Embryo Vitrification and Thawing Procedure

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ABSTRACT

Background:Eggs and embryos can now be stored and thawed more successfully than before. Until recently, "slow freezing" technology also known as cryopreservation was used to freeze embryos and eggs. This has now been supplanted with Vitrification (VTF), a more advanced technology that is generally regarded as the best method for cryopreservation. Hence more study is warranted to get more in-depth knowledge on the VTF and thawing aspects.

Aim: The main aim of this review paper is to understand the concept of Embryo VTF and thawing protocol and specifically about the Kitazato Vitrification (KVTF) process and protocols.

Method: This review paper makes use of historical research materials in the form of journal publications, articles, lab manuals, case studies and other authentic sources to gather information pertaining to Embryo development by understanding what happens after fertilization followed byVTF and Thawing.

Findings and Conclusion: The analysis has been done on aspects of Embryo VTF, rapid freezing, KVTF process, and other related aspects. It was found that the KVTF method gives the best rate for embryo survival and thereby giving a higher success rate on this.

Keywords: Embryo development, VTF, Thawing, Kitazato Vitrification

I. INTRODUCTION:

There has been a rise in interest recently in creating novel, gentler in-vitro fertilization treatment methods that could reduce patients' physical and emotional suffering, improve their comfort, and help cut down on treatment costs (Devroey, P, et al., 2009). The significant feature of oocyte cryopreservation is due to the increase of multiple clinical reproductive technology tools (Galeati et al. 2011), for instance, by permitting. By keeping oocytes before

they are lost, fertility can be increased as they can help prevent age-related changes in ovarian function, diseases, surgeries, chemotherapy, radiotherapy, and so on (Isachenko et al. 2006).

The treatment outcomes and advantages of fresh as well as frozen embryo transfers have been fiercely debated issues. Frozen embryo transfers (FET) with ART, as per several fertility specialists and medical professionals, have a greater conception rate of success than fresh embryo transfers.Ovulation inducement and monitoring are the first steps in both FET and fresh embryo transfers. The female will have an egg retrieval after the uterus is prepared, and the reproductive endocrinologist will fertilize your eggs with those of your spouse or a sperm donor. Three to five days following the egg extraction, the fertilized embryo will be transferred back into the woman's uterus after the eggs and sperm have been harvested and fertilized.

A FET, on the other hand, can take place years after a woman's egg retrieval and sperm fertilization. The fertility specialist will perform a FET in hopes of conceiving by implanting a thawed embryo into the woman's uterusMany reproductive clinics have noted greater rates of pregnancy when frozen embryos are used during the embryo transfer rather than fresh. FETs increased live birth rates for women with polycystic ovarian syndrome-related fertility issues, per a study (Shi et al. 2018). In 2020, a study was additionally done (Stormlund et. al., 2020) in whichno elevated rates of subsequent pregnancies or births among FET users based on the female's normal monthly cycles for periods and hormone levels were found.

A relatively novel method of preserving oocytes by freezing them quickly in a small volume of liquid that contains high quantities of cryoprotectants (CP) is calledVTF. A therapeutic procedure called oocyte VTF enables women to maintain their reproductive potential. VTF requires rapid cooling while minimizing freezing injuries with high CP concentrations (Seet et. al., 2013). This method minimizes damage to oocytes by causing a solution to become a solid that becomes like a glass when the temperature goes low, however, there are no formation of ice crystals (IC) here (Mart'nez-Burgos et al. 2011). Although this lowers the freezing harm caused by IC formation, which provides room for further research, the safety of these approaches in connection to the wellness of the progeny has not fully been established. Moreover, there is a greater chance that toxic, osmotic, and other types of damage will result in the cell shrinking differently during equilibration in CP or medium, which can lead to cell death (Trapphoff et al. 2010). Before going into the VTF protocol and

the Thawing process, it is essential to understand embryo development, to know what happens during and after the VTF process. The next section provides insight into this.

