Live birth after in vitro maturation and vitrification of immature oocytes retrieved from conventional IVF cycle: a case report

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Introduction

Oocyte cryopreservation is gaining increased recognition as a viable alternative to embryo freezing since this technique satisfied several concerns about ethical, religious and social constraints that are associated with the creation and storage of embryos, and allowed donated oocytes to be used following quarantine in a more cost-effective manner and offered women an ideal modality to not only preserve their fertility but also their reproductive autonomy [1]. Despite this, oocyte cryopreservation is technically challenging and it has been more than two decades since the first successful report of oocyte cryopreservation using the slow-freezing method [2].

Although additional reports of successful oocyte freezing and normal pregnancies followed, the slow-freezing method did not become a routine IVF procedure because of its inefficiency and inconsistency [3]. In contrast, vitrification has been reported more recently as an alternative cryopreservation method that is characterized by simplicity, low cost,

and high efficiency for cryopreserving both oocytes and embryos. Oocyte vitrification offers increased success rates in comparison to the slow-freezing method. There are now over 1500 live births resulting from IVF oocyte vitrification reported in the literature [4–6]. In addition, several IVF centers in the world have reported pregnancy rates similar to those of standard fresh IVF treatments [7, 8].

However, very few live births have been reported after cryopreservation of immature oocytes either before in vitro maturation (IVM) [9] or after IVM [10, 11]. The first live birth after slow-freezing of the immature oocytes retrieved from conventional IVF cycles was reported in 1998 [9]. Recently, 4 pregnancies with live birth from immature oocytes retrieved in a natural menstrual cycle, followed by IVM and cryopreservation of the oocytes by vitrification have been reported [10, 11]. However, there has been no report whether the mature oocytes produced by IVM of immature oocytes obtained from conventional IVF cycles can be vitrified and develop into viable embryos. In this case report, we present a pregnancy resulting from transfer of vitrified IVM oocytes generated from immature oocytes of a conventional IVF cycle. To the best of our knowledge, this is the first report of a live birth from vitrification of oocytes produced by IVM of immature oocytes derived from the controlled ovarian hyperstimulation (COH) cycles of a patient in the oocyte donation program.

Capsule This case describes a healthy live birth following vitrification of IVM oocytes generated from immature oocytes retrieved from conventional IVF cycles for oocyte donation.

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Case Report

Before the study, approval was obtained from the Institutional Review Board of the Gunasheela Surgical &

Maternity Hospital. A written consent was signed by the donor and the recipients.

A fertile donor 23 years of age, who had one child of her own, was recruited as a donor. Her basal FSH, LH & PRL were within normal range. She underwent stimulated GnRH agonist/FSH/LH long protocol. Oocytes retrieval was performed 36 h after hCG (10,000 IU/mL). A total of 45 cumulus oocytes complexes were collected. One hour post-collection, the oocytes were denuded with hyase (Cooper Surgical/SAGE, Trumbull, CT) and mechanical pipetting. Metaphase two (MII)-stage oocytes were identified by the presence of the first polar body. After removing cumulus cells, 32 oocytes were at MII-stage and 13 were at Germinal Vesicle (GV)-stage. The 32 MII-stage oocytes were used for one oocyte recipient (42 years old). The oocyte recipient had a fresh cycle and 3 frozen embryo transfer cycles using the fresh 32 MII-stage oocytes. Unfortunately, she did not achieve a pregnancy.

The donor consented to donate her 13 GV-stage oocytes to the second unknown recipient. A written consent was signed by the donor and the recipient.

The 13 GV-stage oocytes were cultured in maturation medium (Cooper Surgical/SAGE, Trumbull, CT) supplemented with the final concentrations of 75 mIU/mL of FSH and LH at 37 °C in an atmosphere of 5 % CO₂ and 95 % air with high humidity for 24 h. Six of them matured to MII-stage and were vitrified using a vitrification method [8, 12] with Quinn's vitrification media (Copper Surgical/SAGE, Trumbull, CT). Briefly, the oocytes were suspended in equilibration medium containing 7.5 % (v/v) ethylene glycol+7.5 % (v/v) DMSO and 12 mg/ml human albumin (HA) for 5–15 min at room temperature, and were then transferred to vitrification medium containing 15 % (v/v) ethylene glycol+15 % (v/v) DMSO+0.6 M sucrose 12 mg/ml HA at room temperature for 45 to 60 s. They were then loaded onto a specially designed vitrification device, the Cryolock (BioDiseno Ltda, Colombia), and were plunged immediately into liquid nitrogen (LN₂) for storage.

