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Semen freezing and male infertility

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Introduction

The first attempts at cryopreservation of sperm go back to the year 1776 AD when the scientist Lazzaro explained that the semen of horses that were frozen in snow recovered part of their motor ability after thawing them. In the year 1866, Italian scientist Mantegazza presented the concept of the Human Sperm Bank to preserve sperm samples after the success of his experiment in cooling human sperm to a temperature of -15 degrees Celsius (1). Good progress in the process occurred through the efforts of the two scientists, Bernstein and Petropavlovski in the year 1937, who introduced glycerol as an agent that helps reduce the effect of freezing on the sperm, which made it possible to prolong the time of freezing the sperm, and this was facilitated by the fact that the process of freezing the sperm became practically possible. As a result, the sperm became stable. The occurrence of expansion the techniques of the dairy industry led to an increase in the processes of artificial insemination of animals, which led to progress in the methods used in the field of sperm freezing (2). The scientist Chang (1940) recorded the first birth in rabbits from embryos kept at a temperature equal to 0 °C, and the scientist Lovelock is considered the founder of modern freezing science for his work from 1950-60 (3).

This was followed by the important discovery of the possibility of using liquid nitrogen to preserve the sperm inside it at a temperature close to -196 degrees Celsius below zero. down to -20 degrees Celsius (4).

A result of the wide progress in the field of reproductive medicine in the past five decades, made the process of freezing human sperm an important tool in the success of clinical intervention for infertility cases, in addition to the success of the development of semen bank (4).

The progress of assisted reproduction techniques and the use of microinjection of sperm into the cytoplasm, and the progress in the techniques concerned with dealing with the female gametophyte, necessitated an increase in demand and the need for techniques for freezing sperm samples and tissues containing sperm cells. (5).

Cryopreservation Processes

Cryopreservation is defined as the process of preserving biological materials at a temperature below zero, such as 80 - or 196 - in which the cellular biochemical reactions responsible for cell death are effectively limited, and the process aims mainly "to try to maintain the viability of cells and functional activity after a period of preservation At a temperature less than zero Celsius, cells kept at a temperature equal to -196 degrees Celsius have a halt in the

phenomenon of cellular diffusion at this point, as well as a reduction in the thermal energy required to complete chemical reactions (6).

The process of freezing sperm has become a typical method that the patient resorts to in a number of cases, including:

1- Exposure to possible infertility that may result of using chemotherapy or radiotherapy to treat cancerous or non-cancerous conditions.

2 - Exposure to possible infertility that may occur due to a surgical operation that may interfere with the case, such as Bladder Neck Surgery in young men, or removal of the second testicle in a patient with a lateral testicular tumor (6).

3- In the case of a continuous decline in the quality of semen as a result of diseases that carry risks related to the generation of a case of loss of sperm in the semen (azoospermia).

4 - In cases of removal of the vasectomy and the individual's desire to have children in the future.

5- In cases of azoospermous patients who have undergone the process of withdrawing sperm from the epididymis or testicular tissues.

6 - Some diseases such as diabetes and immune disorders, may lead to testicular failure.

7- It is considered necessary in countries where the law permits mixed fertilization in intrauterine insemination systems (7).

Types of Cryopreservation Types of Cryopreservation

There are two types of freezing processes: Slow Freezing and Rapid Freezing. All methods aim to achieve one goal, which is to try to preserve the cell for as long as possible from the effects of cooling, cellular ice formation, dehydration and, toxic effects at low temperatures.

Slow Freezing Processes

It was designed by scientists (8), and it includes a series of successive steps that take a time of 2-4 hours, and are carried out either manually or by using a special freezer. - 1 m 0 / min, followed by reducing the temperature from 5 ° C to - 80 °C, and at a rate of reduction 1 - 10 m 0 / min, followed by placing the sample in liquid nitrogen, whose temperature reaches 196 ° C (9).