II. **Embryo Development stages**:

The intricate process through which a fertilized egg becomes an embryo in humans is covered in embryo development studies. The transformation here occurs from a zygote which has one cell intoa multidimensional as well as multi-layerfetus with imperfectly functional organs during the first eight weeks of development. Throughout the first 8 weeks of development, cell signaling, proliferation, and differentiation are essential for sustainable development and enhancing complexity within the embryo. With all of the variables included, the growth of the human fetus is divided into developmental processes week-wise. In the germinal phase of growth that lasts from fertilization to the uterine implant stage the first week is crucial. The following paragraphs provide explanations of key events that occur during this time of human embryogenesis, including gamete estimate, interaction and combining of gametes, fertilization, mitotic cleavage of the blastomere, morula formation, blastocyst (BC) creation, and BC implant.

The growing single-cell zygote will experience a number of mitotic segmentations immediately after fertilization as it journeys for about 4 days to reach the endometrium. In fact, the zygote can be seen with the untrained eye and is much larger than that other bodily cells. The segmenting process results in the formation of blastomeres, thatrestore the usual size of cells by gradually decreasing the cytoplasmic capacity of the zygote. Once cleavage begins, the concepts will proceed through a 2 - celled stage, a 4 - celled stage, a 12-celled stage, and a 16-cell stage. Although the cell count increases swiftly after segmentation, the conceptus keeps its size and shape due to the compact nature of its cells. (Khan et. al., 2022).

The morula first develops as a sixteen to thirty-two cell population 4 days post fertilization, when the zona pellucida (zp) is still covering it. Recent time-lapse microscopy observations suggest that compaction may be a critical survival step for embryos, allowing the embryo to identify and delete blastomeres with chromosomal anomalies. The cavitation of the morula, which occurs when it develops into a BC, depends heavily on the cell in the periphery, or the outer cell. The cell size and embryo itself will start developing from the cluster of cells found in the center of the morula.

BC Formation: When the embryo comes tothis stage, it has grown to between fifty to hundred and fifty cells and is beginning to press against the boundaries of the zp. This results when the fluid is pumping continuously and when the cell division occurs into the interior of the BC by cells in the outer cell mass, which creates a cavity or blastocoel. The expansion helps the zp get thinner and eventually ruptures, allowing the BC to emerge from the zp. BC enlargement also helps to move a cluster of cells to one side internallywhen a lot of fluid enters the area. The inner cell mass (ICM) within the BC is a collection of embryonic stem cells with limitless capability to develop. The cells that will develop into true fetal cells are present in the ICM. The BC embeds into the uterine lining during the process of implantation, which normally takes place in Week 2 of development (Khan et. al., 2022). Now that an overview of the process of embryo development is seen, the focus moves on to how VTF plays a role and what happens in the VTF

III. **VTF Protocol**:

The most recent developments have given rise to a lot of new aspects and avenues for oocyte cryo-preservation in many ways including method, the content of the CP, and so on which has resulted in increased survival of CP oocytes (Pope et. al., 2012). Oocytes can "safely" traverse through the critical temperature range of zero to fifteen degree Celsius thanks to ultra-rapid VTF techniques because no ICs can form there quickly enough to endanger the cell. It is practicable, economical, and morally important to store oocytes at liquid nitrogen (LN) temperature. All of them enable cooling speeds beyond 100,000 C/min (Kuwayama, 2005).Many studies investigating the impact of VTF on multicellular embryos have been published. The protection of endangered species' genetic material and that of important people for purpose of reproduction is made possible by VTF, which is a crucial tool in assisted reproduction (ART) programs (Novak et. al., 2019).

Cryopreservation methods used in ART include both slow cooling and fast cooling. Programmable freezers are used in the slow freezing technique to obtain regulated freezing rates. To stop ICs from forming in cells, a delicate balance must be maintained between the IC production range and that of cellular dehydration. In order to swiftly dehydrate cells using the rapid cooling approach, a high concentration of CPs and additives must be used. The device containing the cells must then be placed directly into LN to achieve asuper-fast cooling value. Quick freezing and VTF are the two categories under which rapid cooling can be classified. VTF is the process of turning a supercooled liquid into an amorphous solid that

resembles glass to avoid the development of ICs when the CP concentration is equal to or more than forty percent. Rapid freezing, not VTF, is the process used when ICs form in the solution during freezing or thawing. In comparison to conventional freezing techniques, VTF has a number of advantages: (1) It takes less time to complete the cryopreservation process; (2) having a higher concentration CP can reduce the timing for which it is being exposed to the CP; (3) It reduces issues caused when penetrating; and (4) It does not require the purchase of costly equipment in slow freeze method and its upkeep (Chen et. al., 2022)

