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ASSISTED REPRODUCTION

Successful live birth from vitrified oocytes after 5 years of cryopreservation

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Abstract
Objective To report a birth of a healthy boy after long-term cryopreservation of oocytes by vitrification.
Design Clinical application.
Setting IVF Center.
Patient A 17 year-old female with secondary pulmonary hypertension caused by transposition of great vessels visited our center in 2002, and she wished oocytes cryopreservation to avoid possible sterility after the following category X medication treatment.
Intervention(s) Vitrified oocytes on Electron Microscope (EM) grids were warmed after 5 years of storage. Surviving MII oocytes were microinjected for fertilization and two embryos were transferred into a gestational carrier day 5 after microinjection.
Main Outcome Measure(s) Survival, fertilization, cleavage, clinical pregnancy and delivery.
Result(s) Eleven out of fourteen oocytes (78.6%) survived warming. Eight Metaphase II (MII) oocytes and 3 in vitro matured oocytes were microinjected; all 11 oocytes (100%) fertilized and 2 embryos were transferred on day 5. A healthy baby boy weighing 3,600 g was delivered at 38 weeks of gestation. Live-birth rates per warmed oocyte and per injected oocyte were 7.1% and 9.1% respectively.
Conclusion(s) Cryopreservation after vitrification with EM grids maintained the developmental competence of oocytes after long-term storage and resulted in a successful live birth.

Keywords Human oocyte cryopreservation · Vitrification · Electron microscope grid · Ethylene glycol · Clinical outcome

Capsule Documentation of a pregnancy after long-term storage of vitrified oocytes.

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Since the first successful pregnancy from frozen human oocyte was reported 20 years ago [1], remarkable technological progress has been made in the area of cryopreservation of human oocytes. The recent increased interest in vitrification has resulted in improved efficiency and pregnancy outcomes [2–13]. Being able to protect and prolong the reproductive capacity by way of oocyte cryopreservation potentially opens the door for many thousands of women who are at a risk of losing ovarian function from radiation, chemotherapy and surgery.

Over the last decade, multiple clinics have reported IVF successes after transfer of embryos derived from frozen/thawed oocytes [5, 14–20]. The authors have recently reported that oocytes from young fertile women retain their reproductive potential after 6 months of vitrification cryopreservation [20] and the pregnancy and implantation rates are comparable to that of frozen embryos. There is evidence that children born from frozen embryos have no significantly increased risk of congenital abnormalities [21–23], however, no such data are available concerning children born from vitrified oocytes after long-term storage.

Here, we report the healthy outcome of a now 2 year old boy who was born after vitrification of oocytes that were stored for 5 years.

Case report

In August 2002, a 17-year-old girl and her mother visited our center inquiring about oocyte cryopreservation after hearing of our program. She had been suffering from pulmonary hypertension caused by transposition of the great vessels. She had undergone many surgeries since birth, yet her clinical symptoms persisted. It was recommended by her cardiologist for her to undergo treatment with Tracleer (Bosentan) to improve pulmonary arterial hypertension. Since Bosentan is a category X medication with unknown effects on the ovary, her mother did not want to take any chance of the medication causing sterility. They requested cryopreservation of her oocytes prior to her medical treatment.

After medical clearance and routine screening tests, the patient underwent a cycle of Controlled Ovarian Hyperstimulation (COH) under a routine GnRH down regulation protocol with recombinant FSH and urinary hMG. On August 23, 2002, she underwent oocyte retrieval that yielded 14 oocytes. All 14 oocytes were vitrified as previously described [2, 20]. Briefly, vitrification was initiated within 3 to 4 h after oocyte retrieval. The oocyte-cumulus complexes were exposed to hyaluronidase (50–80 IU/ml, Type IV-S, Sigma-Aldrich) for a short time for partial removal of cumulus cells 1 h before vitrification. The oocytes to be vitrified were placed in Dulbecco's phosphate-buffered saline (Gibco invitrogen, Carlsbad, CA) supplemented with 1.5 M of Ethylene Glycol (EG, Sigma-Aldrich, Saint Louis, MO) plus 20% Human Serum Albumin (HSA; SAGE IVF) at 37°C for 2.5 min. Then, the oocytes were placed in Dulbecco's phosphate-buffered saline supplemented with 5.5 M of EG and 1.0 M sucrose (Sigma-Aldrich) plus 20% HSA (SAGE IVF) for 20 s. Three to four oocytes were mounted on an EM grid (Ted Pella Inc, Redding, CA) using a fine pipette and excess cryoprotectant solution was removed with underlying paper. The grid holding oocytes was immediately plunged into the container filled with liquid nitrogen and the grid was put into a grid holder using fine forceps. The holder was then capped for secure before immersion in a storage tank.

