Report of four donor-recipient oocyte cryopreservation cycles resulting in high pregnancy and implantation rates

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Objective: To determine the clinical potential of donor-oocyte cryopreservation and thaw techniques for recipient patients.
Design: Institutional review board-approved prospective study of donor oocyte cryopreservation.
Setting: A large, private infertility center.
Patient(s): Four anonymous oocyte donors underwent ovarian hyperstimulation for the purpose of oocyte retrieval and cryopreservation. The oocytes were subsequently thawed, fertilized, and transferred to 4 recipient patients.
Intervention(s): Oocytes were obtained from young donor patients and were cryopreserved with a slow freeze/rapid thaw protocol in which 1,2-propanediol (PrOH) and sucrose were used as cryoprotectants. Oocytes that survived were inseminated using intracytoplasmic sperm injection (ICSI). Resulting embryos were replaced into the recipient patients on the third day post-insemination.
Main Outcome Measure(s): Post-thaw survival rate, fertilization rate, cleavage rate, implantation and clinical pregnancy rates.
Result(s): A total of 79 metaphase II oocytes were frozen, stored frozen overnight in liquid nitrogen, and then thawed. The post-thaw survival rate was 86.1%. Normal fertilization following ICSI occurred in 89.7% of the surviving oocytes. Cleavage was observed in 91.8% of normally fertilized oocytes. A total of 23 embryos were transferred to 4 recipient patients. A clinical pregnancy rate of 75% and an implantation rate of 26.1% were achieved.
Conclusion(s): Human oocyte cryopreservation is an effective technique that can be applied in clinical situations with high oocyte survival and clinical pregnancy rates expected. (Fertil Steril® 2007;87:189.e13–7. ©2007 by American Society for Reproductive Medicine.)

Key Words: Ovary, donor oocyte, cryopreservation, slow freezing/rapid thawing

Although sperm and embryos have been successfully frozen and subsequently thawed for decades, it is only recently that clinically effective human oocyte cryopreservation ("egg freezing") has become a reality. There are two primary groups of women who are likely to benefit from advances in oocyte-freezing technology: [1] those desiring preservation of female fertility prior to compromise by medical treatment (surgical removal of ovaries, radiotherapy, and chemotherapy); and [2] those who plan to delay childbearing until later in life, with the hopes of retaining their reproductive potential.

However, oocyte cryopreservation may also benefit couples undergoing in vitro fertilization (IVF) who have moral and ethical objections to embryo-freezing. Oocyte cryopreservation would be useful in IVF cases in which the semen unexpectedly cannot be obtained on the day of oocyte retrieval (1). Oocyte donor programs could potentially benefit from egg-freezing technology, through the creation of frozen oocyte banks which would allow couples to choose an appropriate donor immediately, without having to delay the process due to searching, screening, scheduling, and synchronization. Banks of cryopreserved donated oocytes would also allow for the quarantine of oocytes until appropriate infectious disease screening of the donor could be completed (2).

It has been nearly 20 years since the first successful pregnancy was reported based on frozen and then thawed oocytes (3). However, only a handful of births were reported over the next decade (4–7). In 1995, Gook et al. reported on the first use of intracytoplasmic sperm injection (ICSI) to fertilize thawed oocytes, increasing the fertilization rate significantly and increasing the number of available embryos for transfer (8). Today, experts estimate that approximately 200 babies have been born worldwide from previously frozen oocytes, and the number of patients with oocytes frozen is most likely in the thousands.

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Routine application of oocyte cryopreservation has been discouraging due to the low overall post-thaw survival rate and subsequent pregnancy rates (PRs) (9). Cryoinjury to the human metaphase II (MII)-stage oocyte, such as meiotic spindle disassociation and/or chromosome instability caused by disassembly and reassembly of the microtubules, was suggested to be the main reason for poor outcomes (10). We present the outcomes of an institutional review board-approved study of donor oocyte cryopreservation involving four anonymous oocyte donors who produced 79 frozen and thawed oocytes, with the resulting embryos being transferred to four recipient couples.