Rapid Freezing Processes

It is one of the modern freezing techniques within this field of reproductive science, and it is also called "Vitrification", which means (transformation into a glass-like state), and it includes a number of methods that have been modified from the slow method, which aims to achieve the same goal, which is to try to preserve the cell from the effects of cooling. The formation of cellular ice, dehydration and, toxic effects that occur at a low temperature. The technique does not need special requirements to achieve this. The method depends on the rapid cooling of the medium of the sample to be frozen by immersion in liquid nitrogen, whose temperature reaches 196-C, which contributes to reducing the chance of crystal formation. Large ice crystals formed during freezing processes (10).

The method was presented for the first time by scientist Sherman, and the method includes direct contact between the sample and liquid nitrogen vapor for a period of 10 - 8 minutes, followed by immersion in liquid nitrogen. Cryoprotectants Substances To reduce the temperature by using high cooling rates quickly, which leads to the ultra-rapid transformation of the medium containing a high concentration of substances that help reduce the effect of the freezing process (4).

The process leads to a rapid increase in the viscosity of the medium, followed by a glass-like state, thus reducing the chance of forming ice crystals and the cellular damage generated by them. It is short, requires inexpensive materials, and has a very fast cooling rate (11).

Vitrification Principles

The method involves exposing the cell to a high concentration of cryoprotectants factors for a short period of time at room temperature, followed by a rapid cooling process. The medium contains factors that reduce freezing damage, whose concentration is usually less than 10% of the cellular medium. This leads to a state of dehydration in the cell because the high osmosis of the freezing agents leads to the state of cellular osmosis of the cells' water, and this makes the cells retain small amounts of water Inside it, this step is followed by placing the sample in liquid nitrogen, and because the cells contain small amounts of water inside, the chance of ice crystals forming inside the cells is reduced (12).

Cryoprotectants Substances

They are chemicals with low partial weight and highly permeable properties used to help protect sperm from the effects of the freezing process, especially the rate of ice crystallization. The most important preservatives used against freezing damage are glycerol, ethylene glycol, dimethyl sulfoxide, and 1,2-propanediol. The principle of its work depends on reducing the freezing point of the material, reducing the amount of salts and solutes present in the liquid phase of the sample, and reducing the formation of ice inside the sperm. (13). Add drops of preservative from freezing damage to an approximate volume of semen gradually with continuous mixing of the sample gently at room temperature, and then It is placed at 37°C for 10-15 minutes to allow for proper equilibration. The medium must interfere with the cells because the effect of preservatives against freezing damage is dependent on the interaction time between them and the cells (13).

Effect of Vitrification on Sperm

The process of freezing sperm includes conducting a number of operations, such as the process of mitigating the medium to be frozen by adding preservatives from freezing damage, as well as the process of immersing the mixture in liquid nitrogen with a temperature of 196-C, and the process of heating and thawing the samples, which in turn includes a number of operations from heating using the water bath and conducting The process of separating the freezing medium by conducting the centrifugation process and adding activation media and others. (14) media activation and others It is believed that each step of these processes induces negative effects that may lead to the generation of damage to the structure of the plasma membranes of the sperm, reducing the motility and fertilization capacity, and nuclear re-condensation. Premature nuclear decondensation. The sperms of mammals are highly sensitive to the condition of lowering the temperature beyond the freezing point of water (0°C), and this is due to the peculiarity in the composition of plasma membranes and intracellular membranes, which are major sites for cellular damage to occur when frozen (15).

The study (16) also showed that the freezing process leads to a significant decrease in the levels of both enzymatic and non-enzymatic antioxidants after freezing and thawing processes, and an increase in the levels of active oxygen species inside the medium.

Increased levels of active oxygen species at the expense of antioxidants lead to an oxidative stress state in which all cellular components of sperm such as lipids, proteins, acid nucleic acids, and sugars are potential targets of the toxic effects of oxidative stress (17).

High levels of active oxygen species and low levels of antioxidants may lead to increased rates of programmed cell death in the sperm cell population. (5).

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