Pregnancy after cryopreservation of donor oocytes and preimplantation genetic diagnosis of embryos in a patient with ovarian failure

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Objective: To describe the successful use of preimplantation genetic diagnosis to assess the prevalence of meiotic errors after oocyte cryopreservation in an oocyte donation cycle.

Design: Case report.

Setting: Private IVF center.

Patient(s): A 42.6-year-old patient with ovarian failure.

Intervention(s): A donor oocyte IVF cycle with cryopreservation of oocytes followed by thaw, fertilization of oocytes, preimplantation genetic diagnosis for selective aneuploidy, and ET.

Main Outcome Measure(s): Preimplantation genetic analysis of chromosomes 13, 16, 18, 21, 22, X, and Y with fluorescence in-situ hybridization.

Result(s): The recipient’s initial serum hCG level was 196 mIU/mL 15 days after oocyte retrieval. An initial ultrasound at the sixth week of gestation revealed two gestational sacs. A second ultrasound 1 week later showed a monochorionic twin in sac A and a singleton pregnancy in sac B. Fetal cardiac activity was visualized for all gestations.

Conclusion(s): This case illustrates the feasibility of cryopreservation of donor oocytes combined with preimplantation genetic diagnosis for clinical use in those settings where there may be an increased risk of spindle-related abnormalities. (Fertil Steril 2004;82:211–4. ©2004 by American Society for Reproductive Medicine.)

Key Words: Cryopreserved oocytes, preimplantation genetic diagnosis, aneuploidy, pregnancy, FISH

Human oocyte cryopreservation is an attractive choice to the range of fertility treatments presently offered. Having frozen oocyte banks would be beneficial in donor programs, allowing for quarantine of oocytes until appropriate infectious disease screening on the donor can be completed. Banks of cryopreserved donated oocytes would facilitate the donation process, which is often complicated by a requirement for donor–recipient synchrony. Oocyte freezing could also be useful in those sporadic IVF cases in which the man unexpectedly cannot provide semen on the day scheduled for follicular puncture and frozen back-up sperm is unavailable.

Various attempts to cryopreserve human oocytes have been performed with contrasting results. Although good fertilization and cleavage rates (1–3), as well as isolated pregnancies (1, 4–8), using either frozen-thawed mature or immature human oocytes have been reported for a number of years, results have been inconsistent. The overall success rates of oocyte survival post-thaw as well as pregnancy rates were very low, discouraging the routine application of oocyte cryopreservation (9). In addition to low survival rates, the human metaphase II (MII) stage oocyte was found to be particularly susceptible to freeze–thaw damage suggesting that several forms of cryoinjury are responsible for the relative lack of success in preserving human oocytes (10). These include damage to the meiotic spindle and to unstably bound chromosomes (11, 12).
Although electron microscopy studies reveal that ultra-rapid freezing of oocytes preserves cell structure comparatively well, embryos developed from frozen eggs show clear evidence of temperature-induced disruption of the oocyte’s meiotic spindle and other subcellular structures (13). These observations confirmed that spindle microtubules are sensitive to freeze-thawing and that cryopreservation could cause chromosomal aberrations during early development. In spite of an apparent normal fertilization with cryopreserved oocytes, unbalanced disjunction may occur in the thawed oocyte giving rise to an aneuploid embryo (10). The lower reproductive efficacy of frozen oocytes compared with fresh ones and the increased risk of aneuploidy have discouraged embryologists from routine freezing of oocytes for reproductive purposes.

Attempts to improve the chance of achieving a pregnancy in women who are at increased risk for oocyte aneuploidy has involved selection of oocytes and embryos by preimplantation genetic diagnosis (PGD). Fluorescence in-situ hybridization (FISH) has been applied to PGD of common aneuploidies (at least XY, 13, 18, 21) with testing in human blastomeres from cleavage-stage embryos (14, 15). Preimplantation aneuploidy screening improved pregnancy outcome by significantly reducing the miscarriage rate and increasing embryo implantation rates for several groups of patients (15). Aneuploidy has been identified as a primary source of the poor pregnancy rate and high miscarriage rate of embryos derived from frozen oocytes. Although births after cryopreservation of oocytes at the MII stage have been achieved, revealing that some thawed oocytes have normal functional meiotic spindles that give rise to euploid embryos, data on the chromosomal normality of embryos derived from frozen oocytes are lacking (16).

