Effect of cryopreservation protocols in relation to human sperm parameters

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Abstract:

Background

The semen cryopreservation has an important role in the male fertility preservation. Certainly, patients who are prone to become infertile due to surgical or medical treatments such as chemo- or radiotherapy for cancer treatment. In fact, semen cryostorage seems to be the only proven method that may offer these couples a chance of having children in the future.

Objectives

This study was aimed to investigate the effects of cryopreservation protocols on human sperm parameters.

Methods

Sixty semen samples with range of age (19-35) years were included in this study. Sperm parameters, were assessed pre- or post-cryopreservation. Cryopreservation protocols were done using SMART medium with either 15% dimethyl sulfoxide(DMSO) or 15% glycerol alone (as control group) or supplemented with either 0.25M or 0.5M of sucrose for treated groups. Crude data were statistically analyzed

Results

Generally, the results of the present study showed that the sperm parameters were significantly deteriorated post-thawing ($P<0.05$) as compared to pre-cryopreservation. Both treated groups (G2 and G3) showed best results as compared to G1 (control groups). In contrast, treated group (G2) with 0.25 M of sucrose appeared better results than the as G3 group that treated with 0.5M of sucrose

Conclusion

Sperm parameters including concentration, motility, and morphology were deteriorated after cryopreservation. Using 0.25M sucrose reduced the impact of cryopreservation.

Key words: Cryopreservation, Human Sperm,Cryoprotectant, Sucrose.

Introduction

Cryopreservation of human spermatozoa is a widespread method applied for fertility preservation in patients undergoing cancer therapy or those who are planning permanent contraceptive measures such as vasectomy (1). In addition, it can become necessary during treatment cycles of assisted fertilization techniques. Despite many advances in the field of cryobiology, cryopreserved spermatozoa still have decreased fertility potential compared to those present in fresh semen samples (2). Sperm cryopreservation has become routine in assisted reproduction but post-thaw sperm survival, motility and fecundity are adversely affected by freezing. Cryopreservation of human spermatozoa has overcome many space and time limitations and now forms an integral part of assisted reproduction technologies (ARTs) (3), although the sperm cryopreservation is a widely used procedure, the consequences of cryopreservation on the structure of sperm chromatin remain unclear. Sperm DNA damage and sperm apoptosis have been considered as potentially useful indices of male fertility. Cellular apoptosis is a normal event that occurs both during and after embryonic development (4). Sperm parameters are affected by cryopreservation. Freezing and thawing induce major damage to

motility and viability (5) of human spermatozoa because formation of intracellular ice crystals (6) and osmotic stress (7). Therefore the aim of this study is to investigate the effects of cryopreservation protocols on the human sperm parameters.

Materials and methods

This study was carried out in the laboratories of Higher Institute of Infertility Diagnosis and Assisted Reproductive Technologies at AL-Nahrain University during the period from October, 2012 to April, 2013. The subjects (no.60; range of age 19-35 years) were advised to undergo a routine semen analysis, pre- and post- sperm cryopreservation technique using two cryoprotectants (CPAs), glycerol and DMSO.
The semen samples were examined immediately after liquefaction according to WHO 2010. Actually, assessment of sperm parameter has been done pre- and post- cryopreservation.

Cryopreservation technique:

After liquefaction of semen sample, 1mL of semen was mixed with (0.7mL) of freezing medium. The sample is initially mixed in a dropwise manner. The cryovial was placed in the cryovial holder. Then, the mixture was left for 10 minutes in the refrigerator when using DMSO as cryoprotectant and at room temperature when using glycerol to avoid cold-shock. Each vial was labeled with code number and date of cryopreservation. Freeze vertically for 15 minutes in the vapour of the liquid nitrogen (8). The cryovial holder that contains cryovial was plunged in the aluminum cane and stored in liquid nitrogen.

Experimental design:

The semen samples were divided randomly into two major groups depends on cryoprotectants were used, DMSO or glycerol. Each group subdivided into three minor subgroup; control (G1), low (G2) and high(G3) concentrations of sucrose.
Control (G1) group indicates SMART medium enriched with either 15% of DMSO or glycerol, while (G2) group contains SMART medium enriched with 15% DMSO or 15% glycerol plus 0.25 M of sucrose to each subgroup, for (G3) group, it consist of SMART medium supplemented with 15% DMSO or 15% glycerol plus 0.5 M of sucrose to each subgroup.

Statistical analysis:

The data were statistically analyzed using SPSS/PC version 18 software (SPSS, Chicago;USA).
Sperm parameters pre- and post cryopreservation using different concentrations of cryoprotactants (DMSO and glycerol) were analyzed using complete randomized design (CRD) (one way ANOVA)(9).

