RESEARCH ARTICLE

Implantation, Pregnancy, and Perinatal Outcomes of Double Vitrified Blastocysts: A Retrospective Observational Study

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Abstract

Objective: To study the effect of double cryopreservation on the potentiality of the blastocyst in terms of implantation, pregnancy, and perinatal outcomes.

Methods: This study was designed with retrospective data from September 2016 to August 2021 where 39 patients (60 embryos) attempting recryopreserved blastocyst transfer. Endometrial preparation was done solely by hormone replacement therapy (HRT), and any other forms of endometrial preparations were excluded.

Results: Our study found that the implantation rate was 88% and the pregnancy rate was 43.5%, overall wherein 64% were singleton pregnancy rate and 23% were twin pregnancy rate. Perinatal outcomes revealed gestational age, birth weight, and number of ventilation days of the neonates.

Conclusion: Our study concluded that it is advisable to do recryopreservation and embryo transfer (ET) in optimal conditions rather than to waste the embryos by transferring them under trivial conditions.

Keywords: Blastocyst, Cryopreservation, Embryo, Infertility, Live birth, Transfer.

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Introduction

In the last 50 years, since the introduction of assisted reproductive technologies (ART), we have witnessed important breakthroughs in cryopreservation. From the introduction of these techniques in animals to its subsequent transition to practice in humans, cryopreservation has become a vital procedure. Spermatozoa was the first human cell to be successfully frozen, which was followed by successful cryopreservation of embryos at various developmental stages.[1,2] The first reported case of live birth of a mouse offspring postcryopreservation was reported in 1972 and since then there has been no looking back.[1] Progressive advancements in freezing technique from slow freezing to vitrification have seen a new wave, where cryopreservation has become an integral part of ART practice today. Segmentation of cycles with freeze-all techniques has become a common practice. Cryopreservation has its benefits; it has helped us move toward safer practices with its pivotal role in preventing the late or early onset of ovarian hyperstimulation syndrome. It has also facilitated single embryo transfer (ET), along with flexibility of transfer timings. It has been hugely popular with patients who want segmentation in their treatment regime. Cryopreservation does increase the cost of an *in vitro* fertilization cycle and requires a learning curve on the part of the embryologist with high-standard quality assurance/quality control in place. Blastocyst vitrification also contributed significantly to the liberal use of preimplantation genetic screening after trophoblast biopsy. Other indications for frozen ET can be patients with polycystic ovary syndrome, low serum progesterone, patients with repeated implantation failure, and patients with a history of ectopic or biochemical pregnancy.

Vitrification differs from typical cryopreservation as it allows solidification without the creation of ice. To define, it is the solidification of a solution at a temperature below its glass transition temperature by using high cooling rates ranging from −15000 to −30000° C/mm rather than ice crystallization.[3] Despite millions of live births occurring after frozen warmed embryos, we still do not know the full effects of this procedure on the embryos. Certain sections of scientists still are skeptical of its epigenetic effects. Many live births occur each year from once frozen and warmed embryos, but it is not an uncommon situation where a situation may demand refreezing and thawing of these embryos twice, due to the sudden cancellation of ET or to save the unexpected supernumerary embryo. The value and safety of refreezing a previously frozen, warmed embryo is then called into doubt. Anecdotal case reports of human pregnancies accomplished *via* frozen ET of refrozen twice-warmed embryos have been published.[4–12]

In this study, we attempted to analyze the chances of implantation with refrozen, twice-warmed embryos. Because the transfer of refrozen twice-warmed embryos is seldom, we attempted to assess implantation rates when at least twice frozen warmed embryos were transferred.

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Methods

On retrospective analysis of the last 5 years, 39 patients who underwent frozen ET using twice frozen and twice warmed embryos comprised the study group. The total number of embryos was 60 in number.

Endometrial preparation for both the groups was solely hormone replacement therapy (HRT) and in the uterus with no preexisting uterine factor; other forms of endometrial preparations were excluded. The ET day was kept between days 14 and 22 of the cycle any transfer <14 or >22 were excluded. Both single and double blastocyst ETs were included in the study, provided the embryos had the same frequency of freezing and thawing.

The study involved 39 patients who were enrolled for revitrified-warmed blastocyst transfer between September 2016 and August 2021. On the day of warming, one or two revitrified-warmed blastocysts were transferred as inclusion criteria. Because the woman had either more embryos warmed than she intended to transfer or for any other medical or social reason, embryos were refrozen in the frozen ET cycle.

Vitrification Warming Protocol

Embryos were put into an equilibration solution (Kitazato, Tokyo, Japan) for 12–15 minutes at room temperature after artificial shrinking of the blastocoel with laser-assisted hatching of zona pellucida. After three to four washes in the vitrification solution, blastocysts were placed in the new vitrification solution.

Then embryos are aspirated by a pipette and expelled into an extremely small droplet which is transferred to the hook at the end of a FiberPlug (already labeled with the patient's name, case ID number, and details of an embryo). Fiberplug is then rubbed 5–7 times to the specially treated surface of a CVM Block (cook, Australia) that has been chilled to liquid nitrogen temperatures. The droplets containing embryos were rapidly vitrified into a glassy bead and were placed into liquid nitrogen immediately after applying the cap of the FiberPlug (CVM HOOK Fiberplug and Sleeve).

