Middle East Fertility Society Journal 23 (2018) 331–334

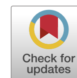
Contents lists available at ScienceDirect

Middle East Fertility Society Journal

journal homepage: www.sciencedirect.com

Original Article

Using two different thawing temperatures and their effect on the motility recovery of human cryopreserved sperms in cancer patients



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ARTICLE INFO

Article history:
Received 5 March 2018
Revised 6 April 2018
Accepted 19 April 2018
Available online 30 April 2018

Keywords:
Cryopreservation
Human sperm
Motility
Temperature
Cancer

ABSTRACT

Human sperm cryopreservation is a widely used technique which helps the male partner for the fertility insurance by preserving their sperms for a long time. Cancer patients suffer from low semen quality especially who undergo chemotherapy or radiation treatments may face complete loss of their sperm production. This study aimed to investigate the effects of different thawing temperature (37 °C & 40 °C) on sperm motility recovery after cryopreservation-involving four types of cancer tumors at variable ages between 16 and 42 years- to help cancer patient for storage their sperms. A detailed semen analysis was collected under guidelines for 30 samples. The samples were then analysed and frozen in liquid nitrogen. The semen samples were subsequently thawed at 37 °C & 40°Cfor 3 min. This was then followed by statistical analysis of the comparative motilities. Results: thawing of cryopreserved human sperm at 40 °C results in a statistically significant increase in motility recovery compared with thawing at 37 °C.
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1. Introduction

Over the past years, due to advanced cancer treatment modalities, young males suffering from different types of cancer developed high chances of cure and survival rates [1]. Many cancer patients present with already poor semen quality before the start of cancer treatment [2].

Cancer treatment in the form of cytotoxic drugs cause more deterioration of semen quality [3]. Most of patients develop azoospermia within weeks after start of chemotherapy. About 25% of them may start spermatogenesis again within two years [4].

Most of these patients need to preserve their spermatozoa for future fertility, as they are still young and need to start or complete their families. New ART modalities like ICSI can offer good chances for those patients to father children [5].

Poor quality semen may be more prone to DNA damage and cell death after cryopreservation than normal semen samples and thus have lower fertilizing capacity [6]. It has been shown that reactive oxygen species (ROS) production impacts membrane fluidity and the recovery of motile, viable spermatozoa after cryopreservation. In addition, the cryopreservation process can reduce the antioxidant activity of the semen fluid making spermatozoa more susceptible to ROS-induced damage [7].

High viability and motility of spermatozoa are important factors for successful fertilization [8]. Thawing procedure is very important as the freezing procedure in terms of its impact on the survival of spermatozoa. The rapid thawing of semen decreases the harmful effects of recrystallization processes and hydration, preventing damage to sperm membrane and cytoplasm. In this case the ice crystals do not have time to be formed and sperm switches directly from the frozen state to the liquid state [9] .

Mostly all cryopreservation protocols use 37 °C as an optimal temperature of thawing .The recently introduced sperm stress tests require incubation of spermatozoa at 40 °C for 4 h and it was found that it is a useful predictor of pregnancy rate in ARTs [10,11].

The purpose of this prospective study was applying different thawing temperatures 37 °C and 40 °C for determining the optimum one on the motility recovery of human cryopreserved sperms of cancer patients.

2. Materials and methods

This study is a prospective single center observational study conducted on 30 men who visited the centre for infertility screen-

Peer review under responsibility of Middle East Fertility Society.
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https://doi.org/10.1016/j.mefs.2018.04.007
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ing attending Nile IVF center during the period from June 2015 and May 2016.

2.1. Experimental design

The study was approved by local ethics committee and informed written consents about the study and expected value and outcome were obtained. Exclusion criteria were the following: patients with clinical varicocele grade II or III, pyospermia, severe low count or received any doses of chemotherapy or radiotherapy before. The patients were subjected to history taking, full examination including general, abdominal and local examination was done followed by evaluation for full semen analysis, hormonal profile (FSH;LH;TOTAL TESTOSTERONE), scrotal duplex to determine testicular size, echogenicity and the presence of varicocele.

2.2. Semen samples

Semen samples were obtained by masturbation and collected into sterile containers, and then they were allowed to liquefy at 37 °C for 30 min. After liquefaction, semen samples were examined for volume, sperm concentration, morphology and motility (semen analysis).

A cryomarker was used to label the straws with the patient's first and last names, an additional unique identifier such as a medical record number or accession number, and the date.

2.3. Freezing semen samples

After analysis, each semen sample was prepared for freezing by drop wise dilution with a common sperm-cryopreservation medium containing glycerol (Sperm Freeze TM Fertipro) (final concentration 7%), citrate, glycine, glucose and antibiotics. After equilibration in a 37 °C water bath for 10 min, the sperm-medium mixture was drawn into 0.25-ml straws. For each patient, ten straws were frozen for future ICSI cycles plus two extra straws to used in our study. All straws were loaded into racks and placed into the chamber of a programmable biological freezer and cooled according to the following program: [(i)cooling rate of – l°C/min from room temperature to +5 °C, (ii) freezing rate of −10 °C/min from +5 °C to −80 °C, (iii) rate of −25 °C/min from −80 °C to −130 °C, (iv) plunge into liquid nitrogen (−196 °C).]

