

Pregnancy after polar body biopsy and freezing and thawing of human embryos

Michael Lee, M.S.,* and Santiago Munné, Ph.D.†

The New England Clinic of Reproductive Medicine, Reading, Massachusetts; and Saint Barnabas Medical Center, Livingston, New Jersey

Objective: To evaluate the outcome of frozen-thawed ET using embryos previously biopsied for preimplantation genetic diagnosis during a fresh ET cycle.

Design: Prospective evaluation.

Setting: Assisted reproductive biology program.

Patient(s): A 31-year-old, G4, P1, TAB1, SAB2 carrier of a balanced RT 45,XX der(14;21)(q10;q10) translocation.

Intervention(s): Preimplantation genetic diagnosis by polar body biopsy. Excess embryos were frozen using the one-step method and then thawed.

Main Outcome Measure(s): Embryo survival after thawing and subsequent pregnancy outcome.

Result(s): Among the 32 mature oocytes, the results of fluorescence in situ hybridization were available for 25 polar bodies. Eleven were unbalanced, 10 were normal (8 fertilized), and 4 were balanced (3 fertilized) for the fresh IVF cycle. Two normal embryos were transferred. Four normal and 3 balanced embryos were cryopreserved. A chemical pregnancy resulted. Four months later, the 7 cryopreserved embryos were thawed; 2 survived (1 balanced and 1 normal) and were transferred. An ongoing pregnancy resulted, and a normal (46,XX) female was delivered.

Conclusion(s): Freezing and thawing of biopsied embryos resulted in a low survival rate. However, this should not be a deterrent to the cryopreservation of extra chromosomally normal embryos because the embryos that do survive are able to implant. (Fertil Steril® 2000;73:645–7. ©2000 by American Society for Reproductive Medicine.)

Key Words: Preimplantation genetic diagnosis, polar body biopsy, frozen-thawed ET, gene translocation

Preimplantation genetic diagnosis (PGD) has been performed successfully in carriers of translocations using a variety of methods. In female carriers of translocations, most pregnancies so far have been achieved using a technique of polar body biopsy in which the resulting first polar body chromosomes are analyzed with painting probes (1). In male carriers, either telomere or breakpoint-spanning probes have been used, or biopsied blastomeres have been forced to a metaphase stage through fusion with oocytes or zygotes.

For robertsonian translocations, enumerator probes can be used to prevent aneuploidy. However, couples who undergo PGD usually prefer to be able to select against balanced embryos; this can be achieved by chromosome painting on polar bodies or metaphase-stage blastomeres, or by breakpoint-spanning probes with interphase blastomeres.

In most cases of PGD, only a few embryos are available for replacement because of chromosomal or genetic abnormalities or because of morphologic or developmental unsuitability. Excess normal embryos are available for cryopreservation in only a few cases. There has been only one report of a pregnancy achieved with cryopreserved embryos that had been biopsied before they were frozen (2). In another study, significantly fewer biopsied embryos survived intact and fewer cells survived in the nonintact embryos after cryopreservation and thawing compared with embryos that were not biopsied (3).

Nevertheless, some embryos did survive, indicating that cryopreservation and thawing can be used for patients who have extra biopsied embryos that are suitable for transfer. However, cryopreservation cannot be used to provide unlimited time to undertake genetic testing of embryos, because many embryos that

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Reprint requests: Santiago
Munné, Saint Barnabas
Medical Center, 101 Old
Short Hills Road, Suite
501, West Orange, New
Jersey (FAX: 201-243-
6235).

* The New England Clinic
of Reproductive Medicine.

† The Institute for
Reproductive Medicine and
Science, Saint Barnabas
Medical Center.

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TABLE 1

Results of PGD in a female carrier of a 45,XX, der(14;21)(q10;q10) translocation.

Oocyte no.	No. of chromatids			Fertilization	Oocyte diagnosis	First cycle disposition	Second cycle disposition
	14	der(14;21)	21				
1	—	—	—	Atretic oocyte	Not analyzed	Discarded	—
2	NR	NR	NR	2PN	NR	Discarded	—
3	NR	0	2	1PN	NR	Discarded	—
4	2	1	0	1PN	Abnormal	Discarded	—
5	0	2	1	1PN	Abnormal	Discarded	—
6	—	—	—	Immature MI	Not analyzed	Discarded	—
7	0	2	0	0PN	Normal	Discarded	—
8	2	0	0	2PN	Abnormal	Discarded	—
9	NR	NR	NR	2PN	NR	Discarded	—
10	0	2	2	2PN	Abnormal	Discarded	—
11	0	2	2	2PN	Abnormal	Discarded	—
12	—	—	—	Immature GV	Not analyzed	Discarded	—
13	—	—	—	Immature GV	Not analyzed	Discarded	—
14	2	0	2	0PN	Balanced	Discarded	—
15	NR	NR	NR	2PN	NR	Discarded	—
16	2	0	1	2PN	Abnormal	Discarded	—
17	2	0	2	2PN	Balanced	Frozen	Thawed: dead
18	0	2	0	2PN	Normal	Frozen	Thawed: dead
19	NR	NR	NR	2PN	NR	Discarded	—
20	2	0	1	2PN	Abnormal	Discarded	—
21	2	0	0	2PN	Abnormal	Discarded	—
22	NR	NR	NR	2PN	NR	Discarded	—
23	0	2	2	2PN	Abnormal	Discarded	—
24	2	0	2	2PN	Balanced	Frozen	Thawed: ET
25	—	—	—	Immature MI	Not analyzed	Discarded	—
26	NR	NR	NR	2PN	NR	Discarded	—
27	0	2	0	2PN	Normal	Frozen	Thawed: dead
28	2	0	0	2PN	Abnormal	Discarded	—
29	2	0	0	0PN	Abnormal	Discarded	—
30	0	2	0	2PN	Normal	Discarded (arrested)	—
31	—	—	—	Immature MI	Not analyzed	Discarded	—
32	NR	NR	NR	2PN	NR	Discarded	—
33	0	2	0	2 cells	Normal	Discarded (parthenogenetic)	—
34	0	2	0	0PN	Normal	Discarded	—
35	0	2	0	2PN	Normal	Frozen	Thawed: ET
36	0	2	0	2PN	Normal	Fresh ET	—
37	0	2	0	2PN	Normal	Fresh ET	—
38	2	0	2	2PN	Balanced	Frozen	Thawed: dead

Note: GV = germinal vesicle; MI = meiosis-I; NR = no result; PN = pronuclei.

