

Successful birth of the first frozen oocyte baby in India

ABSTRACT

We report the first pregnancy and birth in India after the transfer of embryos generated from frozen–thawed oocytes. A 29-year-old woman with previous bad obstetric history and an abnormal karyotype, necessitating donor oocyte programme. Embryos were generated by microinjection of frozen–thawed sperms into thawed human oocytes (intracytoplasmic sperm injection). This resulted in an healthy male baby with a birth weight of 2.54 kg which was born by cesarean section at 35–36 weeks of gestation with normal follow-up. Thus oocyte cryopreservation can be performed with reproducible success leading to a viable offspring.

KEY WORDS: Donor oocyte programme, embryo transfer, intracytoplasmic sperm injection, oocyte cryopreservation, oocyte survival

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INTRODUCTION

The field of Assisted Reproductive Technology (ART) is advancing day by day with newer laboratory techniques being discovered. The area of cryopreservation is one of them. Till date, it has been possible to freeze sperms and embryos for many years, but successful pregnancies from oocytes have been elusive with only few hundred live births worldwide.^[1] The world's first birth through oocyte freezing and thawing was pioneered by Prof. Christopher Chen in Australia, in 1986.^[2]

Oocyte cryopreservation has gained more attention in ART, especially because synchronization between recipient and donor need not be compulsory in the donor oocyte programme, other indications being pre-chemotherapy preservation of fertility, premature ovarian failure, and postponement of childbearing function. The success rates in oocyte cryopreservation have been cited as low and are debatable, with literature review of 21 studies in peer-reviewed journals revealing a mean survival rate of 47%, fertilization rate of 52.5% and mean pregnancy rate/thawed oocyte being 1.52–1.8%.^[3] We report the first successful birth from frozen oocytes in India.

CASE REPORT

The patient was a 29 year old, with a previous bad obstetric history and an abnormal karyotype (46xx, t[3:9] [p21.3;p24], t [7:10] [p22;q22.1]), which necessitated the use of donor oocytes for

the present conception. The reason for the use of frozen oocytes sourced from egg-sharing and egg-donation patients was the non-availability of compatible fresh donors and insistence of the couple to continue the treatment cycle. The couple was then counseled about the use of frozen–thawed oocytes and the expected low success rate for this cycle after obtaining informed consent.

The sourced oocytes were cryopreserved using the slow freezing method. Freezing was initiated 1–2 h post-retrieval, after denuding the cumulus with hyaluronidase and confirming metaphase-II maturity status. Cook's oocyte freezing media (Cook Medical, Queensland, Australia) was used after warming the required quantity to room temperature, the active cryoprotectants being propanediol in increasing concentrations from 0.75 to 1.5 mol/l (solutions 1 and 2 – for 7 min and 30 s, respectively) and then transferred to solution 3 containing 1.5 mol/l propanediol and 0.2 mol/l sucrose [Figure 1] for 5 min. The oocytes were loaded into pre-labeled straws (Cryo-Bio Systems, Rocket Medical PLC, Washington, England), plugged and placed into the cryochamber (Planer Kryo 360, Planer Products Ltd, Burnsville, USA). The initial chamber temperature at +20°C was slowly cooled to –8°C at the rate of –2°C/min. Seeding was manually performed at –8°C. After a hold time of 10 min at –8°C, the straws were cooled slowly to –30°C at a rate of –0.3°C/min and then rapidly to –150°C at a rate of –50°C/min. After 10 min of stabilization, the straws

were transferred to a liquid nitrogen tank and stored until thawing.

The oocytes were subjected to cryopreservation for 6 months. Because a suitable fresh donor was unavailable for this particular patient, we were able to use the cryopreserved oocytes for this couple. The endometrium was prepared with incremental doses of estrogen (Estradiol Valerate 2 mg; German Remedies, Mumbai, India) commencing from Day 2 or Day 3 of the cycle to a maximum of 8 mg/day with initiation of micronised progesterone 400 mg thrice a day, from the day before thawing. The optimum endometrium for transfer was considered to be 10 mm, which was observed in mid-cycle. We decided to thaw at least 10 oocytes in order to enhance the chances of achieving enough good-grade embryos for transfer, success having evaded us earlier. For the thawing process, the straws containing the oocytes were removed from the liquid nitrogen, air dried for 30 s, and then immersed in a water bath at 30°C for 40 s. The oocytes were first passed through solutions 1–2 containing cryobuffer supplemented with decreasing concentration of propanediol (1.0–0.5 mol/l) and increasing concentration

of sucrose (0.2–0.3 mol/l) for 5 min at room temperature, respectively. The oocytes were then transferred to solution 3 (0.3 mol/l sucrose) for 10 min at room temperature followed by 37°C on a warmer plate.