MATERIALS AND METHODS

Institutional review board approval was obtained for this prospective study. Four anonymous oocyte donors with a mean age of 28.3 years were selected and matched with recipients to undergo controlled ovarian hyperstimulation, using a GnRH antagonist protocol. The donors were given a complete medical evaluation in accordance with American Society for Reproductive Medicine donor oocyte guidelines (11) that included a detailed family and personal history, a physical examination, transvaginal basal antral follicle count, vaginal and cervical cultures, Papanicolaou smear, baseline hormonal day 3 profile (FSH, LH, and E_2), serology for HIV, hepatitis B and C, syphilis, cytomegalovirus, and gonorrhea, a urine drug screen, and appropriate genetic testing. Blood type and Rh factor were also determined. Once the donor evaluation was complete, and all informed consents and institutional review board consents were signed and completed, the donor commenced oral contraceptives for 21 days, followed by initiation of gonadotropin (Gonal F RFF Pen; Serono, Inc., Rockland, MA) on cycle day 3 (after withdrawal bleeding) if ovarian suppression was confirmed from a basal hormonal profile and a baseline vaginal ultrasound. Ovarian stimulation was monitored by the measurement of serum E_2 concentration and by ultrasonographic assessment of the follicle diameter every 1–2 days. When the leading follicle reached 14 mm in diameter, a GnRH antagonist (Cetrotide TM; Serono, Inc.) was administered at a daily dose of 250 μg, and 75 IU of r-hLH (Luveris; Serono, Inc.) was added. When at least two of the leading follicles reached 18 mm in diameter, final oocyte maturation was achieved by the administration of 250 μg of recombinant hCG (r-hCG, choriogonadotrophin alfa-Ovidrel; Serono, Inc.). Oocyte retrieval was performed 36 hours after r-hCG administration, with the use of transvaginal guidance and under anesthesia on an outpatient basis.

Four recipient patients diagnosed with impaired ovarian reserve, and with elevated basal FSH levels and prior unsuccessful infertility treatments, were admitted to our assisted reproductive technology program for oocyte donation. All couples gave informed written consent for the replacement of embryos resulting from frozen donated oocytes.

The preparation of recipients and synchronization with oocyte donors were performed using a standard protocol of leuprolide acetate, estrogen, and P. In brief, leuprolide acetate was begun in the midluteal phase of the cycle previous to the transfer at a daily dosage of 1 mg. When the serum estrogen and P levels confirmed down-regulation, the leuprolide dosage was reduced from 1.0 to 0.5 mg daily, and estrogen was commenced using Estrace (micronized E_2; Bristol-Myers Squibb Co., Princeton, NJ) pills at 1 mg orally twice a day for 4 days, and then increased to 2 mg orally twice a day for 4 days, and finally to 2 mg orally three times a day for 4 days. Endometrial response was monitored by transvaginal ultrasound and by serum E_2 levels every 4 days. Upon achievement of endometrial thickness of at least 7 mm with a triple-layer pattern, and serum E_2 levels >200 pg/mL, the recipient was considered ready for transfer. The day after the donor's oocyte retrieval, the respective recipient started 50 mg of daily IM P, 100 mg of doxycicline for 7 days, and 16 mg of methylprednisolone on a daily basis for 7 days. Embryo transfer was performed under transabdominal ultrasound guidance, using a Wallace catheter (Smiths Medical, Portex Ltd., United Kingdom) on day 3 of embryo culture.

A blood test was performed 4 days after ET to measure P and E_2. Progesterone dosage was increased if the result was <20 ng/mL. Eleven days after ET, a β-hCG determination, along with E_2 and P, was performed. If positive (>5 mIU/mL), the recipient repeated the β-hCG determination 2 days later. If negative, the patient would discontinue P. The first ultrasound to confirm the presence of an intrauterine pregnancy was performed 18 days after ET. A clinical pregnancy was defined as the presence of fetal cardiac activity on transvaginal ultrasound.

Oocyte Retrieval and Hyaluronidase

Donor follicular aspiration was performed, and the follicular fluid was examined for the presence of oocytes. Once identified and isolated, oocytes were placed into culture for approximately 1 hour, prior to being denuded through brief exposure to hyaluronidase. Oocytes were checked for the presence of a first polar body in the periviteline space, which classified the oocyte as being in MII. All MII oocytes with normal morphology were cryopreserved. Any immature oocytes were discarded.