Results:

1.Sperm parameters pre-and post eryopreservation

1.1. Using 15% DMSO:

The results of sperm concentration were shown in table (1).The means of sperm concentration were significantly decreased (P<0.05) for control and treated groups post-thawing when compared to the results of pre-cryopreservation, while non significant differences (P>0.05) were noticed in the sperm concentration among the groups post- thawing. From the same table the results of sperm motility (%) significantly decreased (P<0.05) post- thawing as compared to that of pre-cryopreservation. However, sperm motility (%) for G2 group revealed non significant differences (P>0.05) when compared to G3 group, whereas G1 group was significantly decreased (P<0.05) when compared to G2 and G3 groups.
Progressive motility (%) was significantly decreased (P<0.05) post- cryopreservation as compared to that of pre-cryopreservation as illustrated. For the same parameter, G2 group were non significantly different (P>0.05) when compared to G3 group, while for G1 group was significantly reduced (P<0.05) as compared to G2 and G3 groups (table 1). The percentage of non-progressive motility was significantly reduced (P<0.05) for all groups post-thawing as compared to that of pre-cryopreservation as presented in table (1), but they show significantly differences (P<0.05) among them. In general, non-progressive motility (%) for G2 group was the highest as compared to G3 and G1 groups, while, G1group significantly reduced (P<0.05) as compared to G3 group.
Table (1) shows that the immotile sperm (%) was significantly increased (P<0.05) for groups post-thawing as compared to pre-cryopreservation. For the same table, the immotile sperm (%) for G2 group was non significant difference (P>0.05) as compared to G3 group, while G1 group was significantly increased (P<0.05) when compared to both treated groups.
The percentage of normal sperm morphology was significantly decreased (P<0.05) after thawing when compared to pre- cryopreservation. However, both means of G2 and G3 groups revealed non significant differences (P>0.05), while mean of G1 group significantly reduced (P<0.05) in regard to both treated groups as presented in table (1)

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1.2. Using 15% glycerol:

The result of sperm concentration was shown in table (2). The means of sperm concentration were significantly decreased (P<0.05) post-thawing when compared to the results of pre-cryopreservation, while, no significant differences (P>0.05) in the sperm concentration were noticed among the groups post thawing. From same table the result of sperm motility (%) which was significantly decreased (P<0.05) post-thawing as compared to that of pre-cryopreservation. However, the percentage of sperm motility for G2 group revealed non significantly difference (P>0.05) to G3 group, whereas sperm motility (%) for G1 group was significantly reduced (P<0.05) when compared to G2 and G3 groups.
The progressive motility(%)was significantly decreased (P<0.05) post- thawing as compared to that of pre-cryopreservation as illustrated in table (2). For the same parameter, G2 group showed non significant difference (P>0.05) when compared to G3 group, while for G1 group was significantly reduced (P<0.05) as compared to both treated groups. The

Table 1: Effect of cryopreservation protocols using different concentrations of DMSO on sperm parameters post- thawing.

Sperm Parameters	Pre-cryopreservation	Post- cryopreservation		
		Control	Low **0.25Msucrose**	High **0.5M ucrose**
Sperm Concentration (Millions/mL)	43.433 a ±2.13	29.967 b ±1.81	32.967 b ±1.81	31.800 b ±1.88
Sperm Motility (%)	45.300 a ±2.14	2.733 c ±0.71	25.033 b ±2.37	19.800 b ±2.12
Progressive sperm motility (%)	27.700 a ±1.52	0.733 c ±0.25	10.200 b ±1.46	7.167 b ±1.19
Non-prograssive sperm motility (%)	17.600 a ±1.11	2.000 c ±0.50	15.000 ab ±1.33	12.633 b ±1.24
Immotile Sperm (%)	54.033 c ±1.96	96.667 a ±0.94	75.133 b ±2.33	79.867 b ±2.16
Normal sperm morphology (%)	30.200 a ±1.09	19.433 c ±0.89	25.233 b ±1.11	23.367 b ±0.20

Values are expressed as mean ± SEM.
" Different superscripts within each row are significantly different (P<0.05).
" Similar superscripts within each row are non significantly different (P>0.05).

Table2: Effect of cryopreservation protocols using different concentrations of glycerol on sperm parameters post- thawing.

