Effect of Vitamin E on Sperm Motility and Survival in Chilled-Stored Semen

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Abstract

Background:

Sperm cells are well equipped with a powerful defense system of antioxidants, but an imbalance between the production of reactive oxygen species (ROS) and the available antioxidant-defenses result in oxidative stress. Therefore, antioxidants are supplemented extracellularly under *in vitro* conditions. Antioxidants are the main defense factors against oxidative stress induced by free radicals. Vitamin E is believed to be the primary component of the antioxidant system of the spermatozoa and is one of the major membrane protectants against ROS and lipid peroxidation (LPO) attack. It appears to be the first line of defense against the peroxidation of polyunsaturated fatty acids (PUFAs) contained in the cellular and sub-cellular membrane phospholipids because of its lipid solubility.

Objective:

The aim of the current study to evaluate effects of supplementing semen extender with vitamin E, at various concentrations (2, 6 and 12 IU/ml) on sperm motility, survival and morphology on the releases of free radicals and antioxidant enzymes within semen.

Patients ,Materials and Methods:

Twenty two patients were involved in the present study semen samples were obtained, and sperm count was assessed. The antioxidant (vitamin E) was formulated to be tested at three different levels as follow; 2, 6 and 12 IU/ml; Therefore, control and 3 antioxidant-containing extenders were prepared for semen dilution. The sample was divided into 4 aliquots. One volume of semen was added to 5 equal volumes of the designed extender. Extended semen samples (37˚C) were gradually cooled to 4˚C in the refrigerator and stored for 48 hours. A semen sample was taken out after 48h of storage, then warmed to 37˚C and checked for progressive motility, viability and abnormality.

Sperm viability was assessed by Eosin Y. (0.5%)—Nigrosin (0.1%) staining mixture. A total of 200 sperm were assessed under oil immersion with a high-resolution (X100) objective. Sperm morphology was assessed by Hematoxylin- Eosin satin, at least 200 sperm were scored on randomly chosen field, under oil immersion with a high-resolution (X100) objective.

Results:

The highest post-thaw motility and sperm survival (44% and 51.7%) 48 h after storage at 4˚C (Table 1) were attained ($P < 0.01$) in the basal diluents containing 6 IU vitamin E. Likewise, the correspondent percentages of live spermatozoa were 59%. Contrariwise, percentage of sperm abnormalities for the previous treatment was 25% significantly ($P < 0.05$) decrease compares with basal diluents containing 2, 12 IU vitamin E. The lowest ($P \geq 0.05$) survival (23.5%, Table 1) was found in the basal diluents containing 12 IU vitamin. Beyond 6 IU vitamin E, there appears to exert adverse effects on sperm viability and survival.

Conclusions:

For the best protection against the increased free radicals production during chilling-preservation of human semen, extenders must contain 6 IU vitamin E per ml. The intensive production of free radicals in seminal plasma was counteracted by the inclusion of vitamin E at the above mentioned concentrations.

Key words: **vitamin E, sperm, extender.**

Introduction:

Vitamin E is a powerful antioxidant that improves sperm count, quality, and motility, its help keep the sperm membrane healthy and protects sperm from free radical damage [1]. Semen contains various unsaturated fatty acids which are oxidized during preservation resulting in reactive oxygen species (ROS). Gametes are susceptible to (ROS) attack. When manipulated in vitro during assisted reproductive techniques, these cells run the risk of generating and being exposed to supra physiological level of ROS [2]. Defective sperm functions are the most prevalent causes of male infertility and a difficult condition to treat [2] [3]. Many environmental, physiological, and genetic factors have been implicated in the poor sperm function and infertility. Thus, it is very important to indentify the factors /conditions which affect normal sperm functions Among various causes, oxidative stress (OS) has been attributed to affect the fertility status and physiology of spermatozoa [3]. The term oxidative stress is generally applied when oxidants outnumber antioxidants. The imbalance between the production of reactive oxygen species (ROS) and a biological systems ability to readily detoxify the reactive intermediates or easily repair the resulting damage is known as oxidative stress [4]. The main destructive aspects of oxidative stress are the production of ROS, which include free radicals and peroxides. The mechanism by which the free radicals do their damage was ascribed to their ability to catch the electrons from the nucleic acids, lipids and proteins causing the cell damage [4] The production of ROS by sperm is a normal physiological process, but an imbalance between ROS generation and scavenging activity is detrimental to the sperm and associated with male infertility [5]. Physiological levels of ROS influence and mediate the gametes and crucial reproductive processes, such as sperm-oocyte interaction, implantation and early embryo development [6]. Against ROS attack, sperm cells are well equipped with a powerful defense system of antioxidants. Antioxidants are the main defense factors against oxidative stress induced by free radicals [6] [7]. Much work has been done to protect the integrity of the sperm cells of different species from physical and chemical damage during processing and preservation. Enzymatic and non enzymatic antioxidants have been tested on this aspect. The ROS can be neutralized by an antioxidant system, such as Vitamins D, E and C which serve as a defense mechanism against the lipid peroxidation of semen and maintaining sperm motility and viability[7].