A survival check carried out under the inverted microscope revealed eight oocytes that survived the thawing process [Figure 2]. Oocytes with intact zona, clear perivitelline space, and visible polar body were considered as normal and were further cultured in the Cleavage media (Quinn's, SAGE *In vitro* fertilization, Trumbull CT, USA) at 37°C and 5% CO₂ for 2 h before injection. Subsequently, the oocytes were injected with frozen–thawed and prepared sample of husband's sperms. Intracytoplasmic sperm injection [Figures 3 and 4]^[4–9] was the preferred method for fertilization, owing to an expected zona hardening following oocyte cryopreservation. The injected oocytes were further cultured in cleavage media in a Trigas incubator until Pronucleus check 16–18 h later. Five embryos (two and three cells, grade II–III) [Figures 5 and 6] were observed and transferred on Day 2 using a Labotect catheter (Labotect GmbH, Gottingen, Germany). The assessment of embryo grading



Figure 1: Frozen oocytes



Figure 2: Thawed oocytes



Figure 3: Holding egg



Figure 4: Intracytoplasmic sperm injection

was performed as described by Veeck L.^[10] Why five and not the recommended three embryos? Well, considering our previous experience of inadequate embryos for transfer following thawing and negative result for pregnancy, we decided to transfer at least five of this crop, which, as we mentioned, did not have the conventional cleavage or grade as expected (i.e., four cells, grade I-II). Once the serum β -HCG was positive, an ultrasound on the 38th day revealed a single intrauterine gestational sac. Since then she progressed uneventfully, with early fetal screening tests being normal until the 5th month, when she was diagnosed with mild pregnancy-induced hypertension and was treated with anti-hypertensives. During her level-2 scan at 22 weeks, where the fetal screening was normal, a shortened cervix of 1.5 cm was imaged, suggestive of cervical incompetence. Since then, she was admitted with us for careful surveillance of both the mother and the baby. She did moderate physical activities and her blood pressure was well controlled with anti-hypertensives. An elective cesarean was performed at 35–36 weeks on 27/8/2008 owing to severe oligohydramnios. She delivered a healthy male baby weighing 2.54 kg [Figure 7]. Both the mother and the baby were discharged

uneventfully. A general follow-up at 3 months showed normal development for age [Figure 8].

DISCUSSION

We started oocyte freezing in the month of November 2005 and since then have frozen more than 100 oocytes [Table 1]. The only media available were the Irvine Scientific Vitrification media and Cook media and there was a learning curve to be able to successfully freeze and retrieve oocytes. The oocytes frozen with Cook's media were only metaphase II. However, in the initial phase of vitrification with Irvine scientific media, close to 10 oocytes of different patient were frozen at the germinal vesicle and metaphase I, with three maturing to metaphase II in *in vitro* maturation, but no fertilization. Subsequent reports of series of pregnancies and live births have confirmed that mature oocyte cryopreservation is now a viable option in appropriate circumstances.^[1,7,9] Other recent reports show that several babies have been born using oocyte cryopreservation and similar freezing and thawing procedures (slow freezing and rapid thawing).^[11,12] There



Figure 5: Embryos



Figure 6: Embryos



Figure 7: At birth



Figure 8: At 3 months

Table 1: Statistics from November 2005 to November 2008

Total statistics	Total (%)	Cook (%)	Vitrification (%)	Quinn's (%)
No. of oocytes frozen	119	83	29	7
No. of oocytes retrieved	84 (70.58)	62 (74.69)	18 (62.06)	4 (57.14)
No. of oocytes that survived the thawing process	46 (54.76)	29 (46.77)	13 (72.22)	4 (100)
No. of oocytes for which ICSI was performed	46	29	9	4
Total no. of oocytes fertilized	18 (39)	18 (62)	-	-
No. of cases for embryo transfer	5	5	-	-
No. of pregnancies	2 (40)	2 (40)	-	-
Pre-clinical	1	1	-	-
Delivered	1	1	-	-

is still ongoing research with regard to the optimum media and preference of freezing techniques like slow freeze or vitrification, the latter showing promising results. In our experience, the slow freeze method seemed to offer better retrieval rates (74%) and fertilization rates (62%), although vitrification, according to the literature review, seems to offer comparable or even better clinical outcomes.