For warming, the Cryolock was directly inserted into thawing medium of 1.0 M sucrose in HEPES-buffered human tubal fluid (HTF) containing 12 mg/ml HA for 1 min at 37 °C. Warmed oocytes were transferred to diluent medium (0.5 M sucrose) for 4 min. Then they were washed twice in washing medium (HEPES-buffered HTF containing 12 mg/ml HA) for 9 min. Oocyte survival was evaluated based on the integrity of the oocyte membrane and the zona pellucida after warming and culturing for 2 h.

The second couple (female 30 year old and male 38 years old) presented to our fertility clinic with 9 years of primary infertility. The female patient had been diagnosed with severe endometriosis (Stage III) and poor ovarian reserve during preliminary routine fertility investigation and the

male patient was diagnosed with normal spermatozoa sperm analysis.

Before the couple came to see us, she underwent a laparoscopic excision of a big endometriotic cyst in right ovary. She had failed to conceive 5 times in the past with COH cycles elsewhere. When she came to the hospital, she was in premature ovarian failure. Keeping her history of long-standing infertility in mind, and the fact that her husband had a normal semen analysis, we tested her for endometrial tuberculosis (TB). She tested positive for genital TB PCR test (SRL Diagnostics, Mumbai, India). Taking this into account, she was given an anti-TB treatment for 6 months. When she came back after 6 months, her day 3 FSH was 11.2 IU/L and her AntiMullerian Hormone (AMH) level was 0.2 ng/mL (normal range: > 2–6 ng/mL). Her antral follicle count was 3. A scan revealed bilateral endometriomas. The couple was then counseled regarding their reproductive options including oocyte donation cycle. The couple opted for oocyte donation as she had been treated several times for endometriosis. They were counseled for either fresh oocyte donation or frozen oocyte donation cycles. After discussion, the couple opted for vitrified oocyte donation approach for financial reasons and for lack of oocyte donor. Although successful pregnancy results have been reported following IVM oocyte vitrification [9–11], the couple was informed about the limitations of this new approach.

Oocyte warming was performed after 1 year of storage. Vitrified/warmed viable IVM oocytes were inseminated by intracytoplasmic sperm injection using husband's sperm of the second oocyte recipient. Sperm were prepared by 90 % and 40 % pure-sperm separation at 300× g for 20 min. After separation, the sperm pellet was washed twice (300g) with 3 ml of HEPES medium containing 10 % human serum albumin and the motile sperm were collected by the swim-up method.

Fertilization was assessed 16–18 h after ICSI for the appearance of two distinct pronuclei and two polar bodies. Zygotes were cultured in 20 µl SAGE cleavage medium supplemented with 10 % human serum albumin (Cooper Surgical Co., US). Embryos were transferred on day 2 after oocyte warming.

The endometrium of the recipient was prepared using 12 mg of Oestrogen valerate (Progynova, Schering, Berlin, Germany) daily, starting on day 2 of the menstrual cycle. Once the ultrasound scan had confirmed an endometrial thickness of 8 mm or over 8 mm, 200 mg of vaginal progesterone (Uterogestan, Solvay Pharma India Pvt. Ltd., Mumbai, India) was administered daily from the day of oocyte warming and continued, along with Oestrogen valerate, until 12 weeks of gestation, if the pregnancy test was positive.

After warming, all 6 IVM oocytes survived, 4 oocytes fertilized, and three embryos were cleaved.

Three embryos were transferred (two good quality embryos at 4 and 3 cells and one fair quality at 2 cells) on day 2 after oocyte warming. On the day of embryo transfer, the endometrial thickness of the recipient was 10 mm at transvaginal ultrasonogram.

Sixteen days later, the serum β -hCG concentration was 1794 IU/l and 6 weeks after embryo transfer an ongoing intrauterine single pregnancy with fetal heartbeat was confirmed by transvaginal ultrasonography. She went through an uneventful pregnancy and delivered a normal healthy female baby weighing 2,700 g by caesarean section. Examination of the newborn by a pediatrician found no evidence of congenital malformations, and the child has continued to develop normally.

Discussion

To the best of our knowledge, this is the first report of a successful pregnancy and live birth outcome following vitrification of IVM oocytes generated from GV-stage oocytes retrieved from conventional oocyte donor IVF cycles.

IVM oocyte vitrification is generally offered to cancer patients without a partner for fertility preservation with advantages of 1) eliminating costly drugs and frequent monitoring, 2) completing treatment within 2 to 10 days, compared with 2 to 5 weeks required for a stimulated IVF cycle, 3) avoiding the use of hormones in cancer patients with hormone-sensitive tumors, 4) retrieving oocytes at any phase of the menstrual cycle, even in the luteal phase and 5) the ability to harvest immature oocytes from ovarian biopsy specimens [13]. In this study, we applied the IVM oocyte vitrification to an oocyte donation cycle where several GV-stage oocytes were obtained following conventional IVF cycle.