Lee. Polar body biopsy. *Fertil Steril* 2000.

are normal before freezing will be damaged after thawing. We present a case in which pregnancy was achieved after polar body biopsy, PGD, and embryo freezing and thawing.

Embryo biopsy and fixation of biopsied cells was performed at the New England Clinic of Reproductive Medicine and FISH analysis at the Institute of Reproductive Medicine and Science of Saint Barnabas Medical Center. These protocols were in accordance with guidelines approved by the institutional review board of Saint Barnabas Medical Center and the New England Clinic of Reproductive Medicine through Tufts Medical Center, including individual written, informed consent.

CASE REPORT

The patient was a 31-year-old female carrier of a balanced RT 45,XX, der(14;21)(q10;q10), G4, P1, TAB1, SAB 2 translocation. The couple was fertile and the woman's carrier status was ascertained after karyotype analysis of both parents. Her unusual history included one prior term delivery of a son with Down syndrome. Thereafter, she had three more pregnancies, two ended in spontaneous abortion (one of which was a confirmed translocation) and one in therapeutic abortion after chorionic villi sampling revealed Down syndrome.

The patient underwent a regimen of gonadotropin stimu-

lation that included GnRH agonist suppression followed by the administration of exogenous gonadotropins. She received leuprolide acetate beginning in the midluteal phase of the menstrual cycle before stimulation. This was followed by the administration of three ampules of gonadotropins beginning on cycle day 2.

The dosages were adjusted based on her response using established criteria; they generally were decreased when the lead follicles were >14 mm in maximum diameter. The criteria for hCG administration were the presence of ≥ 2 follicles of 18–20 mm in maximum diameter or a single follicle of ≥ 20 mm in diameter. Oocyte retrievals were done transvaginally under ultrasonography. All visible follicles were aspirated.

Six hours or less after oocyte retrieval, the first polar bodies from fresh metaphase II oocytes were biopsied by partial zona dissection in an area immediately in front of the polar body. A microneedle with an inner diameter of 15 μm and a tip beveled at 45 degrees was used to penetrate the opening in the zona and draw out the polar body by suction. Polar body fixation was performed as described previously (1), without modification. The embryos then were cultured in S1 medium (Scandinavian IVF Science, Gothenborg, Sweden) under mineral oil at 37°C and 5% CO₂ in air for 3 days. Embryo transfers were performed on the afternoon of the third day after retrieval.

The fixed polar bodies were taken by airplane to The Institute for Reproductive Medicine and Science at Saint Barnabas Medical Center in Livingston, New Jersey for analysis according to previously published protocols (1). In brief, 1 μL of whole chromosome painting (WCP) probe specific for chromosome 14 labeled in Spectrum Green (Vysis), 1 μL of WCP probe specific for chromosome 21 labeled in Spectrum Orange (Vysis), and 2 μL of locus-specific probe for chromosome 21 labeled in Spectrum Aqua (Vysis) were concentrated to a volume of 31 μL and mixed with 7 μL of WCP hybridization buffer (Vysis, Downers, IL). This hybridization solution was codenatured with the glass slides containing the fixed polar bodies by placing the mounted slides on a hotplate at 73°C for 5 minutes.

The slides then were sealed with rubber cement and placed in a moist chamber at 37°C for overnight hybridization. Afterward, all slides were washed in 1 \times SSC for 2 minutes at 71°C and counterstained with diaminophenylindole in antifade solution. The slides were observed under a

fluorescence microscope (Olympus BX60; NY-NJ Scientific, Middlebush, NJ) with a triple-bandpass filter to visualize Spectrum Green, Spectrum Orange, and Spectrum Aqua simultaneously, and the image was recorded with an image analysis system (Metasystems, Belmont, MA).

RESULTS

Table 1 shows the results of PGD of the first polar bodies analyzed. Of the 38 oocytes retrieved, 5 were immature and 1 was atretic. Of the remaining 32 mature oocytes, the results of fluorescence in situ hybridization were available for 25 polar bodies. Eleven were unbalanced, 10 were normal (8 fertilized), and 4 were balanced (3 fertilized). One polar body was lost and the chromosomes were too compacted in the others to produce clear results.

On day 3 of development, 2 normal embryos were transferred. Four normal and 3 balanced embryos were frozen using the one-step cryopreservation method (4). The patient had an initial β -hCG level of 10 mIU/mL that increased to a maximum of 18 mIU/mL and then declined to zero.

Four months later, the patient elected to undergo a frozen-thawed ET cycle. All her frozen embryos were thawed; only two survived, a normal three-cell embryo with two intact cells, and a balanced four-cell embryo with three intact cells. These two embryos were transferred, and one implanted. The patient delivered a normal (46,XX) baby.

DISCUSSION

Our findings in this case support those of a previous report (3) in which freezing and thawing of biopsied embryos resulted in a low survival rate. However, this should not be a deterrent to the cryopreservation of extra chromosomally normal embryos because the embryos that do survive are able to implant.

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