CONCLUSION

Oocyte freezing can be welcomed as an emerging and useful technique, especially in donor programmes and in young women with cancer for fertility preservation options. Worldwide, there have been only few hundred babies born through this technique owing to the sensitive morphology and cryokinetics of the oocyte.^[13,14] While we still conduct comparative research between the two freezing methods, namely slow freezing and vitrification, this little bundle of joy signals yet another landmark achievement for our country while serving as a ray of hope to many young women in need of oocyte freezing technology.

REFERENCES

- Borini A, Bafaro MG, Bonu MA. Pregnancies after freezing and thawing: Preliminary data. *Hum Reprod* 1998;13:124-5.
- Parmegiani L, Cognigni GE, Bernardi S, Ciampaglia W, Infante F, Pocognoli P, *et al.* Freezing within 2 h from oocyte retrieval increases the efficiency of human oocyte cryopreservation when using a slow freezing/rapid thawing protocol with high sucrose concentration. *Hum Reprod* 2008;23:1771-7.
- Borini A, Sciajno R, Bianchi V, Sereni E, Flamigni C, Coticchio G. Clinical Outcome of oocyte cryopreservation after slow cooling with a protocol utilizing a high sucrose concentration. *Hum Reprod* 2006;21:512-7.
- Fabbri R, Porcu E, Marsella T, Rocchetta G, Venturoli S, Falmigni C. Human Oocyte cryopreservation: New perspectives regarding oocyte survival. *Hum Reprod* 2001;16:411-6.
- Antinori S, Dani G, Selman HA. Pregnancy after sperm injection into cryopreserved human oocytes. *Hum Reprod* 1998;13:157-8.
- Polak de Fried E, Notrica J, Rubinstein M, Marazzi A, Gómez Gonzalez M. Pregnancy after human donor oocyte cryopreservation and thawing in association with intracytoplasmic sperm injection in a patient with ovarian failure. *Fertil Steril* 1998;69:555-7.
- Porcu E, Fabbri R, Seracchioli R, Ciotti PM, Magrini O, Flamigni C. Birth of a healthy female after intracytoplasmic sperm injection of cryopreserved human oocytes. *Fertil Steril* 1997;68:724-6.
- Yang DS, Blohm PL, Winslow L, Cramer L. A twin pregnancy after microinjection of human cryopreserved oocyte with a specially developed oocyte cryopreservation regime. *Fertil Steril* 1998;S239:357.
- Young E, Kenny A, Puigdomenech E, Van Thillo G, Tiverón M, Piazza A. Triplet pregnancy after intracytoplasmic sperm injection of cryopreserved oocytes: Case report. *Fertil Steril* 1998;70:360-1.
- Veeck LL. *Atlas of the Human Oocyte and Early Conceptus*. Vol 2. Baltimore: Williams and Wilkins; 1986. p. 121.
- Fosas N, Marina F, Torres PJ, Jove I, Martin P, Perez N, *et al.* The births of five spanish babies from cryopreserved donated oocytes. *Hum Reprod* 2003;18:1417-21.
- Levi Setti PE, Albani E, Novara PV, Cesana A, Morreale G. Cryopreservation of supernumerary oocytes in IVF / ICSI cycles. *Hum Reprod* 2006;21:370-5.
- Kuleshova L, Gianaroli L, Magli C, Ferraretti A, Trounson. A Birth following vitrification of a small number of human oocytes: Case report. *Hum Reprod* 1999;14:3077-9.
- Levi Setti PE, Albani E, Morreale G, Bianchi S, Novara P, Cesana A, *et al.* Supernumerary oocyte cryopreservation in 303 IVF/ICSI cycles. *Hum Reprod* 2004;19:178-9.

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