Sperm samples were stored frozen in liquid nitrogen for at least 24 h. After that, the two extra straws were thawed by removing them from the liquid nitrogen container, followed by 3 min at 37 °C in a warming chamber and the other one at 40 °C.The primary outcome was the assessment of recovery of total and progressive motility.

2.4. Statistical analysis

Data were statistically described in terms of mean standard deviation (SD), median and range, or frequencies (number of cases) and percentages when appropriate. Comparison of numerical variables between the study groups was done using Mann Whitney *U* test for independent samples. For comparing categorical data, Chi square test was performed. Exact test was used instead when the expected frequency is less than 5. *P* values less than 0.05 was considered statistically significant. All statistical calculations were done using computer programs SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows.

3. Results

The average age of the 30 cancer patients who was included in this study, determined as 28.38 ± 6.44 and the average of sperm count was 32.34 ± 21.31. The progressive motility (PR) before cryopreservation and non-progressive motility were determined as a standard .the average was 13.59 ± 12.91 for progressive motility and 24.66 ± 11.95 for non-progressive motility.

For Hodgkin Lymphoma, the average mean progressive motility recovery after thawing at 40 °C was 5.11% ± 4.22, compared to 2.67% ± 3.57 at 37 °C thawing temperature, showing significant difference with P value <0.05 (Fig. 1).

For Non Hodgkin Lymphoma, thawing at 40 °C showed recovery of progressive motility with 10.69% ± 4 compared to 4.31 ± 3, showing also a significant difference with P value <0.05.

In patients with leukaemia, thawing at 40 °C and 37 °C showed recovery of progressive motility with 10.6% ± 1.15 and 4.31% respectively. But due to low patient number of leukaemic patients, no significance was reached. P value >0.05.

Also, no significant difference was found in patients with seminoma due to small patient sample. Progressive motility recovery at 40 °C thawing temperature was 15 ± 4 and for 37 °C was 7.5 ± 2.8. P value >0.05

The average of cryopreserved sperm motility at 40 °C was 4.76 (for progressive motility) and 11.17 (for non-progressive motility)

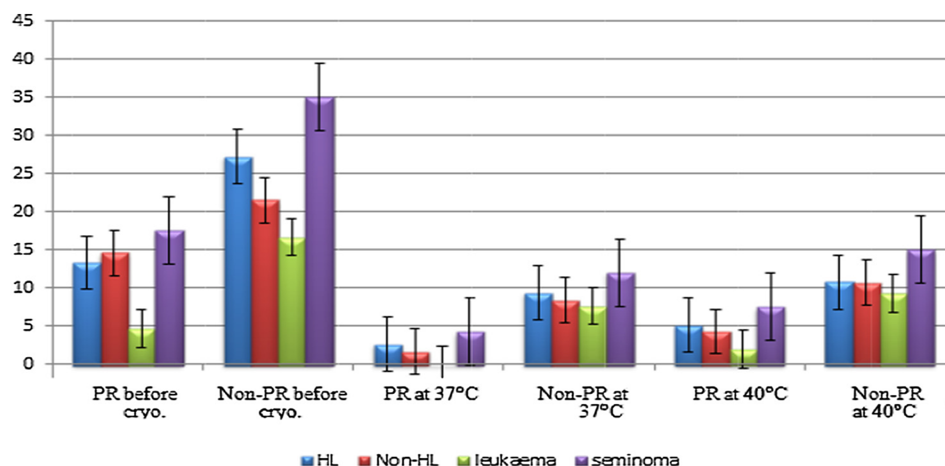

Fig. 1. Descriptive analysis of progressive and non-progressive motility of the types of cancer before cryopreservation, at thawing temperature 37 °C and at thawing temperature 40 °C with error bars.

Table 1
Describe PR and Non-PR of the types of cancer before cryopreservation and at thawing temperature 37 °C and 40 °C Table 1. Descriptive analysis of progressive and non-progressive motility of the types of cancer before cryopreservation, at thawing temperature 37 °C and at thawing temperature 40 °C .

Type	HL "n = 10" Mean ± Std. Error	Non-HL "n = 13" Mean ± Std. Error	leukemia "n = 3" Mean ± Std. Error	seminoma "n = 4" Mean ± Std. Error
PR before cryo.%	**13.33 ± 2.76**	**14.62 ± 4.75**	**4.67 ± 0.33**	**17.5 ± 4.78**
Non-PR before cryo.%	**27.22 ± 4.65**	**21.54 ± 2.62**	**16.67 ± 3.33**	**35 ± 6.45**
PR at 37 °C	**2.67 ± 1.19**	**1.69 ± 0.44**	**0 ± 0**	**4.25 ± 2.17**
Non-PR at 37 °C	**9.34 ± 1.72**	**8.38 ± 0.836**	**7.67 ± 1.33**	**12 ± 2.71**
PR at 40 °C	**5.11 ± 1.41**	**4.31 ± 0.843**	**2 ± 0**	**7.5 ± 1.44**
Non-PR at 40 °C	**10.78 ± 1.59**	**10.69 ± 1.11**	**9.33 ± 0.67**	**15 ± 2.04**

while the average in the 37 °C case was 2.17 for (for progressive motility) and 9.10 (for progressive motility), which means that the effect of thawing at 40 °C was better than thawing at 37 °C and had a high average of motility recovery. The P value was <0.05 which confirmed on the significant result

There was a significantly higher sperms with PR and Non-PR of patients with HL and Non-HL when thawing occurs at 40 °C than those when thawing occurs at 37 °C (Table 1).

