HUMAN SPERM CRYOPRESERVATION- A REVIEW

Abstract:

Cryopreservation (CP) was first introduced sometime in the 1960s and since then process of CP of human sperm has been one of the effective procedureswith respect to male fertility used in variety of casesprior to treatment for preserving donor and participant sperm cells prior to reproduction therapies like ART (Assisted Reproductive Therapy/Technology). CP of sperm is a common procedure for preserving male fertility in situations, of infertility due to reasons like cancer therapies and many more. While the implications of cryogenics on cells have been widely established, several literature are there on whether CPimpacts sperm chromosomal integrity or the usage of a specific and unique freezing-thawing strategy.The purpose of this article is to discuss and bring to focus about the effects of freezing and thawing on human spermatozoa and the changes in acrosome integrity, Mitochondria, DNA integrity, plasma membrane integrity.

Keywords: Cryopreservation, Human Sperm, Acrosome Integrity, Mitochondria , DNA & Plasma Membrane

INTRODUCTION:

For at least 135 years, researchers have attempted to keep mammalian spermatozoa fertile for longer periods of time. In 1866, an Italian physician named P. Mantegazza developed the concept of a human sperm bank to retain semen specimens after observing the survival of human spermatozoa that had been cooled to –150°C[1].ART has been used in millions of times in fertility treatments to enable child birth since the first baby via test tube in the world(Louise Brown), born on 25th July in the year 1978. This wasdue to the ground-breaking work of Dr. Robert Edwards and Dr. Patrick Steptoe. CPof sperm has become common in all ART labs since then. [2].

The Image of Human Sperm is shown below:

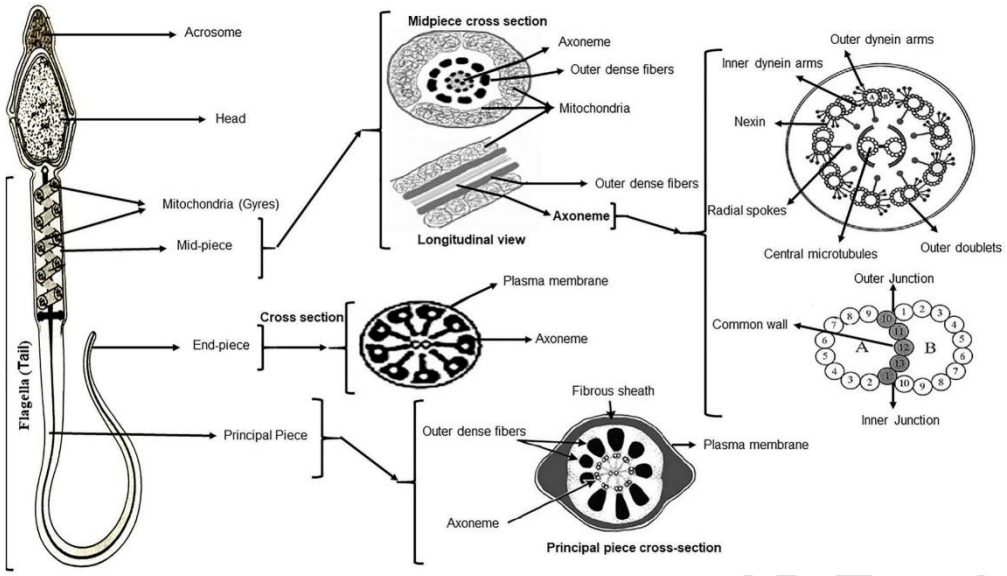

Fig. 1. Human Sperm[15]

Multiple ART programmes employCPfor male fertility protection in cases, for example, prior to cytotoxic chemotherapy [3], or some surgical proceduresmay lead to testicular failure or dysfunction. Patients are assured something similar to "fertility insurance" by freezing their sperm prior to treatment, allowing patients to have kids of their own using intrauterine conception or in vitro fertilisation techniques.

In cases related to cancer therapy etc that may cause harm, resulting in sterility or reduced fertility or any irreversible damage to germ cells as a result of adjuvant therapy, semen cryo-storage seems to be the only option that is reliable and established. Several factors determine the risk related with therapy, such as the age of the patient at the time of treating, the dosage, the therapy site, as well as the type of treatment [4]. CP is also recommended in circumstances where testicular damages are present due to issues that are not malignant like any autoimmune conditions, diabetes mellitus etc.[5]. CP is required in countries where heterologous fertilisation is legal and in donor insemination programmes so that infectious agents such as the human immune-deficieuse purposes [6]. Cryo-storage of sperm is also used to prevent repeated biopsies or aspirations in azoospermic individuals that have had spermatozoa removal or interventional spermatozoa aspiration [7]. Furthermore, CP is done in patients as a routine prior to beginning an ART, when Individuals preserve the semen sample in advance of oocyte retrieval to avoid problems caused by missed ejaculation owing to specific emotional states or other commitments [8]. Finally, freezing of male gametes is usually suggested to prevent infertility in subjects exposed to dangerous chemicals which may interfere with gamete formation for various reasons [8].