Sperm parameters	Pre- cryopreservation	Post- cryopreservation		
		Control	Low **o.25M sucrose**	High **0.5Msucrose**
Sperm Concentration (Millions/mL)	45.633 a ±1.96	34.067 b ±1.88	36.833 b ±1.80	36.767 b ±1.59
Sperm motility (%)	45.333 a ±2.43	1.933 c ±0.44	27.000 b ±2.25	24.333 b ±1.79
Progressive sperm motility (%)	24.667 a ±1.89	0.600 c ±0.15	11.333 b ±1.20	8.167 b ±0.87
Non-prograssive sperm Motility (%)	20.667 a ±1.52	1.333 c ±0.32	15.667 b ±1.22	16.167 b ±1.16
Immotile Sperm (%)	54.700 c ±2.43	98.100 a ±0.44	73.000 b ±2.25	75.667 b ±1.79
Normal sperm morphology (%)	32.167 a ±1.04	19.967 c ±0.82	25.433 b ±0.88	24.733 b ±0.79

Values are expressed as mean ± SEM.
" Different superscripts within each row are significantly different (P<0.05).
" Similar superscripts within each row are non significantly different (P>0.05).

percentage of non-progressive motility was significantly decreased (P>0.05) post- thawing when compared to pre-cryopreservation as illustrated in the same table. However, G2 group revealed non significant difference (P>0.05) when compared to G3 group, while for G1group it was significantly reduced (P<0.05) as compared to both treated groups (Table 2).

Table (2) shows that the immotile sperm (%) was significantly increased (P>0.05) post - thawing as compared to pre-cryopreservation. For the same figure, the immotile sperm (%) for G2 group was non significantly different (P>0.05) as compared to G3 group, while G1 group was significantly increased (P<0.05) when compared to both treated groups.

The percentage of normal sperm morphology was decreased significantly (P<0.05) post-thawing when compared to pre-cryopreservation. However, both means of G2 and G3 groups showed non significant differences (P>0.05), while mean of G1group significantly reduced (P<0.05) in regard to the both treated groups as noticed in (2).

Discussion:

In the present study, semen cryopreservation using rapid freezing technique was selected as a method for several factors including simple, easy and fast technique, as well as have better results than slow Cryopreservation that includes very long process, and requires an expensive cooling apparatus and a large amount of liquid nitrogen. Rapid cooling, on the other hand, yields some of the success of slow cooling and avoids the hour or multihour long process of cooling to -196 °C. The goal of rapid cooling is to provide an intracellular environment which, when cooled rapidly, avoids most ice crystallization (10).

Cryopreservation medium, SMART medium, was used in this study which consists of a base medium, protein source like albumin or human serum albumin in the medium which is important for sperm viability and activity. The acidity (pH) of CM is maintained by bicarbonate buffer system, sodium chloride, carbohydrates, and sodium pyruvate along with glucose added to the CM as a source of energy (11). Other study was improved sperm activation and

Oocyte fertilization (12).

Different types of cryoprotectants were used in the present study, while some of these cryoprotectants penetrating as (DMSO, glycerol); sucrose was used also as non penetrating cryoprotectant. The goods for using glycerol is a colorless, odorless, viscous, sweet- tasting liquid that is soluble in water and low in toxicity, The glycerol substructure is a central component of many lipids. Glycerol is compatible with other biochemical materials in living cells and is frequently used in cell preservation to reduce damage caused by ice crystal formation (13), widely used for sperm and blastocysts. It has low toxicity, but is slow to cross membranes which can contribute to osmotic shock, while, DMSO is a clear and colorless liquid (13), small in size and has a double, hydrophilic and hydrophobic character. The favourable binding of water molecules to DMSO induces a dehydration of the lipid bilayer, which is reflected in the decrease of solvation of the polar head groups (14).

The positive effects from this study appeared in both penetrating CPAs DMSO and glycerol that are treated with 0.25M of sucrose better than treated with 0.5 M of sucrose. In the current investigation, the percentage of post-thawing sperm concentration and non progressive motility for 0.5M sucrose treated group with 15%glycerol was significantly increased ($P<0.05$) as compared to 0.5M sucrose treated groups with 15% DMSO, because glycerol are less toxic than DMSO. Furthermore, glycerol has been used successfully as DMSO cryoprotectant in the freezing of diluted semen for many years (15).

However, there are several potentially damaging events within the cryopreservation technique which can lead to the loss of sperm functions. These include chemical and/or osmotic toxicities (16) of the high concentrations of cryoprotectants required to reduce ice formation as used in the present study. Further damaging factors are related to low temperature. It was largely reported that several damaging processes could occur during freezing-thawing of human spermatozoa, such as thermal shock with formation of intracellular and extracellular ice crystals, cellular dehydration, and osmotic shock (17).

In the present study, production of ROS during cryopreservation may be the main cause of cellular damage (18). 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 (19).

From the results of present study, it was concluded that the sperm parameters including concentration, motility, and morphology were deteriorated after cryopreservation.Also using Glycerol as cryoprotectant has better results than DMSO during semen cryopreservation. Furthermore, sucrose is necessary to protect sperm from cryodamage during cryopreservation.

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