In January 2007, she returned to our center with a plan to use the frozen oocytes to get pregnant. She was 22 years old and had been married for three years. She was working full time as a physical therapist. Although her physical symptoms had improved, her medical condition was not stable enough to carry a pregnancy, therefore her 34 year-old cousin volunteered to be a Gestational Carrier (GC). The GC had three children of her own and was in good health. After a thorough medical evaluation and screening, the GC was found to be a good candidate to carry the pregnancy.

After coordinated efforts between us and the GC's local gynecologist, endometrial induction was achieved using estrogen transdermal patch (2 patches/week) (Vivelle; 0.1 mg, Novartis Pharmaceutical, East Hanover, NJ, USA). Once the endometrium reached adequate thickness (12 mm), progesterone (100 mg/day) was added. The GC flew out to our center for the Embryo Transfer (ET) procedure a day before the scheduled ET.

Oocyte warming took place on July 18, 2007. For warming, the EM grid holding the oocytes was taken from the grid holder with fine forceps under liquid nitrogen, then immediately transferred sequentially into Falcon plastic culture dishes (two-well; Beckton Dickinson, Franklin Lakes, NJ) containing 1.0 ml of warming solutions of 1.0, 0.5, 0.25, 0.125, 0 mol/L of sucrose at intervals of 2.5 min at 37°C. The oocyte-cumulus complexes were detached from the grid by pipetting after the last warming step. Eleven out of fourteen oocytes survived warming. Eight mature oocytes (Metaphase II) and 3 of in vitro matured oocytes were microinjected (ICSI) with her husband sperm and all 11 oocytes were fertilized. After five days of incubation in sequential culture media (G1.3 and G2.3; Vitrolife, Englewood, CO), one blastocyst and one morula were obtained. The rest of the embryos were arrested between 4 to 8-cell stages.

The clinical summary and detailed accounts of the progression of the vitrified oocytes are shown on Table 1.

Table 1 Clinical summary of vitrified warmed oocytes

Age (y)	17
No. of vitrified oocytes	14
No. of warmed oocytes	14
No. of survived oocytes (MII+GV) (%)	11 (78.6)
No. of metaphase II (MII) oocytes	8
No. of GV stage oocytes	3
No. of microinjected oocytes	11 [a]
No. of fertilized oocytes (%)	11 (100)
No. of cleaved embryos (%)	9 [b] (81.8)
No. of transferred embryos	2
Clinical Pregnancy	Yes
No. of implantations (%)	1 (50.0)
No. of delivered infant	1
Gender (g)	Boy (3,600)
No. of implantation per warmed oocytes (%)	1/14 (7.1)
No. of implantation per injected oocytes (%)	1/11 (9.1)
Live-birth per warmed oocytes (%)	1/14 (7.1)
Live-birth per injected oocytes (%)	1/11 (9.1)

[a] Three GV oocytes were in vitro matured 24 h after warming

[b] Two embryos obtained from in vitro matured oocytes did not develop to cleavage stage

Ultrasound guided ET was performed on July 23, 2007. A positive serum ß-hCG test result was obtained on August 1, 2007 and a single implantation was confirmed by fetal cardiac motion approximately 4 weeks after the ET. The pregnancy resulted in a normal spontaneous delivery of a healthy male infant at 38 weeks of gestation, weighing 3,600 g, with apgar scores of 8/9. The boy is currently 2 years old and in good health.

Discussion

Fertility preservation is one field of reproductive medicine that has attracted much interest in recent years. One of the ways to protect and prolong a woman's reproductive function is to cryopreserve oocytes while she is young and healthy. Recent improvements in cryopreservation and vitrification have occurred and now nearly 500 live births have been reported using cryopreserved oocytes [24]. Recent studies reported high pregnancy and implantation rates using vitrified oocytes obtained from young fertile women [20]. However, establishing the safety of this technology is urgently needed and the ASRM still lists oocyte cryopreservation as an experimental procedure. The majority of reported births have been from oocytes that had been cryopreserved for a short period of time (around 6 months), although a live birth using cryopreserved oocytes with the slow freezing method has been reported after 6 years of storage [14].

This case report documents that vitrified oocytes (using EG, sucrose and EM grid) maintained developmental competence after 5 years of cryopreservation in liquid nitrogen with a resultant live birth with no known congenital abnormalities. In addition, live-birth rates per warmed oocyte (7.1%) and per injected oocyte (9.1%) were higher than that (5.1% and 7.2%) of the author's previous study [20]. Like any other new technology, safety and efficiency of oocyte vitrification will need to withstand the test of time. As oocyte cryopreservation is growing in popularity, it is imperative to establish a registry to document its safety and effectiveness.

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