ROLE OF COQ10 SUPPLEMENTED TO THAWING SOLUTION IN IMPROVEMENT HUMAN SPERM PARAMETERS POST-CRYOPRESERVATION

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ABSTRACT

Background: Cryopreservation techniques are highly developed field of Assisted Reproductive Technologies (ARTs) to help infertile couples to overcome infertility. Coenzyme Q10 (CoQ10) is a vitamin – like substance is present in most eukaryotic cell, and produced in the mitochondria primarily. Objective: To study the effects of CoQ10 supplemented to thawing solution on sperm parameters post-cryopreservation technique. Materials and Methods: One hundred and eight males were participated in this study. The mean age for all males included in this study was (29.94 ± 0.65) years and duration of infertility was 2-14 years. Semen samples were collected and seminal fluid analysis was done according to WHO (2010) All groups contain 1mL from semen sample mixed with 0.7 mL of cryopreservation solution. All groups after thawing were divided into 3 subgroups (GA: control group; without honey bee), (GB: contain 5 μm from CoQ10) and (GC: contain 10 μm) Results: There was significant increase (P<0.05) in the progressive sperm motility (%), progressive sperm motility (%) grade (A), progressive sperm motility (%) grade (B) and normal sperm morphology when compared among groups (GA: control group), (GB:5 μm CoQ10 ),(GC:10 μm CoQ10] Significant reduction (P<0.05) in the percentages of sperm immobility (%) when compared to control group with GB and GC. In the present study appeared that the direct swim-up technique resulted in significantly (P<0.05) better results for sperm parameters than the swim-up technique from all types of ejaculates classified according to male infertility factors. The study proved that there was significant (P<0.05) improvement in sperm
parameters. **Conclusion:** 10µM COQ10 obtained best results for human sperm parameters post-thawing compared to other groups. Best technique in this study direct swim-up technique compared with centrifuge technique.

**KEYWORDS:** Human, Sperm, Infertility, Cryopreservation, Coenzyme Q10.

**INTRODUCTION**

Cryopreservation is widely used in many assisted conception units to preserve male fertility, for example before cytotoxic chemotherapy, radiotherapy or certain surgical treatments that may lead to testicular failure or ejaculatory dysfunction.[1] Freezing and thawing involve damage to the plasma membrane and acrosome of human spermatozoa as evidenced by significant ultra-structural changes demonstrated by electron microscopy.[2] This damage is exacerbated during the cryopreservation process by a decrease in the lipid content of the membranes, which contain a high proportion of polyunsaturated fatty acids; the decrease in lipid content indicates lipid peroxidation in the presence of reactive oxygen species.[3] Coenzyme Q10 (Co Q 10) is essentially a vitamin or vitamin-like substance. CoQ10 likewise is found in small amounts in a wide variety of foods and is synthesized in all tissues.[4] The biosynthesis of CoQ10 from the amino acid tyrosine is a multistage process requiring at least eight vitamins and several trace elements. Coenzymes are cofactors upon which the comparatively large and complex enzymes absolutely depend for their function.[5] CoQ10 is a component of the mitochondrial respiratory chain, play role both in energy metabolism and as antioxidants for cell membranes and lipoproteins.[6,7] Therefore, the present study was aimed to investigate the effects of CoQ10 supplemented to thawing solution on sperm parameters post- cryopreservation technique.

