the abnormal semen and reduced the time required for the process of capacitation of sperm compared with this process inside the body(Dugan et al., 1997). Techniques used to activate the human sperm are designed similarly to what happens inside the body in terms of separation of sperm and seminal plasma and sperm selection of a progressive movement(Yogev et al., 2000). In this study, swim up from pellet was used to prepare semen samples of asthenozoospermic patients. The results were showed a significant improvement in progressive motile sperm and normal morphology of sperm while there was great decrease of sperm concentration after activation. The reason is probably that the good movement and form normal sperm can reach to the top of the tube where weak sperm and white blood cells remain at the pellet. Sperm, which has good speed is able to swim to the top and the resistance of gravity(Stovall et al., 1994). Makleret al., 1998 mentioned that sperms which have normal morphology able to reach to upper part while the sperms which have abnormal morphology are stay in the bottom of tube. The

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The present study was conducted to determine the effect of vitamin E supplementation on sperm parameters, particularly progressive motility and normal morphology, to improve cryopreservation outcomes.

**REFERENCES**


[17] Sinan et al., (2009) reported that addition of α-tocopherol in fresh and stored boar semen samples significantly increased the percentages of motile spermatozoa and sperm velocity (VAP, VSL, and VCL), linearity and straightness in each treatment

In conclusion, the present study was reaveled the positive role of vitamin E as antioxidant in the minimize damage which result from freezing and thawing processes that to purpose the maintainence of sperm motility and cryosurvival rate.

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**REFERENCES**


AUTHORS

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