After validating the details of the patient and embryos on the fiber plug, the Fiberplug cover was removed from the device and placed straight into 0.5 mL of prewarmed thawing solution (Kitazato, Tokyo, Japan) for 1 minute. The blastocysts were then moved into 500 L of diluent solution two for 3 minutes on a heated platform, followed by 500 L of washing solutions one and two for 5 minutes each. After warming, all embryos were cultured in preequilibrated continuous culture media (Irvine Scientific, CA, USA) and allowed to recover for at least 2 hours before determining whether the embryos were transferred or not; a blastocyst with >70% intact cells and some reexpansion blastocoel cavity was considered to have survived.

The embryo was carefully put onto an ET catheter (Cook Medical, USA) and carefully transported to the patient's uterus under sonography monitoring.

For recryopreservation, embryos were incubated for around 2 hours and allowed to expand after warming, before second vitrification. The warming protocol was applied as per the above protocol.

Patient's Preparation for ET

After a transvaginal scan confirmed that the endometrial thickness was <5 mm, the patient was started on E2 valerate (T Progynova 2 mg/thrice a day, German Remedies Ltd) until her endometrial thickness was 8–10 mm, and then her estrogen supplementation was supplemented with injection progesterone (Injection Strone, Serum Institute of India Pvt Ltd) 50 mg/day intramuscularly for 6 days for blastocyst transfer. After gently warming the embryo, it was carefully put onto an ET catheter (Cook Medical, USA) and transferred to the patient's uterus under sonography monitoring.

Assessment of Pregnancy

At 14 days following ET, the serum beta-human chorionic gonadotropin (β-hCG) level was measured to determine pregnancy; 100 μ/mL or higher β-hCG was considered positive.

After 48 hours repeat β-hCG test was done to check for doubling titer.

At 6–7 weeks, biochemically pregnant patients were assessed for the presence of fetal cardiac motion by transvaginal ultrasonography.

The outcomes measured were survival rate following thawing along with implantation and pregnancy rate. If a postwarmed blastocyst had >70% intact live cells and some reexpansion blastocoel cavity, the embryo was considered alive. The presence of a sac in the uterus was used to calculate the clinical pregnancy rate.

At the conclusion of the first trimester, a viable pregnancy rate was defined as a pregnancy that was still going on. The number of gestational sacs per embryo transferred was used to calculate the implantation rate.

Results

Our study revealed that 39 patients had 60 embryos recryopreserved and rewarmed for ET. From the total of 60 rewarmed embryos, 53 embryos survived and were transferred to patients. A total of 17 patients had positive β-hCG.

According to our findings, the rewarmed embryos had an 88% survival rate and a 43.5% implant rate. Out of 11, 17 patients had a singleton pregnancy (64%) and four had a twin pregnancy (23%).

One patient had chemical pregnancy and one patient had experienced miscarriage.

The perinatal outcomes revealed gestational age, birth weight, and number of ventilation days. Low birth weight (LBW) at birth was defined as <2500 gm. A total of five neonates were in the category of LBW, no preterm delivery was observed and no neonates experienced ventilation.

Discussion

Embryo cryopreservation is an important component of assisted reproductive technology that helps to increase cumulative pregnancy rates and reduce repeated gestations.[13] The majority of infertility clinics have now completely switched from slow freezing to the vitrification process, which is internationally approved and has the advantage of a shorter procedure time and a higher success rate.[14–17] The advancement of culture media and cryopreservation procedures, as well as the enhancement of the embryo scoring system, has substantially enhanced the embryo selection process and reduced the number of supernumerary embryos that are discarded. Son et al.[13] and Hashimoto et al.[3] documented pregnancy and birth after the transfer of twice-vitrified blastocysts, and Takahashi and Araki published a similar result.

The health of children born through assisted reproduction has always been a source of concern.[18,19] The epigenetic effects of vitrification on a child born through ART are still a major concern.[18] There have been few case reports of revitrification published, proving the safety of it.

Multiple publications of revitrification have been published but most of them were frozen initially at 2PN or cleavage stage and the second vitrification of blastocyst stage. But we could not come across a large study on revitrified blastocyst and its implantation and pregnancy potential which makes this a rare study. Proving that embryos at the blastocyst stage could be revitrified and utilized for ET in rare cases and subsequently could lead to live birth.

Our study aimed to address the effect of double vitrification and thawing on the grading and implantation potential of a blastocyst and its perinatal outcomes.

All neonates born from those revitrified embryos were of normal gestational age and weight, with no abnormalities recorded. This is a modest study; however, larger studies do guarantee the safety of recryopreserved embryos.

The only limitation of this study is that only data for revitrified and rewarmed embryos were taken and no controls were taken for comparison. Difficulties during ET such as severe cervical stenosis, large pockets of endometrial fluid or blood on the day of ET, gross endometrial injury during transfer, or some social reasons on/or medical illness in the recipient which requires a cancellation, that is, one must revitrify the blastocyst rather than do a suboptimal ET and cause embryo wastage along with the trauma of negative outcomes to your patient.

Conclusion

It is known that vitrified embryos keep back their developmental potential postwarming. Recryopreservation of the embryo may not hinder further competency for implantation.

Cryopreserved warmed embryos may be recryopreserved in critical conditions, by vitrification with better survival and implantation potential.

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