VTF prevents the production of ice and creates a glassy state where molecules' translational motions are stopped without the suspension fluid's structural reorganization. To obtain a glass-like solidification of the embryos, rapid cooling rates, and high CP concentrations are required. When cooling at rapid rates to extremely low temperatures, the VTF solutions do not freeze. The very little amount of VTF media that must be chilled to extremely low temperatures (-192 to -1960C) in different kinds of cryostorage devices is what determines whether VTF will be successful. Hence, VTF is the significant increase in viscosity that solidifies a solution at low temperatures (Fahy, 1984). The abrupt temperature change can damage the embryos' plasma membranes and disturb the cellular skeleton. In comparison to slow freezing, VTF has proven to be a straightforward, affordable, reliable, and consistent cryopreservation technique. In some experiments, Pronuclear stage embryos that were vitrified displayed BC formation rates of thirty-one percent and cleavage rates of more than eighty percent on day 2 (Al-Hasani et. al., 2007). The success rate of in vitro fertilization (IVF) using FET and gametes is improved by VTF. On comparing conventional and cleavage stage embryos, Loutradi et al. (2008) conducted a meta-analysis and systematic review and discovered that VTF had a ninety sever percent survival rate while slow freezing had an Eighty-four percent survival rate. VTF has been shown to be better to slow freezing procedures due to higher clinical pregnancy rates and implantation rates (Balban et. al., 2008).The Kitazato VTF and Thawing process and its associated media compositions and process are studied in this research. The following sections give an insight into it.

IV. **KitazatoVitrification** (KVTF):

One of the leading companies in advancing and advancing VTF is Kitazato. Its most significant contribution to this sector has been the creation of the acclaimed Cryotop® (CT) Method, the industry standard for vitrifying oocytes and embryos at all developmental stages. The unique VTF container known as CT® is made of a thin, fine film strip that is enclosed to

a rigid plastic handle for the least amount of substance required to get the maximum rates of cooling and warming, which results in over 90% post-thaw survival. The CT® Process is straightforward, dependable, widely safe, and simple for anyone to use. The CT® Method has been used in over one lakh fifty thousand clinical instances in over ninety countries and two thousand two hundred ART facilities after more than ten years on the market. Several scientific journals attest to their great outcomes (kitazato – ivf.com, 2020).

A. Device Description:

In order to vitrify and thaw embryos for ART operations, the CT® VTF and Thawing Kits (TK) are made up of a collection of 5 media. The Equilibration Solution (ES) and VTF Solution (VTFS), both of which contain the CPs ethylene glycol and dimethyl sulfoxide, are two media components included in the CT® VTF Kit. Embryos get their first exposure to the ES and subsequently to VTFS during VTF. This technique can replace water by penetrating CPs through BC-stage embryos before VTF and LN preservation. A 1.5 ml vial of ES, two 1.5 ml vials of VTFS, four CTequipment, and two Repro Plates are included in the CT® VTF Kit.

The vitrified PN through BC stage embryos in the CT® TK are defrosted and CPs are removed gradually using three mediums. The TS (Thawing Solution), DS (Dilution Solution), and WS (Washing Solution) make up the CT® TK. Two four ml vials of TS, one 4.0 ml vial of DS, one four ml vial of WS, one Repro Plate, and two thirty-five mm dishes are already included in the CTTK. Gentamicin is present in all of the media in the CT® VTF Kit and CT® TK. These kits use aseptic filtration on the media while using radiation sterilization on the plates and storage containers.

B. Advantages:

This Method offers the best Warming Rate on the market for both Open System and Closed System due to its protocol and the ground-breaking design of its apparatus. Many studies have demonstrated that one of the key elements in raising survival rates is the warming pace. The figure below, gives the warming rates from different devices as taken from the FDA.

