Successful Day 5 Embryo Transfer and Pregnancies Resulting After Transport of Embryos by Air for Biopsy and Genetic Analysis

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Purpose: Case studies of four in vitro fertilization (IVF) cycles where embryo transport by commercial airline followed by biopsy and genetic analysis with subsequent culture to Day 5 and resulting ongoing pregnancy.

Method: Retrospective clinical case study of 4 patients requiring preimplantation genetic diagnosis (PGD) testing. Normally fertilized embryos were transported in a battery-powered portable incubator by commercial airline following evaluation for fertilization under controlled conditions from the Center for Assisted Reproduction, Bedford, Texas to the Reproductive Genetic Institute, Chicago, Illinois. Following Day 3 embryo biopsy and genetic analysis, embryos were transported back to the Center for Assisted Reproduction for Day 5 embryo transfer.

Results: Ongoing clinical pregnancy resulted for all patients receiving embryo transfer.

Conclusion: These results demonstrate the feasibility of embryo transport by air for centers that do not have the in-house capabilities to perform genetic analysis. With successful pregnancies obtained through extended culture to Day 5, embryos requiring genetic analysis can be successfully transported by air, tested, and returned to the initial facility for embryo transfer without time restriction.

KEY WORDS: Clinical pregnancy; embryo transport; extended culture; PGD.

INTRODUCTION

Clinical pregnancies have been reported for traditional in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) cases when oocytes and/or embryos are transported by both ground and/or by air (1–3). Transport of gametes or embryos have become a popular means of achieving pregnancy in programs that provide infertility services but do not have an on-site IVF laboratory. In addition, many programs lacking the resources to establish an IVF laboratory utilize a central site where human assisted reproductive technologies (ART) can be performed. Advantages of this include a reduction of laboratory and personnel expenses associated with operating an ART laboratory. In addition to evading the cost associated with laboratory setup and maintenance, laboratory certification and labor intensive reporting is avoided. Other benefits of having a central ART laboratory site include consistent and strict quality control and concise laboratory protocols at a central location.

Just as some infertility programs are in need of an off-site IVF laboratory to perform assisted reproductive technologies, even more programs lack a genetic testing laboratory or the capability to perform preimplantation genetic diagnosis (PGD). Although the Center for Assisted Reproduction provides complete IVF services encompassing surrogacy and donor oocyte; however, it does not currently perform genetic testing.

Transport of embryos for the purpose of genetic testing with the intent to travel with embryos from one location, genetically test them at another, then return them to the original center for embryo transfer

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is a timely process. Blastomere biopsy from a 6–8-cell embryo on Day 3 leaves little time to biopsy, analyze, then transport back to original site for Day 3 embryo transfer. Recently, with the advent of sequential media embryos can be cultured successfully to Day 5 and Day 6 (4,5). This allows embryos requiring genetic analysis to be successfully transported by air, biopsied, analyzed, and returned to initial center for embryo transfer without time restriction.

In these case reports we will demonstrate the feasibility of embryo transport by air for centers that do not have the in-house capabilities to perform particular genetic analysis.

MATERIALS AND METHODS

This report demonstrates the ability to successfully transport embryos by air for genetic analysis and return the embryos for Day 5 transfer. Patients were advised to consider IVF with ICSI followed by PGD. All patients underwent controlled ovarian stimulation with GnRH analog and pure follicle stimulating hormone (FSH), using a conventional down regulation protocol. Upon confirmation of two dominant follicles (mean diameter ≥ 16 mm) and a peak estradiol level (E_2), human chorionic gonadotropin (hCG) was administered with oocyte retrieval occurring 36–37 h later. Following retrieval (Day 0) oocytes were placed into human tubal fluid (HTF; Irvine Scientific, Santa Ana, CA) +12% synthetic serum substitute (SSS; Irvine Scientific, Santa Ana, CA) and cultured for approximately 6 h. Upon oocyte examination sperm injection was performed on Metaphase II oocytes 42 h post-hCG following hyaluronidase treatment, using previously described techniques (6,7). Oocytes were cultured overnight in 100 μ l media drops of G1.2 (IVF Science, Gothenburg, Sweden) with oil overlay in a humidified incubator with 5% CO₂ and 95% air at 37°C. Fertilization was evaluated 16–18 h following sperm injection (Day 1). Intramuscular luteal support with 50 mg progesterone in oil was initiated on Day 1 following retrieval.

