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 procedures with respect to male fertility used in variety of cases prior 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 literatures are there on whether CP impacts 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 the effects of freezing and thawing on human spermatozoa and the changes in Acrosome integrity, mitochondria, DNA integrity, and plasma membrane integrity. In this age of technological advancements, after being used to treat infertility, CP acquired popularity in human medicine. It improves the effectiveness of assisted reproductive treatments by allowing all retrieved and/or fertilised cells to be saved for later use. Couples who conceive during their first treatment cycle can donate their unused frozen embryos to research under the CP programme. It enables people who are losing their fertility to maintain their reproductive cells and, in the future, conceive via assisted methods. It can be used by women who want to postpone childbirth or have a family history of early menopause. Similarly, the varied usage in critical applications makes CP the need of the hour.

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. The first baby via test tube in the world is Louise Joy Brown, born on 25th July 1978. This was due to the ground-breaking work of Dr. Robert Edwards and Dr. Patrick Steptoe. CP of sperm has become common in all ART laboratories since then. [2].

The Image of Human Sperm is shown below:

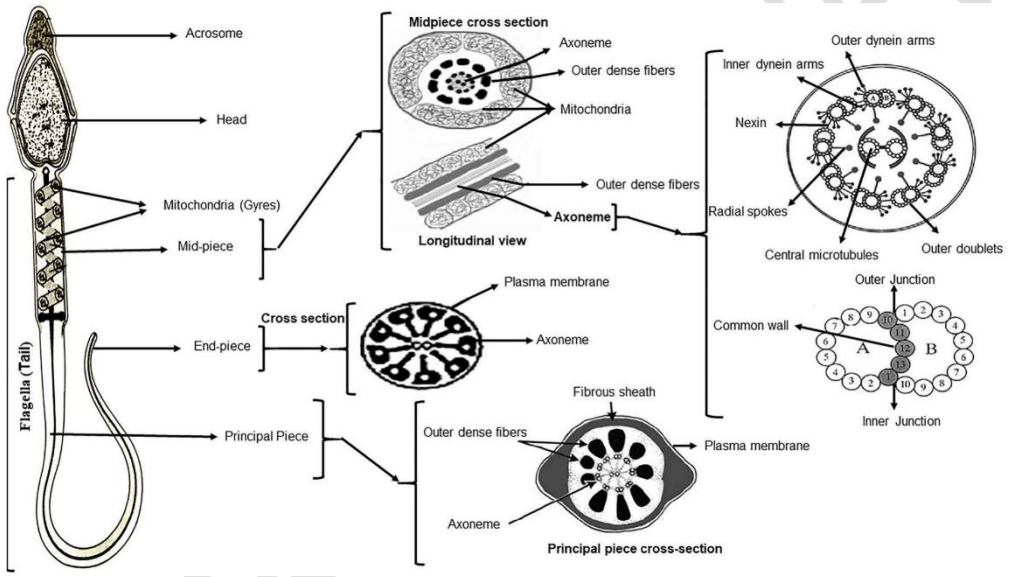

Fig. 1. Human Sperm[15]

Multiple ART programmes employ CP for male fertility protection in cases, for example, prior to cytotoxic chemotherapy[3], or some surgical procedures may 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 that may cause harm, resulting in sterility or reduced fertility or any irreversible damage to germ cells can occur as a result of adjuvant therapy and semen cryostorage 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 heterogonous fertilisation is legal and in donor insemination programmes so that we prevent infectious agents such as the human immune-deficiency purposes [6]. Cryostorage of sperm is also used to prevent repeated biopsies or aspirations in azoospermic individuals who 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. It involves 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 amount 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, and freezing temperatures ranging from -70 to -99^0 C [15] .

CLASSIFICATION OF CRYOPRESERVATION:

CP procedures can be broadly divided into four 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 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 acrosomeintegrity, mitochondria, DNA integrity and 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, total acrosin activity and Acrosomal status were assessed before and after CP human spermatozoa. In this investigation, three CP methods were experimented with, where it was found that post preservation acrosin activity and the percentage of intact acrosome decreased. The magnitude of reduction of 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 acrosome (27%) or overall acrosin activity (43%). These data suggested that cell death may have resulted in Acrosomal damage. Motile spermatozoa possessed 96% intact acrosome 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 acrosome 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]. Some of the issue observed during CP of human spermatozoa, include thermal stress with the formation of ice in extra and 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]

The efflux of water across a membrane is impeded by rapid cooling, resulting in substantial intracellular ice formation and 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 and 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 and phospholipids [25]. Even though large concentrations of polyunsaturated fatty acids and cholesterol give the plasma membrane enhanced fluidity at lesser temperatures [26], cooling alters lipids membrane phase and 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 glycoprotein or lipids like glycolipids [19].

Mitochondria, also known as the power house provides energy for movement of sperm and is present between the plasma membrane and the nine fibrous columns [27, 28].

The ATP (Adenosine Tri Phosphate) in the mitochondria goes to the microtubules for increased motility. [30].Variations in fluidity of 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 based on 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 programmed 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 reduced with the addition of genistein [35], resveratrol [36], and ascorbic acid [37] to the seminal fluid during CP. Motility was enhanced and ROS reduced by use of vitamin E [38], ascorbate, and catalase [39].

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 component in fertility management and its success has a significant impact on ART. Though the significance of different methods of CP is reported, a comprehensive multi centre study comparing the CP methods with large sample is essential.

The main advantages of CP lies in the aspects of long term preservation of the sperm without its deterioration. It major use in the fertility treatments which have been a boon for many couples who are unable to conceive naturally.

A baseline In the proper technical measures need to be employed to provide maximum protection to the sperm cells and proper sperm selection methods to be employed after CP needs to be established. Advance techniques like microfluidic sperm sorting device and magnetic activated cell sorting (MACS) method have been established as reliable, safe and fast methods for identifying and selecting functional sperms [40]. Application of such techniques in conjunction with normal sperm selection approaches in ART has the potential to improve the conception rate particularly in patients who depend on CP.

GLOSSARY:

[1] Azoospermic: When the male ejaculate has no sperm.

[2] Cryodamage: It essentially implies the damage due to cold.

[3] Cryonics: freezing a person who has died of a disease with the hopes of resurrecting them at a later date when a cure for the ailment has been discovered.

[4] Cryoprotectant: a chemical that stops tissues from freezing or prevents cell damage during freezing.

[5] Cryostorage: storage at extremely low temperatures through cryopreservation

[6] Gamete: An organism's reproductive cells

[7] Motility: The ability of an organism by which they can move independently using the metabolic energy.

[8] Seminal plasma: Seminal plasma is a complex fluid made up of secretions from the seminal tract's organs and tubules as well as the testicles' seminiferous tubules.

[9] Spermatozoa (sperm): Male sex cells carrying the genetic material of a male.

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