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

Dr.SahibYahya AL-Murshedi

Assist. prof. phD. Department of Urology / College of Medicine/University of kufa

***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 (cryopresevation with sperm freezing medium for one month) , and fourth part (cryopresevation 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 resultes were showed significant increase ($P < 0.05$) of progressive motile sperm and normal sperm morphology but it was observed a siginficant 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 (Mokovtsev*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 (Dohle*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 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) (Bolle*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 midium for one month) , and fourth part (cryopreservation with sperm freeze medium plus vitamin E (40 umol/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 ;Julavijitphong*et 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 37ºC for 30 minutes up to 60 minutes for liquefaction .Sperm concentration ,sperm motility ,and sperm morphology were assessed acocdinge to WHO guidelines (1999)

Sperm preparation by swim up from pellet .

0.5ml of semen specimen was transfered from a plastic cup to a sterile 15 ml conical . The specimen was mixed with IVF medium(fertiprobelgium)(1-2ml) by using a sterile pasture pipette..The tubes were centrifuged at 3000 rpm for 10 minutes.Carefullydiscardthe supernatant and resuspendthe 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 transfered to 1.8 ml cryovial (Thermo scientific, Denmark) ,then sperm freeze medium (containg 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 removaled 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 transfered 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 procecss in improvement of sperm motility and normal mophology for sperm . it was observed a significant increase ($p < 0.05$) of progressive motile sperm and sperm normal mophology after activation by swim up from pellet and IVF medium compared with before activation while the results were revealed asignificante 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

Sperm parameters	Before activation n=25	After activation n=25
Sperm concentration Million/ml	**53.54** ± **12.835** (a)	**8.20 ±2.986** (b)
Progresseve motile sperm %	**36.80 ±5.842**+ (a)	**60.76 ±6.10 (b)**
Normal morphology percent %	**33.00±5.400** (a)	**50.70** ±**5.18** (b)

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

Sperm parameters	Before freezing and thawing n=25	After freezing and thawing n=25
Sperm concentration Million/ml	**8.20 ±2.98** (a)	**7.2400** ±**2.81** (a)
Progresseve motile sperm %	**60.76 ±6.10 (a)**	**22.79 ±6.47** (b)
Normal morphology percent %	**50.70** ±**5.18** (a)	**49.50 ±4.78** (a)

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 was showed the positive role of vitamin E in improvement of sperm motility and cryosurvival rate . progressive motile sperm and cryosurvival rate were have a significant increase ($p < 0.05$) while sperm concentration and sperm normal morphology were have not significance deffrenceses ($p < 0.05$) after the addition of vitamin E compared to that in the freezing without vitamin E . (Table 3).

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

Sperm parameters	Cryopreservation with out vitamin E	Cryopreservation with vitamin E
Sperm concentration Million/ml	**7.24 +2.81** (a)	**7.40 +3.**11 (a)
Progresseve motile sperm %	**22.79 +6.47**(a)	**41.34 +6.05 (b)**
Normal morphology percent %	**49.50 +4.78** (a)	**48.50 +5.10** (a)
Cryosurvival rate	**36.35 + 9.33** (a)	**67.42 + 6.87** (b)

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 with out vitamin E during the cryopreservation process.