Oocyte Cryopreservation

Dehydration and cryopreservation of oocytes were performed with the use of a modified slow-freeze protocol with Oocyte Freeze (Medi-Cult, Jyllinge, Denmark). The cells were briefly washed in a human tubal fluid medium and then equilibrated in a solution containing 1.5 M 1,2-propanediol (PrOH) for 10 minutes. The oocytes were then equilibrated in a 1.5-M concentration of PrOH and 0.3 M sucrose for 15 minutes. Oocytes were then slowly cooled at a rate of -2°C/min to a temperature slightly below the melting point of the solution (-7°C), at which point the solution was "seeded" to

TABLE 1

Oocyte cryopreservation and laboratory results.

Oocyte freeze–thaw outcome	Total	Mean per patient (SD)	Percentage
Frozen metaphase II oocytes	79	19.8 (9.5)	N/A
Oocytes that survived cryopreservation and thaw	68	17.0 (7.0)	86.1%
Normal fertilization (2 pronuclei)	61	15.3 (6.9)	89.7%
Cleaved embryos	56	14.0 (6.8)	91.8%

Barritt. Oocyte cryopreservation. Fertil Steril 2007.

avoid supercooling (9, 12). Oocytes were then cooled to −30°C at a rate of 0.3°C/min, and finally, they were plunged into liquid nitrogen at −30°C.

Oocyte Thawing

Thawing was performed with Oocyte Thaw (Medi-Cult), after storage of the cryopreserved oocytes overnight in liquid nitrogen, by rapidly warming in a water bath at 30°C to avoid devitrification. The dehydrated cell was exposed to hypotonic conditions to facilitate rehydration and cryoprotectant removal, while preventing osmotic shock. Sequential washes exposed frozen oocytes to decreased levels of PrOH from 1.0 M for 5 minutes, to 0.5 M for 5 minutes, and finally to a concentration of 0.0 M PrOH for 30 minutes before the cells were returned to normal oocyte culture medium.

Intracytoplasmic Sperm Injection

Evaluation of oocyte morphology, cell membrane, and perivitelline space was performed after oocytes were thawed. Oocyte survival was defined as an oocyte with an intact cell membrane, normal ooplasma, and zona pellucida, as well as a perivitelline space of normal size. Using fresh sperm from the recipient's husband, ICSI was performed on the oocytes that survived and had been in culture for 3 hours post-thaw. Fertilization was analyzed on day 1, approximately 17–19 hours after the microinjection of spermatozoa, and normal fertilization was considered to be the presence of two pronuclei and two polar bodies. Embryonic development was assessed on day 2 and day 3 of development. Embryos were discarded if they arrested at any point in their development, or displayed grossly abnormal morphology. Embryo transfer was performed under transabdominal ultrasound guidance, with the use of a Wallace catheter (Smiths Medical, Portex Ltd., Kent, United Kingdom) on day 3 of culture. Assisted hatching was performed with acidified Tyrode's solution on all embryos that were transferred.

RESULTS

Oocyte donors underwent stimulation an average of 8.75 (±1.26) days. The mean cumulative dose of gonadotrophins was 2,128.12 (±592) IU. The mean peak E_2 was 2,384.25 (±766) pg/mL. In total, 91 oocytes were retrieved, of which 79 (86.8%) were at the MII stage. All 79 MII oocytes were frozen, and all were thawed 20–24 hours after cryopreservation. The survival rate of oocytes after thawing was 86.1% (68/79). Intracytoplasmic sperm injection was performed on all surviving oocytes, and normal fertilization was confirmed in 89.7% of surviving oocytes (61/68). Cleavage occurred in 91.8% of those oocytes that fertilized normally (56/61) (Table 1). In total, 23 embryos were transferred to four recipients. Clinical pregnancy was confirmed in 3 of 4 recipients (75%). In total, six individual implantations were confirmed by ultrasonography, resulting in a 26.1% implantation rate (6/23). The mean number of embryos transferred was 5.8 (±2.1) (Table 2). One singleton, one twin, and one triplet pregnancy resulted (Table 3).

DISCUSSION

Although nearly two decades have passed since the first pregnancy was achieved following human oocyte cryopreservation (3), it is only recently that scientific advances and medical interest have brought this technology to the

TABLE 2

Implantation and clinical pregnancy rates.

Clinical outcome	Total	Mean per patient (SD)	Percentage
Transferred embryos	23	5.8 (2.1)	N/A
Embryo implantation	6	1.5 (1.3)	26.1%
Clinical pregnancy	3	0.75 (0.5)	75.0%

Barritt. Oocyte cryopreservation. Fertil Steril 2007.

TABLE 3

Clinical data about recipient patients.

