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Original Article

Study of human sperm motility post cryopreservation

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ABSTRACT

Background: Cryopreservation of spermatozoa is a widely used technique to preserve the fertility of males. It can also benefit the armed forces personnel who are to be sent for long recruitments, while leaving their families behind. This study, apart from studying the effects of freezing and thawing, reveals the effect of the post thaw interval on the motility of the human spermatozoa and thus widens the insemination window period.
Methods: A detailed semen analysis was carried out as per the WHO guidelines for 25 samples. The samples were then washed, analysed and frozen in liquid nitrogen. The semen samples were subsequently thawed and similarly analysed after 20 min and 40 min of thawing. This was then followed by statistical analysis of the comparative motilities.
Results: Motility of sperms is found to decrease after cryopreservation. However, the study revealed that after thawing a significant increase in the motility of the sperms was noted with the progression of time ($p < 0.05$).
Conclusion: By simulating conditions similar to the in vivo conditions for the post thaw semen samples, we can safely wait, confirm the parameters like motility and count, and then inseminate the samples instead of blindly inseminating them immediately after thawing.

Introduction

Cryopreservation is a process where cells, whole tissues or any other substances susceptible to damage caused by chemical reactivity or time are preserved by cooling to sub zero temperature. Semen cryopreservation is a process to preserve sperm cells. Cryopreservation of tissues began with the freezing of Fowl sperms, which during the year 1957 was cryopreserved by a team of scientists in UK. The process was applied to humans during 1950 with pregnancies obtained after insemination of frozen sperm. Cryopreservation of spermatozoa is at present, the most valuable and used way to preserve reproductive function in men undergoing

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gonadotoxic treatment such as chemo- or radiotherapies.[1] In addition sperm cryopreservation is increasingly used even in cases of other disorders, such as auto immune diseases and myelodysplastic syndromes requiring treatments that may affect reproductive functions. Moreover, sperm cryopreservation is offered to patients with severe oligospermia or ejaculatory disorder for intracytoplasmic sperm injection (ICSI).Also, some non-malignant diseases such as diabetes and autoimmune disorders may lead to testicular damage, and cryopreservation is also advisable in these conditions.[2] It can be a boon in the armed forces scenario, for the couples who are unable to stay together due to their field or ship recruitments.

The first reported effects of low temperatures on spermatozoa were recorded by Lazaro Spallanzani in 1776, and the first to discuss the possible uses of sperm banks was the Italian neurologist, physiologist and anthropologist, Paolo Mantegazza. He wrote the following in 1866: "It might even be that a husband who has died on a battle-field can fecundate his own wife after he has been reduced to a corpse and produce legitimate children after his death".[3]

Cryopreservation is known to have detrimental effects on the sperm structure and function. Recovery of an optimal number of functionally intact spermatozoa from thawed samples has always been the main objective of semen cryopreservation technology.[4] The principle involved is the prevention of intracellular ice crystal formation and optimal dehydration of the cells. For this a large variety of glycerol and non − glycerol based cryoprotectants have been used. Cryoprotectants act by decreasing the freezing point of a solution by increasing the amount of salts and solutes present in the liquid phase of the sample, thereby decreasing ice formation within the spermatozoa.[5]

Human uterus is considered as the best natural incubator for inseminated semen samples. Therefore most of the clinicians believe in inseminating semen samples immediately after thawing. This is because they fear that if the sample is kept in the in vitro conditions for a long time, the sperms might lose their functional capacity. The purpose of this prospective study was to carry out computer assisted semen analysis of the frozen sperm sample after thawing and predict their cryosurvival and evaluate the progressive motility recovery rate of the frozen spermatozoa 20 and 40 min post thawing.

Therefore by this study we intended to reconsider the ideal time for insemination after thawing of the semen sample.

Materials and methods

Subject selection: 25 patients who were enrolled at the ART centre for their own semen analysis were selected after a verbal informed consent. Semen samples were produced by masturbation into 110 ml sterile containers (code 351006, BD Biosciences, USA) from these 25 patients after 3 days of abstinence. As per the inclusion criteria, samples with a volume ≥2 ml, total sperm count ≥15 million/ml, motility ≥50% were included in the study and samples with round cells ≥1 million were excluded.

Assessment of spermatozoon count and motility: After leaving the sample for 20 min at 37 °C for liquefaction to occur, the motility of spermatozoa was evaluated using the Computer Assisted Semen Analysis (CASA), (ISAS, Proiser SL, Valencia, Spain). The motility of each spermatozoon was graded as "a," "b," "c," or "d" according to the WHO laboratory manual for semen parameters [WHO 1999 guidelines].[6] (Table 1). As per this classification, the sperms with type 'a + b + c' motility were taken as the total number of motile sperms and the ones with type 'd' motility were taken as the total number of immotile sperms. Spermatozoa concentrations were evaluated using a Makler chamber (Sefi Medical Instruments, Haifa, Israel) (Fig. 1) as per the WHO 2010 guidelines. Samples with concentrations of 15×10^6/ml or less (oligozoospermia)[6] and round cells of $>1 \times 10^6$/ml were excluded from the study. Minimal clumping and agglutination seen on visual analysis was ignored.

Semen preparation protocol: The sample was transferred to a11 ml conical tube (Code CE 0543, Nunc, Denmark). It was mixed with 4 ml of gamete buffer media (Code K SIGB, William A Cook, Australia Pty. Ltd.) (Fig 2) and centrifuged for 10 min at 1500 rpm. After centrifugation a pellet containing the sperms was formed at the bottom of the tube. The supernatant was discarded, 2 ml of the gamete buffer media was again added without disturbing the pellet and the sample was left for swim up in the incubator for 20 min at an inclination. The motile sperms left the pellet and swim into the supernatant. The swim up containing the motile sperms constituted the post wash sample which was then analysed by CASA.

Sperm freezing and thawing protocol: 1 ml of sperm freezing media (CodeHSISC-20 William A Cook, Australia Pty. Ltd.) was added drop by drop to the post wash sample (taking about 3–4 min to prevent osmotic shock to the sperms), with constant shaking to ensure thorough mixing of the two. The suspension formed was transferred to the 1.8 ml cryovials (Code CE 0543, Nunc, Denmark). The sample was subjected to sequential cooling first at room temperature for 10 min, then in the refrigerator (4 °C) for 10 min. Following this, the samples were frozen by static-phase vapour cooling and then plunged into liquid nitrogen (−196 °C) (Fig. 3).

After cryopreservation for at least 60 min, the sample was thawed at room temperature. During the procedure, universal safety precautions were taken and cryogloves were worn. The cryovial was opened keeping the mouth of the vial away from the face as sudden expansion of the air in the vial may lead to

Table 1 – Sperm motility – 4 groups (WHO 1999 guidelines).

WHO category	Code	Corresponding velocity
Rapid progressive	a	>25 μm/s (≥1 monitor square, or 5 sperm head lengths
Slow progressive	b	5–24 μm/s
Non progressive	c	<5 μm/s (<1 sperm head length)
Immotile	d	–