In this article we share our initial experience with oocyte cryopreservation combined with the use of PGD to assess the prevalence of meiotic errors after oocyte cryopreservation in an oocyte donation cycle. Intracytoplasmic sperm injection (ICSI) was used for insemination purposes to maximize normal fertilization when eggs survived the cryopreservation. Preimplantation genetic diagnosis was used for selection and transfer of chromosomally normal preimplantation embryos to the recipient.

**CASE REPORT**

A 24.9-year-old anonymous donor was stimulated using a recombinant FSH/GnRH antagonist/low-dose hCG protocol. Gonadotropin stimulation was started on day 3 of the cycle after oral contraceptive (OC) pretreatment with dose adjustments based on the individual’s ovarian response. Because the recipient and her husband were unavoidably detained (stranded due to a snow storm), the oocytes could not be inseminated. To salvage the cycle, the oocytes were stripped of cumulus cells with hyaluronidase and maturation verified as 12 metaphase II and 1 degenerated oocytes.

The 12 viable oocytes were cryopreserved 6 hours after retrieval (VOR) using a modified two-step dehydration protocol of 1,2 propanediol and sucrose (17). Cooling was performed using a controlled rate freezer in which the temperature was slowly reduced to −7°C at a rate of −2°C/min whereupon ice crystallization was induced manually with a chilled cotton swab. After seeding, cooling proceeded at −0.3°C/min to −37°C, after which oocytes were plunged directly into liquid nitrogen at −196°C for cryostorage.

Oocytes were thawed the following day at 37°C in a water bath and cryoprotectant was removed by passage of the oocytes through six serial dilutions of 1,2 propanediol/sucrose. Finally the oocytes were cultured in IVF medium at 37°C in an atmosphere of 5% CO₂ in air.

After thawing, the oocyte morphology, cytoplasm, cell membrane, and perivitelline space were evaluated. The oocytes were considered to have survived if their zona pellucida and cell membrane were intact, the perivitelline space was of a normal size, and there was no evidence of cytoplasmic leakage or oocyte shrinkage. All 12 oocytes survived the thaw procedure and were allowed to equilibrate for 4 hours before ICSI insemination (normalized day 0). The ICSI was performed with fresh ejaculated sperm from the recipient’s husband and 17 hours after microinjection, the presence of pronuclei was verified (day 1). Nine oocytes fertilized normally and exhibited normal cleavage 24 hours later. Embryonic division and morphology was evaluated every 24 hours thereafter.

On day 3, two embryos had arrested but all nine embryos underwent blastomere biopsy. Individual embryos were placed into calcium/magnesium-free HTF-HEPES media (SAGE In Vitro Fertilization, Inc., Trumbull, CT) for embryo biopsy 68 hours after insemination. Embryos were positioned so that a nucleated cell was adjacent to the anticipated biopsy site. A 25- to 30-μm hole was opened in the zona pellucida with a series of 3–5 single pulses from an infrared 1.48-μm diode laser using a 1.0-millisecond pulse duration at 100% power (Hamilton-Thorne Research, Beverly, MA) (18). The nucleated blastomere was removed through the opening by applying gentle pressure with a blastomere biopsy pipette (Conception Technologies, San Diego, CA) to the adjacent intact zona pellucida to eject the blastomere (John Stephens, personal communication).

Immediately after the biopsy, each biopsied embryo was rinsed thoroughly and placed into extended culture in G2 blastocyst culture media (Vitrolife, Gothenburg, Sweden) while awaiting the outcome of FISH analysis. The isolated blastomeres for FISH analysis were fixed individually on glass slides by the addition of multiple drops of 3:1 methanol:acetic acid after preincubation in hypotonic solution. Two rounds of FISH (16, 19) were performed on fixed blastomeres using probes for chromosome 13, 16, 18, 21, 22, X, Y (Vysis, Downer’s Grove, IL). A total of three embryos were assessed as normal for the chromosomes
TABLE 1

PGD diagnosis and developmental status of embryos derived from cryopreserved oocytes.