Prior to transport of embryos for genetic analysis on Day 1 or Day 2, normally fertilized oocytes containing two pronuclei (2PN) or multicell embryos were resuspended in preequilibrated G1.2 and individually placed, into snap-top culture tubes (Falcon, 2036) for transport. In order to ensure an airtight seal, the cap of each tube containing G1.2 was wrapped with laboratory parafilm. Each culture tube was then placed in a thermal controlled (37°C) dry heat portable incuba-

tor (LEC Instruments, LEC960). Due to the absence of phenol red in the G1.2 and G2.2 media, a snap-top tube containing preequilibrated HTF media was sealed with parafilm and placed in the portable incubator to serve as an indirect quality control measure to ensure no gas exchange occurred during transport. All materials used for embryo culture, including the portable incubator, were quality control tested where $\geq 60\%$ one-cell mouse embryos reached blastocyst by Day 5. Generally, all materials including culture media were forwarded to the Reproductive Genetics Institute prior to embryo transport where technicians at the Reproductive Genetics Institute prepared culture dishes utilizing the same protocols, materials, and culture media as what is used at the Center for Assisted Reproduction. Zygotes were transported almost 1,000 miles by air from the Center for Assisted Reproduction, Bedford, Texas to the Reproductive Genetics Institute, Chicago, Illinois. Transport from the Center for Assisted Reproduction to Dallas/Fort Worth International Airport, nonstop flight interval, and transport from Chicago O'Hare International Airport to the Reproductive Genetics Institute took approximately 5 h. Upon arriving in Chicago, the zygotes/multicell embryos were removed from the culture tubes and placed in fresh media drops of preequilibrated G1.2 under oil. The control tube containing HTF was visually inspected after each trip for a change in media color and in all cases, the stable equilibrated color indicated no gas exchange during transport.

Following PGD on Day 3 and overnight culture at the Reproductive Genetics Institute each genetically normal embryo was evaluated for embryo progression and morphology and then resuspended in preequilibrated G2.2 (IVF Science, Gothenburg, Sweden) and individually placed in a snap-top culture tube. These tubes were wrapped with laboratory parafilm and loaded into the portable incubator along with a tube of HTF for quality control and transported to the Center for Assisted Reproduction. Once returned, each Day 4 embryo was recovered from the transport tubes and placed in fresh media drops of preequilibrated G2.2 under oil. The tube of HTF was evaluated, and again no color change was visually observed indicating no gas exchange. Each normal embryo was evaluated for embryo progression and morphology upon return to the Center for Assisted Reproduction. The next morning (Day 5), each embryo was again observed for progression and morphology. Subsequent embryo transfer occurred utilizing an abdominal ultrasound (5 MHz) to assist intrauterine placement

of the embryo transfer catheter (Edwards-Wallace catheter; Marlow Technologies, Willoughby, OH). Ongoing pregnancies were confirmed with the use of an ultrasound to detect the presence of an intrauterine sac with fetal cardiac activity.

RESULTS

Patient #1

The first patient, a 30-year-old female who had difficulty conceiving despite 3 years of unprotected intercourse, demonstrated a history of recurrent miscarriage with three conceptions ending in spontaneous abortion (SAB). The initial pregnancy was conceived via natural cycle ending in SAB at 5 weeks gestation. The second pregnancy was conceived after clomid/intercourse (IC) treatment ending with a SAB occurring at 9 weeks gestation. Genetic abnormalities were detected in the fetal tissue of the second pregnancy. The third pregnancy was conceived following luteinizing hormone (LH) timed intercourse ending in a third SAB at 6 weeks gestation. Further blood chromosome testing on this fetus along with parental blood analysis revealed an unbalanced translocation between chromosomes 7 and 11 in one partner.