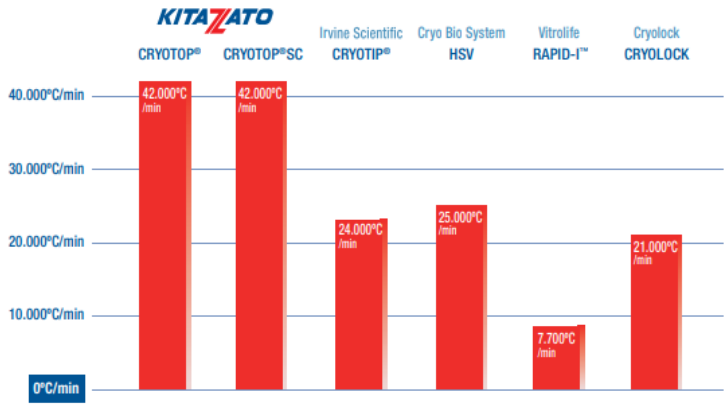

Figure. 1 Warming rates (*Kitazato-ivf.com, 2020)*

This is all made possible by the small amount needed to continue VTF. The loading of specimens with a capacity of 0.1 l is permitted by both CT® and CT® SC; this minimal amount enables the lowering of the concentration of "CP agents," enhancing the chance of VTF.

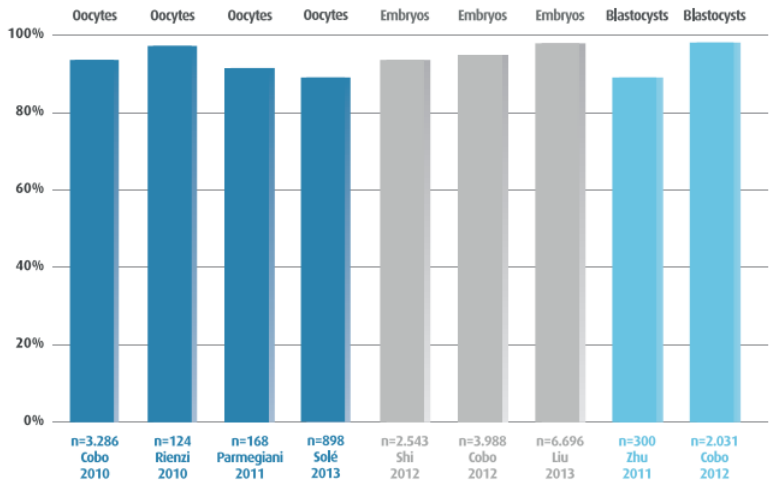

Figure. 2 CT Survival rates in Humans (*Kitazato-ivf.com, 2020)*

Excellent survival rates for oocytes and embryos at all developmental stages have been documented in a vast number of clinical publications using the largest research populations in the industry. The clinical information provided includes published articles that specifically mention the VTF procedure utilised for the preservation of embryos and BCs from female individuals using the CT® and VTF/Thawing media with HPC. Some of literature gives conclusive evidence on the advantage of KVTF method and its increase in chances of preganacy. One of the study's findings indicate that clinical pregnancies made up forty two percent of all transfers, while live births made up thrity percent of all transfers (Kato et. al.,

2012). Another study indicated that clinical pregnancies accounted for fifty nine percent of all pregnancies, whereas continuing pregnancies accounted for forty eight of all pregnancies (Ku et. al., 2012). Finally, another study's findings indicated that clinical pregnancies accounted for forty nine percent of all cycles and ongoing pregnancies for forty six of all cycles (Inoue and Yelian, 2014).

V. Conclusion:

The outcomes of the different studies on performance testing show that the CT® VTF Kit and CT® TK are equally safe and efficient to the predicate equipment, and they support the finding of considerable equivalence. Being the finest procedure for oocyte cryopreservation, the CTapproach is now being employed in an increasing number of facilities throughout the world for oocyte VTF. Nearly all of these labs have shown considerably higher survival, in vitro development, and subsequent conception rates than were previously attained with classical freezing. The approach has only lately gained international recognition, though, therefore there are still a limited number of published reports. It is very likely that shortly, publications from other groups will also be published with similar findings, demonstrating the utility of the method for human oocyte cryopreservation. Using the CT technique, more than three hundred healthy babies have so far been produced from vitrified oocytes. The available data from several laboratories point to the high viability and parity of developmental capacity of oocytes vitrified using this approach (Kuwayama, 2007). This mounting research demonstrates that CTVTF may provide remedies for women with diverse infertility issues and may help compensate for women's reproductive disabilities.

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