	1	2	3	4
Age	45.2	42.8	46.7	41.3
Previous children	–	–	–	–
Previous IVF cycles	4	–	4	–
Previous oocyte donation cycles	–	–	1	–
Embryos transferred	4	8	7	4
Pregnancy	Yes	Yes	Yes	No

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forefront. Advances in laboratory performance and quality control have improved results in all areas of assisted reproductive technologies. Specific improvements in oocyte cryopreservation, thawing, and fertilization techniques have introduced new possibilities to patients desiring preservation of reproductive potential. Our study reports on the first series of cryopreserved donor eggs to be successfully fertilized and transplanted into recipient patients at our center.

Several articles have been published reporting on the successful freezing, thawing, and transfer of human oocytes (12–15). In general, these described cryosurvival, fertilization, and implantation rates that were lower than those achieved with the use of fresh oocytes. An analysis by Sonmezer and Oktay of 21 studies on oocyte cryopreservation from peer-reviewed journals found that the mean oocyte survival rate after thaw, mean fertilization rate, and mean PR per cryopreserved and thawed oocyte was 47%, 52.5%, and 1.52%, respectively (16).

We had several aims in our study. We intended to gain experience with oocyte cryopreservation techniques in preparation for offering this technique clinically to those requiring egg-freezing for medical or elective indications. We also wanted to minimize the variables likely to complicate this technique in either medically compromised or older patients. The oocyte survival rate of 86.1% was well above the rate in most reports, as were fertilization and cleavage rates of 89.7% and 91.8%, respectively. Implantation rates have been reportedly low (10%–19%) (15), and thus a greater number of embryos is needed for transfer, as well as a greater number of frozen and thawed oocytes to achieve a pregnancy. We also aimed to calculate an ideal number of oocytes required to increase the likelihood of a pregnancy. In 1999, Porcu et al. reported on the need for close to 100 frozen oocytes to achieve one pregnancy (16 pregnancies with 1,502 frozen oocytes) (17). Tucker et al. reported on the need for 62 frozen oocytes per pregnancy (five pregnancies with 311 frozen and thawed oocytes) (18). Yang et al. required 17 frozen oocytes to achieve one pregnancy (seven pregnancies from 120 frozen and thawed oocytes) (19). In 2003, Fosas et al. reported on the need for 22 frozen donor oocytes to achieve one pregnancy (four pregnancies using 88 frozen and thawed oocytes) (20). In our study, 3 out of 4 patients became pregnant, with a total of six embryos implanting and resulting in ongoing pregnancies, representing the need of only 13 frozen and thawed oocytes to achieve an implantation (six fetal heartbeats from 79 frozen and thawed oocytes). The clinical PR in this report is 75%, which is, to our knowledge, the highest ever reported following oocyte cryopreservation.

With improving techniques and expanding indications, cryopreservation of the female gamete offers the ability to protect and extend reproductive capacity. There are special circumstances, without doubt, where it could be extremely helpful. Whenever possible, oocyte cryopreservation is preferred over ovarian tissue freezing because it is less invasive and has resulted in numerous live births. However, contraindications to ovarian stimulation and oocyte freezing may exist. There may be limited or no other clinical options for girls and women who face a high likelihood of diminished or absent ovarian reserve resulting from necessary disease treatments. When ovarian tissues are frozen, no ovarian stimulation is needed, and therefore time restrictions are fewer, and no risk of stimulating estrogen-sensitive cancer due to ovarian stimulation exists. One approach to avoid estrogen-receptor stimulation involves oocyte retrieval and subsequent cryopreservtaion during a natural cycle. This technique is less than ideal because typically, no more than a single oocyte can be obtained (21). To ameliorate this issue, alternative protocols for ovarian stimulation, such as the use of an aromatase inhibitor (22), have been under investigation for induction of ovulation in breast cancer patients (23).

From these data, we can conclude that human oocyte cryopreservation is an effective technique that can be applied in clinical situations. Use of a donor egg model allows the study of this technology in a more controlled setting by limiting confounding variables. In addition, it suggests that the creation of cryopreserved donor oocyte banks may be an effective way of collecting and allocating appropriate oocytes to a recipient population, without the need for synchronization of cycles or the creation of supernumerary embryos destined to be cryopreserved. Furthermore, our initial experience gives us confidence that we can begin to offer this technology to patients both for medical indications and possibly for those desiring preservation of their reproductive potential.

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