Thirteen oocytes were retrieved including two atretic, nonviable oocytes (Day 0). Upon oocyte examination prior to ICSI, of the 11 intact oocytes, 7 oocytes were Metaphase II, 1 oocyte was Metaphase I, and the remaining 3 oocytes were germinal vesicles (GV). Sperm injection was performed on Metaphase II oocytes. Four oocytes were normally fertilized indicated by two pronuclei (2PN), one oocyte was abnormally fertilized indicated by three pronuclei (3PN), and two were nonfertilized indicated by zero pronuclei.

Embryos were transferred to Reproductive Genetics Institute on Day 1. The four normally fertilized embryos were evaluated on Day 2 (approximately 48 h post ICSI) and had progressed to the 4-cell stage. Following an additional 21.5 h of culture, each 4-cell embryo had divided again; three embryos had reached the 8-cell stage, the last embryo reaching the 6-cell stage. Embryo biopsy was performed, one blastomere was removed from each of the three 8-cell embryos and two blastomeres were removed from the 6-cell. The embryos were then placed in equilibrate G2.2. Following genetic testing, three embryos were diagnosed as having a chromosomal unbalanced translocation. Of the three abnormal embryos, two showed

an unbalance derivative 7 and one embryo showed an unbalance with derivative 11. One embryo was normal.

The normal embryo was returned to the Center for Assisted Reproduction on Day 4. Once returned to the Center for Assisted Reproduction subsequent single embryo transfer of a developing blastocyst stage embryo was performed on Day 5. An ongoing pregnancy was confirmed with the use of an ultrasound to detect the presence of an intrauterine sac with fetal cardiac activity 27 days following oocyte retrieval. Resulting preterm delivery of a baby girl weighing 3 lb. occurred at 29 weeks gestation.

Patient #2

The second patient, a 38-year-old female had difficulty conceiving despite 2 years of unprotected intercourse. Blood chromosome testing revealed an unbalanced translocation between chromosomes 14 and 22 in one partner. Following egg retrieval five oocytes were recovered. Upon oocyte examination prior to ICSI, two oocytes were Metaphase II oocytes and the remaining three oocytes were GV. Sperm injection was performed on Metaphase II oocytes. Two oocytes were normally fertilized (2PN). Due to the low number of pronuclear stage embryos the patient elected to postpone PGD and cryopreserve the two embryos at the 2PN stage for later testing. The two 2PN stage zygotes were frozen in propanediol, using previously described slow cooling protocols (8). Second retrieval yielded four oocytes, three oocytes were Metaphase II oocytes and the remaining oocyte was a GV. Again, two oocytes were normally fertilized utilizing the ICSI procedure. The two frozen embryos from the initial cycle were thawed and transported for genetic testing along with the two fertilized oocytes from the fresh cycle.

The four normally fertilized embryos were evaluated on Day 2 (approximately 48 h post-ICSI) and had progressed to the 2–4-cell stage. Embryos were transported to the Reproductive Genetics Institute on Day 3. Following an additional 25 h of culture (Day 3), each of the four embryos had divided; two embryos had reached the 8-cell stage, one embryo had reached the 6-cell stage, and one had reached the 5-cell stage. Embryo biopsy was performed and one blastomere was removed from each of the four embryos. The embryos were then placed in equilibrate G2.2. No chromatin was found following fixation of the frozen/thawed embryos, therefore, no results were obtained and the embryos were discarded. Genetic

testing of the two fresh embryos showed no genetic abnormality.