**MATERIALS AND METHODS**

This study was conducted in the High Institute of Infertility diagnosis and ARTs at Al-Nahrain University. The period of study was from November- 2014 to June- 2015. One hundred and eight men were included in the present study, the mean age years 29.94 ± 0.65 were ranged between 18-44 years. The duration of infertility ranged from 2 to 14 years. The semen analyses were done according to the World Health Organization standard criteria (2010) The container must be labeled with the following information, name, age, abstinence period and time of sample collection. The specimens were placed in an incubator at 37 ºC for 30 minutes to allow liquefaction. Freezing technique was made by using Sperm Freeze TM
kit (Belgium) Sperm Freeze is a 15% glycerol based cryoprotectant in HEPES buffer. It contains 0.4% human serum albumin (HIV and Hepatitis negative) In the present study, the CoQ10 was added to thawing solution for all groups except control group (GA): GB: contain 5 µM CoQ10 and GC: contain 10 µM CoQ10. Then mixing the CoQ10 with the thawing solution. Semen sample divided into three subgroup GA: contain thawing solution only, GB: contain thawing solution and 5 µM CoQ10 and G3: contain thawing solution and 10 µM CoQ10. Stock solution (A) was prepared by dissolving 0.8633 gm. in 10 ml of DMSO. Then, stock solution (B) prepared by take 0.1 ml from Stock solution (A) diluted with 0.9 ml of SMART medium. Low concentration of treated group (GB: 5 µM) was prepared by adding 0.05 ml of stock solution (B) to 0.95 ml of SMART medium. However, addition 0.10 ml of stock solution (B) to 0.90 ml of SMART medium to prepare high concentration group (G3; 10 µM)

RESULTS
The effect of different concentrations of CoQ10 supplied to thawing solution using direct technique and centrifugation technique post-thawing as shown in figure (1) which explains the results of progressive sperm motility (%) post-thawing. Non-significant differences (P>0.05) were assessed for the control group using centrifugation swim-up technique when compared to direct swim-up technique. Similarly, non-significant differences (P>0.05) were appeared for (GB; 5 µM.CoQ10) using centrifugation technique when compared to direct swim-up technique. In the same figure, a significant increase (P<0.05) was noticed for (GC; 10 µM.CoQ10) using centrifugation swim-up when compared to direct swim-up technique.

![Figure 1: human progressive sperm motility (%) post-thawing when using different concentrations of CoQ10 and both sperm techniques.](image-url)
* Means with different superscripts within each columns are significantly different (P<0.05)

NS: Means with similar superscripts within each columns are non-significantly different (P>0.05)

Figure (2) shows the results of progressive sperm motility (%) grade (A) post thawing. A significant increase (P<0.05) for control group (GA) was assessed for centrifugation swim-up technique as compared to direct swim-up technique. However, non-significant differences (P>0.05) were observed for GB (5µM.CoQ10) using centrifugation technique as compared to direct swim-up technique. In the same figure, a significant increase (P<0.05) was assessed for GC (10µM.CoQ10) using centrifugation swim-up technique as compared to direct swim-up technique.

![Figure 2: human sperm motility grade A (%) post-thawing when using different concentrations of CoQ10 and both sperm techniques.](image)

Figure (3) shows the result of progressive sperm motility (%) grade (B) post-thawing. Non-significant differences (P>0.05) were assessed for the control group using centrifugation technique as compared to direct swim-up technique. In the same figure, a significant increment (P<0.05) was appeared for GB (5µM.CoQ10) and GC (10µM.CoQ10) was assessed for centrifugation swim-up technique as compared to direct swim-up technique.
Figure 3: human sperm motility grade B (%) post-thawing when using different concentrations of CoQ10 and both sperm techniques.

* Means with different superscripts within each columns are significantly different (P<0.05)

NS: Means with similar superscripts within each columns are non-significantly different (P>0.05)

Figure (4) shows the results of immotile sperm motility (%) post-thawing. Non-significant differences (P>0.05) were assessed for control group (GA) using direct swim-up technique as compared to centrifugation swim-up technique. However, a significant reduction (P<0.05) was noticed for GB (5µM.CoQ10) and GC (10µM.CoQ10) for direct swim-up technique as compared to centrifugation swim-up technique.

Figure 4: percentages of immotile human sperm post-thawing when using different concentrations of CoQ10 and both sperm techniques.