Patients, Materials and Methods:

1. Semen collection

Twenty two patients were involved in the present study at Al-Iraqia University college of Medicine from September 2015 to January 2016. Semen samples were obtained, and sperm count was assessed. Semen were evaluated and included in the study if the following criteria were met: volume and the progressive motile sperms percentage.

2. Semen Dilution

Basic Semen Extender

The basic extender (control) comprised of 3.63 g Tris (hydroxy methyl aminomethane), 0.50 g fructose, 1.99 g citric acid (monohydrate), 5 ml egg yolk, 1 ml antibiotics solution containing 100,000 IU penicillin and 100,000 µg streptomycin

and glass-distilled water up to 100 ml and pH was adjusted to 6.8 – 7[7].

Antioxidants-Containing Extenders

The antioxidant (vitamin E) was formulated to be tested at three different levels as follow:

2, 6 and 12 IU/ml; Therefore, control and 3 antioxidant-containing extenders were prepared for semen dilution. Treatment groups (G) were; G1 (Control, basic diluents), G2 (basic diluents containing 2 IU vitamin E/ml), G3 (basic diluents containing 6IU vitamin E/ml), G4 (basic diluents containing 12 IU vitamin E/ml).

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Semen preparation

Semen samples were obtained of good quality and were counted. The sample was divided into 4 parts. One volume of semen was added to 5 equal volumes of the designed extender. Extended semen samples (37˚C) were gradually cooled to 4˚C in the refrigerator and stored for 48 hours. A semen sample was taken out after 48h of storage, then warmed to 37˚C and checked for progressive motility, viability and abnormality.

Semen Assessment

Sperm motility was examined by microscope. To evaluate this parameter, one drop of semen was placed on a slide and spermatozoa with progressive motility counted used X40 objective lens.

Sperm viability was assessed by Eosin Y. (0.5%)—Nigrosin (0.1%) staining mixture. Dead cells were stained by the Eosin, whereas the live cells retained their cellular membrane integrity preventing the stain to enter cells(Figure 1). Nigrosin served as a background stain to provide contrast for the unstained (white) live cells. A total of 200 sperm were assessed under oil immersion with a high-resolution (X100) objective equipped with correct adjustment of the bright field optics. Unstained spermatozoa which appeared white were classified as “live” and those that show any pink or red color were classified as “dead” [8]. Sperm survival after 48 h storage was estimated by dividing the percentage of progressive motility at 48 h by the percentage of initial progressive motility.

Sperm morphology was assessed by Hematoxylin- Eosin satin, at least 200 sperm were scored on randomly chosen field, under oil immersion with a high-resolution (X100) objective(Figure 2).

Results:

The highest post-thaw motility and sperm survival (44% and 51.7%) 48 h after storage at 4˚C (Table 1) were attained ($P < 0.01$) in the basal diluents containing 6 IU vitamin E. Likewise, the correspondent percentages of live spermatozoa were 59%. Contrariwise, a percentage of sperm abnormalities for the previous treatment were 25% significantly ($P < 0.05$) decrease compares with basal diluents containing 2, 12 IU vitamin E. The lowest ($P > 0.05$) survival (22.4%, Table 1) was found in the basal diluents containing 12 IU vitamin. Beyond 6 IU vitamin E, there appears to exert adverse effects on sperm viability and survival (Table 1).

Table (1) : Effect of concentration of vitamin E in semen extender on percentage of sperm, progressive motility, and abnormality and survival rate after 48 h storage at 4˚C.

Antioxidant-Extender	% Initial Motility	% Progressive Motility after 48 h (4˚C)	% Live perm after 48 h (4˚C)	% Abnormality after 48 h (4˚C)	% Sperm Survival
Control (C)	85± 3.0	20 ± 2.0	25 ± 5.0	44 ± 4.0	23.5
C + 2 IU E/ml	85± 3.0	38 ± 5.0*	46 ± 2.0*	35± 3.0*	44.7*
C + 6 IU E/ml	85± 3.0	44± 4.0**	59 ± 4.0**	25 ± 5.0*	51.7**
C + 12 IU E/ml	85± 3.0	19 ± 3.0	25± 5.0	38± 5.0*	22.4

*(P < 0.05) significant ** (P < 0.01) High significant

Figure (1) Eosin –Nigrosin staining; live sperm are unstained; dead sperm are stained pink or red (100x).