TECHNIQUES IN CRYOPRESERVATION:

Two major freezing methods employed are Slow freezing (SF) and Fast Freezing (FF) used in CP.

A. Slow Freezing:

Behrman and Sawada's [9] SF technique involves gradually cooling sperm over a period of 2–4 hours in two or three phases, with the help of a freezing unit.

In manual mode the temperature of the sperm is reduced while adding a CPT stepwise and then immersing the samples in liquid nitrogen at 196°C [10][11]. The ideal initial cooling rate has been found as 0.5–1°C/min [12]. The sample is then frozen at a rate of 1–10°C/min from 5°C to 80°C. The item is subsequently immersed in liquid nitrogen at a temperature of 196°C.

The samples are stored in Programmable freezers using a plate, cooled by liquid nitrogen. Liquid nitrogen is injected into the tank, and once configured, the machine employs software data recording to drop from 20°C through 80°C by 1.5°C/min, then at 6°C/min, with the liquid nitrogen temperature remaining at 196°C at the completion of the forty minute operation [13]. They have also been utilised to improve the consistency of freezing operations and also doesn't require manual operational monitoring, making it easy to use.

B. Rapid Freezing:

Sherman [14] was the first to propose RF. In this eight to ten minutes of direct contact between the straws and nitrogen vapours and immersing in liquid nitrogen at 196°C. There is a heat gradient inside nitrogen vapours as a function of distance and volume of the liquid below. The sample is initially mixed with an equal amounts of cold cryoprotectant droplet wise; the mixture is next kept into the straws and stored at 4°C for ten minutes. Then, the straws are put 15–20 cm above the level of liquid nitrogen (80°C) for fifteen minutes before being immersed in liquid nitrogen. IT is then cooled down horizontally. This method has a number of disadvantages like reduced repeatability, freezing temperatures ranging from 70 to 99 degrees Celsius [15] etc.

CLASSIFICATION OF CRYOPRESERVATION:

CP procedures can be broadly divided into four types based on cell types [16]:

1) Slow Freezing:

The method of SF has been discussed earlier which is the basic method based on a slow process.

2) Vitrification

- The Cryoprotectant (CPT) is made up of three main components, Polyvinylpyrrolidone (PVP), Dimethyl Sulfoxide (DMSO), and Glycerol.
- It is necessary to utilise CPT concentrations that are high enough to avoid ice crystallisation.
- HEPES is added to the CP to buffer the pH and to make the it an isotonic mitochondrial solution.

3) Non-freezing storage at subzero temperatures

- It works on the basis of a mechanism for cooling that makes a changeable magnetic field.
- The sample would be chilled to a super cooled condition even if CPTs were not used.

4) Preservation in a dry environment.

- This is the method when the long duration storage is possible without freezers or use of chemical like liquid Nitrogen.
- The loading cells that have the protective disaccharide trehalose increases cell survival when water level is not high.

EFFECTS OF CRYOPRESERVATION:

The effects of CP are to be discussed with respect to different aspects like acrosome integrity, Mitochondria, DNA integrity, plasma membrane integrity.

A. Acrosome Integrity & CP:

The acrosome is a one-of-a-kind organelle membrane present on top of the sperm nucleus's front region and has stayed remarkably intact throughout evolution. This acidic vacuole includes a number of hydrolases that allow sperm to penetrate the egg's coverings when it is released [17].

According to a study [18] conducted as early as 1991, before and after CP human spermatozoa, total acrosin activity and acrosomal status were assessed. In this investigation, three CPmethods were experimented with, where it was found that post preservation acrosin

activity and the percentage of intact acrosomes decreased. The measured value of reduced magnitude was only marginal but statistically connected, showing that acrosomal depletion was a factor in the lowering of acrosin activity. On average, motility decreased more than the proportion of sperm cells with undamaged acrosomes (27%) or overall acrosin activity (43%). These data suggested that cell death may have resulted in acrosomal damage. Motile spermatozoa possessed 96% intact acrosomes after thawing, but their acrosin activity was substantially lower than motile, unfrozen sperm cells. These data not only back up the theory that CP causes Acrosomal loss, but they also reveal that spermatozoa with intact acrosomes that sustain CP have reduced total acrosin activity.

CP has been linked to sperm shape and function modifications as Sperm cells tend to be less vulnerable to CP degradation than other cell types due to their fluid membrane and low water content.[19]. During CP of human spermatozoa, a some issues include thermal stress with the formation of ice in extra& Intra cellular regions, drying of cell, and osmotic shock. [20].