* Means with different superscripts within each columns are significantly different (P<0.05)
NS: Means with similar superscripts within each columns are non-significantly different (P>0.05)

Figure (5) explains the results of normal sperm morphology (%) post-thawing was a significant reduction (P<0.05) for the control group were assessed for direct swim-up technique as compared to centrifugation swim-up technique. Similarly, a significant reduction for GB (5µM.CoQ10) and GC (10µM.CoQ10) were assessed for direct swim-up technique as compared to centrifugation technique.

**Figure 5:** human normal sperm morphology post-thawing when using different concentrations of CoQ10 and both sperm techniques.

* Means with different superscripts within each columns are significantly different (P<0.05)

NS: Means with similar superscripts within each columns are non-significantly different (P>0.05)

**DISCUSSION**

In this work, CoQ10 was selected and supplemented to SMART medium as thawing solution for several factors including presences of CoQ10 with in most body fluids and tissues\(^8\), has a role in oxidation stress as antioxidant\(^9\), activation of some metabolic pathways, enhancement of motility\(^10\), and production of ATP in cooperation with mitochondria.\(^{11}\) The biosynthesis of CoQ10 from the amino acid tyrosine is a multistage process requiring at least eight vitamins and several trace elements. Moreover, coenzyme Q10 is an essential cofactor in the electron transport chain and serves as an important antioxidant in both mitochondria and lipid membranes. CoQ10 is also an obligatory cofactor for the function of uncoupling proteins. Furthermore, dietary supplementation affecting CoQ10 levels has been shown in number of organisms to cause multiple phenotypic effects\(^{12}\). Therefore, CO enzyme Q10 plays an
essential role in the electron transport in oxidative phosphorylation and so in the mitochondrial production of ATP, the energy in each of our cells and all of our life processes. 96% of all aerobically produced energy is produced by CoQ10. On the other hand, coenzymes are cofactors upon which comparatively large and complex enzymes absolutely depend for their function. Coenzyme Q10 is the coenzyme for at least three mitochondrial enzymes (complexes I, II and III) as well as enzymes in other parts of the cell.

In the current study, there were superior results for human sperm parameters post-thawing when using 10 µM CoQ10 than using 5 µM CoQ10. The main and principal factor is the direct and/indirect roles of CoQ10 on sperm plasma membrane, metabolism and physiology. Coenzyme Q10 is a non-enzymatic antioxidants that is related to low density lipoproteins and protects against peroxidative damage. It is an energy promoting agent and enhances sperm motility. It is present in sperm mid piece and recycles vitamin E and prevent its pro-oxidant activity. In general, oxidative stress has deleterious effect on the physiology of the spermatozoa such as lipid peroxidation, DNA damage, and also has been associated with destruction of sperm motility. The principle means of oxidative stress to impair sperm motility is by alteration of the membrane fluidity.

Two sperm assessment techniques including direct swim-up and centrifugation swim-up techniques were applied. Therefore, different results in the sperm parameters were achieved. In this study, it is assumed that one of the major deleterious effects of freezing/thawing is the formation of reactive oxygen species. Radical oxygen species are blamed for deleterious effects such as declines in spermatozoal motility and peroxidation of the plasma lipid membrane, and therefore, native semen were used for freezing to decrease sperm damage. Although there are several studies suggesting that seminal plasma has a protective role during the freezing processes, there are also papers reporting advantages of swim-up or other separation methods. Other studies claim that there is no significant difference between washed and native sperm for freezing.