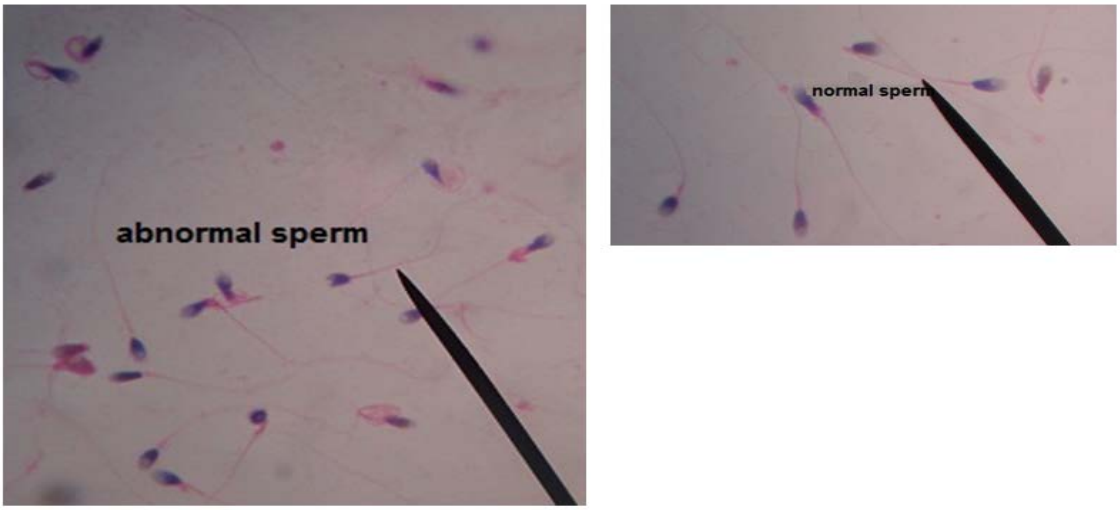

Figure (2) sperm stained with Hematoxylin- Eosin (100x)

Discussion:

Due to the high speed of the sperm, depending on the generation of energy by the mid-piece mitochondrial oxidative phosphorylation, a high concentration of free radicals are produced inside and outside the sperm cells [9] [10]. The increase of the production of ROS might damage the sperm cell membrane resulting in lower sperm motility and survival after storage at low temperatures [11] which leads to diminishing the sperm penetration of the cervical mucus *in vitro* [12]. Addition of either enzymatic or non-enzymatic specific antioxidants would impact a beneficial reduction to the free radicals. Vitamin E (α-tocopherol) and vitamin C (ascorbic acid) were traditionally used for a long time as antioxidants. Vitamins E was considered as electron trapping molecules, and considered to be the main component of the antioxidant system of spermatozoa, one of the major protectors of the membranes against ROS and lipid peroxidation attack [13]. Because of its solubility in lipids, vitamin E might serve as the first line of defense against the peroxidation of the polyunsaturated fatty acids on the membranous phospholipids structure [14] [15]. The best sperm motility in the current tested was found at the vitamin E concentration in semen extender must not exceed 6 IU/ml to achieve the best post-thaw motility and survival with the highest antioxidant activity, beyond 6 IU vitamin E, there appears to exert adverse effects on sperm viability and survival (Table 1). At a similar level, many studies found better post-thaw sperm parameters of frozen ram semen [16] Also, found protective effects of low level of vitamin E (0.3 mM) on frozen dog sperm [17]. Above

this level there found deleterious effects on sperm characteristics [18]. This observation was also confirmed on post-thaw quality of bull semen [19].

Also, the concentration of vitamin E up to 1.0 mM offer protection against membrane oxidative stress of frozen ram sperm [20]. Moreover, the cycle of freezing and thawing has been reported to be responsible for a decrease in the level of antioxidants such as glutathione (GSH) or superoxide dismutase (SOD) in human and bovine spermatozoa [21] [22]. This decrease in antioxidants would enable ROS to cause sperm damage during the storage at low temperatures. The determinant role of superoxide dismutase (SOD) in the antioxidant defense systems has been known since 1968. It is well known that superoxide ion (O2–) is the starting point in the chain production of free radicals. At this early stage, superoxide dismutase inactivates the superoxide ion by transforming it into hydrogen peroxide (H2O2). The latter is then quickly catabolised by catalase and peroxidases into dioxygen (O2) and water (H2O). Different studies have confirmed that the production of H2O2 under the action of SOD is the triggering factor in the natural antioxidant defense mechanisms. SOD therefore seems to be the key enzyme in the natural defense against free radicals [23].

The present study showed that Abnormal morphology of sperm increase significantly with the storage at low temperatures, percentages of sperm abnormalities in the basal diluents containing 6 IU vitamin E was 25% significantly ($P < 0.05$) decrease compare with basal diluents containing 2, 12 IU vitamin E. changes detected at the light level in the morphologies of the sperm head, midpeice, or acrosome, by contrast, abnormal morphologies of the principal piece, flagellar defects characterized as looped or coiled tails. This particular flagellar defect probably results from extreme osmotic stress. Rapid changes in osmolarity often occur during chilling - thawing which cause deformations of membranous structures associated with flagellum [24].

Conclusions:

For the best protection against the increased free radicals production during chilling preservation of human semen, extenders must contain 6 IU vitamin E per ml. The intensive production of free radicals in seminal plasma was counteracted by the inclusion of vitamin E at the above mentioned concentrations. Further studies are required to test the impacts of inclusion of antioxidants in the freezing media on subsequent sperm fertilization *in vivo* and *in vitro.*

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