There was a non significant difference between thawing at 37 °C and 40 °C in both leukemia and seminoma patients regarding PR and Non-PR. In both types, the sample size was too small.

There was a highly significant difference regarding PR and Non-PR of all patients regardless their type of cancer when thawing occurs at 40 °C than those when thawing occurs at 37 °C (being higher in the former ones)

4. Discussion

Steliarova-Foucher et al. documented an increase in the rate of survival among cancer patients because of the advanced therapies and techniques. And most of the cancer patients' cases in men are at young age ranged from 20 to 40 [12]. Therefore, the focus on saving the patient's life shifted to improving the quality of life after treatment. Male infertility due to cancer treatments may be temporary or permanent and can range from mild to severe. So cryopreservation of sperms is mandatory prior chemotherapy for fertility insurance [13]. Based on that, this study included four types of cancer patients which are Hodgkin lymphoma, non-Hodgkin lymphoma, leukemia and seminoma. The age of patients ranged from 16 to 42 years as the young age has a serious priority for sperm cryopreservation. The post-thawing revealed a high significant result at Hodgkin lymphoma, non-Hodgkin lymphoma patients but the result of leukemia and seminoma was non-significant.

Sperm cryopreservation is considered an essential procedure in ART. But during the freezing and thawing process, a sperm cryo-damage may occur. However, there are no published controlling protocols to minimize this damage. The sperm membrane may be exposed to physical and chemical stress because of the freezing and thawing procedure which may lead to negative effect on the fertilizing ability [14–16]. This study selected the fast-thawing rate at 40 °C for 3 min and resulted in no cell damage with high motility recovery.

Mortimer reported that the survival of spermatozoa with qualified function during freezing and thawing depends on many factors. The thawing temperature is a critical factor among these factors [14]. In the current study, the fast thawing rate was responsible for these improved results, because the rapid thawing prevents the ice crystals formation and minimizes the membrane damage [17]. Regarding the thawing temperature effect, several authors assumed that the cell damage degree may be associated with using higher temperature in thawing process [18].

The major result emerging from this study was the increase in motility recovery observed after thawing of cryopreserved human sperm at 40 °C compared with the thawing temperature at 37 °C. This finding was observed after cryopreservation of 30 semen sample of cancer patients with different tumor types. In accordance with Martinez-Soto study, better sperm motility was achieved after thawing at 40 °C and reported that the percentage of spermatozoa with fast-linear movement is increased to 28.05% at 40 °C compared to the percentage 17.26% after thawing at 37 °C with p-value <0.01 [19]. Sperm thawing at 40 °C resulted in a significant increase in sperm motility recovery compared with thawing at 37 °C in our study. Calamera and his group documented that Sperm thawing at 40 °C could be a safe way for enhancement the motility recovery after cryopreservation human sperms [20].

Chatterjee and his group explained the potential increase of motility when using 40c as a thawing temperature compared to 37, is that high thawing temperature results in fast rate of sperm antioxidant enzyme recovery. At thawing, two major opposing forces control cell damage (1) production of oxygen radicals (2) recovery rate of sperm antioxidant enzymes. Therefore, higher thawing temperature results in rapid neutralization of free oxygen radicals. Another explanation for increased final motility when thawing at higher temperature, may be related to the increased biosynthesis of ATP in the spermatozoa axoneme [21].

This study revealed motility observations which are in concordance with data documented previously, where an increase in motility recovery was documented when a high thawing rate was applied [22,23]. However, other studies contradicted that and found no difference in motility recovery when different thawing temperatures were used [24,25]. The differences effects on the motility recovery reported in this study showed that the thawing process optimization is essential to ensure a fast-linear movement of sperms. And that was performed by raising the thawing temperature to 40 °C and fast-thawing rate.

5. Conclusion

In summary thawing of cryopreserved human sperm at 40 °C results in a statistically significant increase in motility recovery compared with thawing at 37 °C. This increase in motility recovery refers to be related to a more rapid and complete recovery of membrane integrity and permeability. Incubating the sperm at 40 °C for a short time within the thawing process doesn't appear to be harmful to as it was thought. Thawing of human sperm at 40 °C for 3 min may become a beneficial for the recovery of functional human spermatozoa after cryopreservation. Further studies are still essential to assess whether thawing the sperm at40 °C have similar fertilization and pregnancy rates in IVF compared with thawing at the standard temperature of 37 °C.

Conflicts of Interest

The authors have no conflicts of interest to declare.

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