Centrifugation swim-up technique for sperm parameter assessment, used in the present study, decreased sperm concentration and activity as a result of ROS release and consequently causes oxidative stress. ROS production during cryopreservation may be the main cause of cellular damage. Normally, a balance is maintained between the amount of ROS produced and that scavenged. Sperm damage appears when this equilibrium is disturbed. A shift in the levels of ROS towards pro-oxidants in semen can induce an oxidative stress on spermatozoa,
in order to scavenge ROS and reduce their destructive action under normal physiological conditions, and seminal plasma.\cite{24} However, the primary cause of cellular damage during cryopreservation is the formation of intracellular or extracellular ice crystals. During the freezing process, the cooling rate plays an important role in determining the extent of cryoinjury to the spermatozoa.\cite{26} It has largely been reported that chilling injury can modify the structure and integrity of plasma membranes\cite{27} mainly composed of phospholipids and cholesterol.\cite{28} Even though high concentrations of cholesterol and polyunsaturated fatty acids give more fluidity to the membrane at lower temperature,\cite{29} during cryopreservation the cooling process causes phase transition of membrane lipids and impairs membrane protein function. Furthermore, the production of reactive oxygen species leads to an increase in lipid peroxidation after cryopreservation.\cite{30} In the current study, results of the semen cryopreservation were significantly decreased in the means of sperm concentration, sperm motility (%) and progressive sperm motility (%) post-thawing processing compared to pre-cryopreservation. These results can be attributed to the cryopreservation process itself, since it cause a change in osmotic pressure that led to alteration in ion concentration, showed the impact on mitochondrial activity then caused failure in energy production and finally caused decreased in motility. Whereas, there was a significant increase (P<0.05) in the mean of immotile sperm percentage after cryopreservation. This result is in agreement with other studies done by Rahana\cite{31} and Rasheed.\cite{32} Post-thawing sperm motility and progressive activity are routinely only about 50% of pre-freeze values as mentioned by other studies Nijis\cite{33} and Anger.\cite{34} The quantitative motility assessments showed reductions in progressive motility (25-75%)\cite{22} A recent study showed that the addition CoQ10 caused significant changes on sperm progression in each separation time. The mechanism by which CoQ10 significantly affected the sperm progression, is still to be determined. The possible explanation is the involvement of the regulation of energy production as it is concentrated within the mitochondrial. Furthermore, coenzyme Q10 is an energy promoting agent, and reduces super oxide anion.\cite{35} Cryopreservation increases the percentage of dead or membrane damaged sperm. The process of cryopreservation is known to cause more production of reactive oxygen species, as antioxidant defenses are reduced in the process. Hence, addition of antioxidants may have beneficial effects on spermatozoa function during the cryopreservation.\cite{36} In this study, among control and treated groups post-thawing showed that the sperm concentration as well as other sperm parameters were decreased significantly (P<0.05) after the six month freeze-thawing procedure with liquid nitrogen. Present results are in agreement with. Thitikan and Somboon.\cite{37} According to Januskauskas and Zillinskas
and RuizPesini et al, sperm deformity induced by cryopreservation was believed to be mainly associated with mitochondrial damage in human spermatozoa, mitochondrial enzymatic activities were shown to be correlated with spermatozoal motility.[38] These studies are in agreement with the present results which reveal there was a significant decrease of sperm motility post-thawing. Also, the study performed by Hu Jm explained the effect of sucrose on sperm motility after adding cryoprotectant containing sucrose to the semen sample then the motility of the post-thaw human sperm was compared before and after cryopreservation and concluded decreased sperm motility after cryopreservation, and the sucrose was a feasible sperm cryoprotectant.[39] Present results showed a significant increase (P<0.05) in the percentage of normal sperm morphology for all groups post-thawing. According to Axnér, they concluded that the percentage of morphologically normal epididymal spermatozoa is not correlated with cryopreservation induced sperm damage using the described freezing protocol.[40] A study performed by Rasheed explained the effect of cryopreservation on the normal morphology and concluded that the normal sperm morphology (%) was highly significantly increased after thawing and sperm preparation than before preparation.[32] These results are in agreement with present results. This functional impairment is due to the structural damage in the flagella caused by alteration in permeability and membrane fluidity.[41] Furthermore, the percentage of normal sperm morphology was significant decreased (P<0.05) compared with before cryopreservation. This result goes with that of other studies.[42]

CONCLUSION
From results of the present study, it was concluded that the 10□M COQ10 presented best results for human sperm parameters post-thawing compared to other groups. Best technique in this study direct swim-up technique compared with centrifuge technique.

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