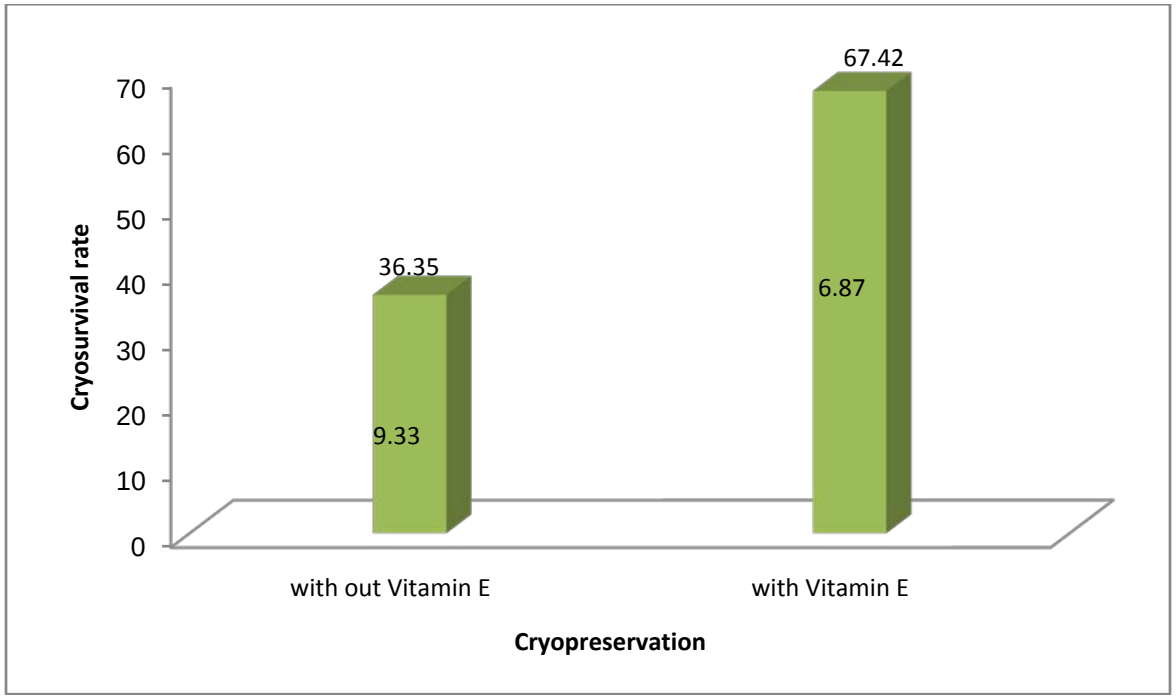

Figure 1: Cryosurvival rate of sperm with and with out vitamin E during the cryopreservation process.
The numbers inside the column are represent SD

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 improvment 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). Makler*et 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

present study was showed the negative influence of cryopreservation on sperm parameters especially the progresseve motility and normal morphology for sperms .may be the reason in that is the freezing of sperm and the addition of the freezing media lead to decline in the movement of sperm and change the structure and morphology of sperm. the pLasma membrane of sperm contains high quantity of lipids in it structure.The plasma membrane of the sperm contains high quantity from gel or fluid lipids .when these lipids are fluids , the plasma membrane of sperm become functional able.Two factor affect the change of plasma membrane permeability are the ratio of the phospholipid to the cholesterol and the degree of tempreature . (Medeiros *et al* .,2002). The freezing and thawing processes cause of unreverse changes of the ability of plasma membrane to passage of the ions ,solvent and materials from and into the cells that lead to decrease of functional ability of sperm,these changes called cold shock.(Dziekonska*et al* ., 2009). The effect of the cryopreservation on mitochondria , plasma membrane and the formation of ice crystal outside cell lead to decline on sperm motility and sperm normal morphology .(Sinan*et al* ., 2008).The cryopreservation process cause of increase of the levels of reactive oxygen species and decrease of the levels of antioxidants therefore this study was aimed to prevent the harmful effect of cryopreservation by addition of vitamin E as antioxidant to freezing medium .The results of this study were showed a significant improvment of progressive motile sperm after the addition of vitamin E (40 umol/l) to freezing medium .The reason of that due to the protective role of vitamin E against a cryopreservation processess and it role of maintenance on sperm viability . our study results were agreed with another studies. Ansari et al .,(2010) reported decline of the damaged sperms after the addition of mixture of antioxidant to freezing medium , so Mohammed *et al*.,(2012) noted significant improvement of progressive motile sperm and sperm viability after the addition vitamin E during the cryopreservation processess .another study was mentioned increase of sperm motility percent after the addition of vitamin E to freezing medium (Taylor *et al* ., 2009) . in the present study ,there is significant improvement of cryosurvival rate was recorded after the addition vitamin E to freezing medium that may be the improvement of sperm motility led to increasing of cryosurvival rate because the positive correlation between them. One of The studies was revealed that addition of 40 µmolTrolox before freeze to cryopreservation media improve all human sperm motion kinetics. Mohammed *et al*.,(2012), So jeong *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 processess that to purpose the maintenace of sperm motility and cryosurvival rate.

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AUTHORS

First Author – Dr.SahibYahya AL-Murshedi /assist. prof. phD. Department of Urology / College of Medicine/University of kufa.Sahib.almurshidi@uokufa.edu.iq