<table>
<thead>
<tr>
<th>Embryo no.</th>
<th>Stage day 3</th>
<th>Stage day 6</th>
<th>PGD diagnosis</th>
<th>Disposition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2-cell/arrest</td>
<td>Biopsy — no results</td>
<td>Discard</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>8-cell</td>
<td>Hatching blast</td>
<td>Trisomy 13</td>
<td>Discard</td>
</tr>
<tr>
<td>3</td>
<td>8-cell</td>
<td>8-cell/arrest</td>
<td>Monosomy 18</td>
<td>Discard</td>
</tr>
<tr>
<td>4</td>
<td>8-cell</td>
<td>Hatching blast</td>
<td>Monosomy 16</td>
<td>Discard</td>
</tr>
<tr>
<td>5</td>
<td>3-cell/arrest</td>
<td>Hypertriploid xxy</td>
<td>Discard</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>5-cell</td>
<td>Normal female</td>
<td>Transfer</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>8-cell</td>
<td>Normal male</td>
<td>Transfer</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>8-cell</td>
<td>Normal male</td>
<td>Transfer</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>8-cell</td>
<td>Hatching blast</td>
<td>Nullisomy XY</td>
<td>Discard</td>
</tr>
</tbody>
</table>


analyzed and were deemed suitable for embryo transfer or cryopreservation. The remaining abnormal embryos were continued in extended culture to assess development potential (Table 1).

The recipient’s endometrium was prepared to receive the embryos through the oral administration of estrogens (E) followed by IM P supplementation. The endometrial response was monitored by vaginal ultrasound by measuring endometrial thickness. The patient elected to transfer all three normal embryos and on day 4 these embryos were replaced using abdominally guided ultrasound ET technique.

The patient’s initial serum β-hCG level was 196 mIU/mL (15 days after VOR). Her initial ultrasound (+26 VOR) showed two sacs with two heartbeats. A second ultrasound (U/S + 36 VOR) revealed a monochronic twin in sac A and a singleton pregnancy in sac B.

DISCUSSION

This case illustrates the feasibility of cryopreservation of donor oocytes combined with PGD for clinical use in those settings where there may be an increased risk of spindle-related abnormalities. These encouraging results indicated that it is possible not only to achieve a high survival rate of cryopreserved human oocytes but also to successfully fertilize these oocytes and obtain a high cleavage rate with satisfactory embryo development.

Preimplantation aneuploidy screening can be used to assist in the identification of IVF embryos most likely to result in a successful pregnancy. Preferential transfer of the embryos characterized in this manner has improved outcomes for certain groups of IVF patients (14, 15). Avoidance of chromosomally abnormal births is of special concern for women undergoing oocyte donation. Concern has been expressed that oocyte freezing may induce damage to the meiotic spindle that would lead to chromosomal abnormalities in embryos derived from frozen/thawed oocytes (13).

Disruption of the cytoskeleton and spindle microtubules is thought to be the chief cause of chromosomal anomaly in thawed mature eggs (11). With a few exceptions, aneuploid preimplantation embryos are morphologically indistinguishable from euploid embryos and consequently the usual assessments carried out in IVF clinics before embryo transfer do not allow them to be detected.

In this study, we introduced the use of PGD to identify and preferentially transfer only embryos with normal chromosomal screening after donor oocyte cryopreservation. We were able to perform a diagnosis using FISH for chromosomal analysis and embryo transfer within 24 hours of cell biopsy, allowing transfer on day 4 after biopsy on day 3. The results presented here indicate that it is possible to cryopreserve human oocytes for an oocyte donation program and that PGD could be an efficient method of achieving a satisfactory outcome in terms of implantation and pregnancy.

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References