The production of internal or external ice crystals is the principal source of cellular injury during CP and the cooling rate determines the extent of cryoinjury during the freezing process [21].

Due to efflux of water across a membrane is impeded by rapid cooling, substantial intracellular ice formation occurs, resulting in super-cooling. This has a negative impact on cell survival. A slow cooling rate, on the other hand, causes water to flow out of the internal environment and into the external environment, increasing concentration of solutes and osmotic pressure. This condition produces cell volume changes associated to water movement, dehydration, and toxic damage due to the high solute concentration.[22]. Cryoinjury can occur both during the thawing and freezing processes, as the ice melts or recrystallized [22]. In frozen samples, recrystallization of extra- and intracellular ice happens as smaller ice crystals, with a recrystallization rate that increases with temperature. [23].

B. Mitochondria & Plasma membrane Integrity & CP:

According to a research, the injury caused due to chilling seems to alter both the integrity and the structure of plasma membranes [24], that are made up of cholesterol &phospholipids[25].

Even though large concentrations ofpolyunsaturated fatty acids& cholesterol give the plasma membrane enhanced fluidity at lesser temperatures [26], cooling alterslipids membrane phase &protein activity during the process. In the outer layer of cell, plasma membrane has

glycocalyx (carbohydrate-rich membrane), that has chains of oligosaccharide that bind to essential proteins in the plasma-membrane like glycoproteins or lipids like glycolipids. [19].

Mitochondria, also known as the power house provides energy for movement of sperm and ispresent between the plasma membrane and the nine fibrous columns [27, 28] or oxphosin the mitochondria [29] that give most energy.

The ATP (Adenosine Tri Phosphate) in the mitochondria goes to the microtubules for increased motility. [30].Variations in fluidity the mitochondrial membrane can affect mitochondrial function and cause reactive oxygen species to be produced (ROS) [20]. Sperm cell damage in plasma membrane and peroxidative damage mediated by high ROS concentrations has been linked to axonemal structural degeneration. [31].

C. DNA Integrity &CP:

While the effects of cryonics on sperm cell's fertilisation ability, motility, morphology, and viability have been thoroughly reported. In the literature, there is no consensus if or not CP leads to DNA damage, and also the damage it causes. Studies have found significant changes in the DNA integrity of the sperm after CP [6], however some other researchers have found the opposite [32]. This discrepancy between the researches can be explained partially due to the reason that the findings are not to a large number of samples, as well as the use of

(1) Different freezing procedures,

(2) Different DNA integrity tests, and

(3) Varied semen preparation methods employed prior to CP

For example, Donnelly and colleagues [7] Before and after CP, 50 males were tested for DNA integrity in both semen and processed sperm samples (by methods of gradient centrifugation or direct swim up).They discovered that freezing sperm in seminal plasma improves DNA integrity after freezing. This could be due to the high antioxidant content of seminal plasma.

In a study by Petym and colleagues [33], cryodamage on sperm chromatin was studied using two different methods of liquid nitrogen vapour against computerised programme freezer. They examined 50 sperm samples and DNA damage increased upon freezing using liquid nitrogen.

Fragmentation of the DNA is due to the release of apoptosis-inducing substances from the mitochondria [34]. DNA damage seems reduced in case of genistein [35], resveratrol [36], and

ascorbic acid [37]addition to the seminal fluid during CP.Motility seems enhanced and ROS reduced by use of vitamin E [38], ascorbate, and catalase [39], though they do spermatozoal viability is not changed nor reduce DNA damage.

CONCLUSION:

Sperm CP is frequently used to preserve sperm cells before ART, chemotherapy, vasectomy, or surgery caused infertility, to assure the restoration of a small number of sperm cells in extreme infertility of males. Sperm CP is an important in fertility management and its success appears to have a significant impact on ART.

Even though the advantages of CP are multi-fold, the effects of CP on the human sperm require more research. This suggests that a multi-center result with many semen specimens to process using different freezing procedures for further studies. However, the baseline is that proper technical measures need to be employed to provide maximum protection to the sperm cells and proper sperm selection methods can be employed after CP. Some of techniques like Microfluidic sperm sorting device and magnetic activated cell sorting (MACS) method can be experimented with to reduce the effect of CP. In a study conducted [40] MACS appeared to be a reliable, safe, and fast approach for identifying functioning sperm. When used in conjunction with normal sperm selection approaches in ART, this strategy has the potential to improve conception rates. These studies give hope for many people who have no other option than to depend on CP. More research needs to be done on the various aspects related to CP& how it can be made most efficient which is something that can be done in the future.

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