In the conventional IVF cycles, about 20 % of retrieved oocytes are immature, either at Germinal vesicle breakdown (GVBD)- or GV-stage. However, it is still controversial to use those immature oocytes for clinical purposes. In general, embryos produced from the immature oocytes are usually deprioritized for transfer, even in oocyte donation program, unless there are not enough embryos from in vivo matured oocytes for an appropriate embryo transfer. Actually, it is difficult to know whether embryos derived from the immature oocytes contribute to pregnancies or live births. Shu et al. [14] recently reported that although immature oocytes from stimulated cycles can be normally fertilized and used to increase the number of embryos available for transfer, the embryos cannot be efficiently translated into pregnancies. This might be attributed to a poor quality oocyte caused by asynchrony in the cytoplasmic and nuclear maturation of the oocyte. In general, embryo developmental potential generated from immature oocytes in conventional IVF cycles is lower than that of sibling in vivo matured oocytes [14, 15].

Another possible explanation could be that the embryos produced from immature oocytes may not be synchronized with the endometrium, compared to the embryos produced from sibling mature oocytes. Oocyte/embryo cryopreservation is a way to synchronize the embryo and endometrium. In fact, the first pregnancy and live birth was achieved after preparing the endometrium for a thawing cycle using frozen/thawed immature oocytes obtained from conventional IVF cycles [9]. Therefore, the clinical significance of using immature oocytes in stimulated cycles needs further investigation.

Theoretically, there are two approaches for preserving immature oocytes: oocyte cryopreservation at the MII-stage (after IVM) and oocyte cryopreservation at the GV-stage (before IVM). Although the first pregnancy and live birth was obtained after freezing GV-stage oocytes (before IVM) [9], it seems that vitrifying oocytes at MII-stage after IVM is better than at the GV-stage in terms of survival rate and embryo developmental potential after warming even though they were similar in the rates of in vitro maturation and fertilization [16, 17]. In a recent study, the maturation rate of the GV-stage oocytes that had survived after vitrification/warming was only 10.6 % (7/66) after 24 h culture [18]. Chian et al. [10, 11] reported few successful pregnancies and live births after vitrifying MII-stage oocytes following IVM of immature oocytes retrieved in an unstimulated menstrual cycle. Based on these results, we vitrified MII-stage oocytes after IVM of immature oocytes which, contrary to Chian's report, were donated from a conventional IVF oocyte donation cycle and we had a 46.2 % (6/13) maturation rate and a 100 % survival rate (6/6) after warming. Two good and one moderate quality embryos developed on day 2.

Chang and colleagues [12] compared survival and embryo development after vitrification between oocytes matured in vivo and in vitro in oocyte donation IVF cycles and reported that there was no difference in survival rates between oocytes matured in vivo and in vitro. However, embryo development was better in embryos produced from in vivo matured oocytes than that from in vitro ones. However, the ability to implant or result in a pregnancy of the embryos produced from vitrified IVM oocytes was not tested in that study. Therefore, we demonstrated in this case that the embryos produced from vitrified IVM oocyte obtained from conventional IVF cycles had the ability to implant and result in a healthy live birth.

In this case, our oocyte maturation rate was not good (46.2 %, 6/13). An important aspect of the ICSI procedure involves the removal of cumulus and corona cells a few hours after oocyte retrieval, which allows a precise determination of nuclear maturation status. Cumulus-oocyte communication via the gap junction plays a critical role in oocyte maturation and embryo development. It has been shown that the retention of attached cumulus cells can help achieve higher rates of oocyte maturation of human oocytes

[15, 19]. However, immature oocytes were completely denuded of surrounding cumulus cells in this study, to visualize their maturation status prior to ICSI. Therefore, the transfer of low molecular substrates, which are essential for completion of maturation, could then be interrupted and resulted in low in vitro oocyte maturation rate in this study. Recently, Son et al. [20] demonstrated how to evaluate the maturity of oocyte maturation while identifying COC without removing cumulus cells using spreading method at the time of oocyte retrieval. The technique could be very useful in isolating GV-stage oocytes with intact cumulus cells while identifying COC to achieve a better oocyte maturation rate for future studies.

In conclusion, this case report implies that the IVM oocytes derived from immature oocytes in COH cycles can survive after vitrification/warming and develop into viable embryos. Further studies are needed to define the optimal IVM conditions for human immature oocytes from stimulated cycles. This study could help cancer patients to vitrify a larger number of MII-stage oocytes in cases where only few follicles have a larger diameter after conventional IVF stimulation for the fertility preservation program as well as for the oocyte donor program.

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