The two normal embryos were returned to the Center for Assisted Reproduction on Day 4. Once returned to the Center for Assisted Reproduction subsequent embryo transfer of one developing blastocyst stage embryo and one developing compacting morula was performed on Day 5. An ongoing pregnancy was confirmed with the use of an ultrasound to detect the presence of an intrauterine sac with fetal cardiac activity 29 days following oocyte retrieval. Resulting full term delivery of a baby girl weighing 7 lb. 7 oz. occurred at 39 weeks gestation.

Patient #3

The third patient, a 41-year-old female who had difficulty conceiving despite 4 years of unprotected intercourse, demonstrated a history of recurrent miscarriage with three conceptions ending in SAB. The initial pregnancy was conceived via natural cycle ending in SAB at 8 weeks gestation. The second pregnancy was conceived after clomid/IC treatment but ended with a SAB occurring at 8 weeks gestation. The third pregnancy was conceived again following clomid/IC treatment resulting with a third SAB at 8 weeks gestation. Chromosome analysis on the patient demonstrated a double aneuploidy for trisomy 13 and trisomy 16. The additional chromosome 13 was derived from a Robertsonian translocation involving both the long arms of chromosome 13 and chromosome 14.

Ten oocytes were isolated following retrieval. Upon oocyte examination prior to ICSI, of the 10 intact oocytes, 8 oocytes were Metaphase II and the remaining 2 oocytes were GV. Sperm injection was performed on Metaphase II oocytes. Seven oocytes were normally fertilized (2PN) and one was nonfertilized. Embryos were transported to the Reproductive Genetics Institute on Day 1 following evaluation for fertilization. At the Reproductive Genetics Institute embryos were evaluated on Day 2 (approximately 48 h post-ICSI) and had progressed to the 2–4-cell stage. Following and additional 26 h of culture, each embryo had developed to the 4–8-cell stage. Embryo biopsy was performed by removing one to two blastomere from each embryo. Following genetic testing it was found only one embryo was normal.

The normal embryo was returned to the Center for Assisted Reproduction on Day 4. At the Center for Assisted Reproduction subsequent single embryo transfer of a developing blastocyst stage embryo was

performed on Day 5. An ongoing pregnancy was confirmed with the use of an ultrasound to detect the presence of an intrauterine sac with fetal cardiac activity 40 days following oocyte retrieval. Resulting full term delivery of a baby boy weighing 7 lb. 12 oz. occurred at 40 weeks gestation.

Patient #4

The fourth patient, a 33-year-old female demonstrated a history of recurrent miscarriage with 11 conceptions ending in SAB. Thirteen pregnancies were reported over a 4-year period resulting in 11 miscarriages, 1 therapeutic abortion and 1 full term delivery. Genetic testing on the patient showed a translocation of chromosome 5 and 14.

Five oocytes were retrieved. Upon oocyte examination prior to ICSI, of the five intact oocytes, four oocytes were Metaphase II, one oocyte was a GV. Sperm injection was performed on Metaphase II oocytes. Two oocytes were normally fertilized (2PN) and two were nonfertilized. The normally fertilized embryos were evaluated on Day 2 (approximately 48 h post-ICSI) before transport and had progressed to the 4-cell stage. Both normally fertilized embryos were transported to the Reproductive Genetics Institute on Day 2. Following and additional 26 h of culture, each 4-cell embryo had divided again and reached the 7-cells stage. Embryo biopsy was performed on each 7-cell embryo by removing one blastomere. Following genetic testing, both embryos were determined to be abnormal. One embryo was diagnosed and confirmed as unbalanced derivative 5. The second embryo was confirmed monosomy 14 and unbalanced derivative 5. No embryo transfer was performed. Both embryos stalled on Day 5 and were discarded at the multicell stage.

DISCUSSION AND CONCLUSION

Several reports have demonstrated successful oocyte and embryo transport by ground. The majority of all transport reports are of oocyte or embryo transportation from one satellite office where the oocyte retrieval is performed to a central clinic offering ART procedures where embryo culture and transfer is completed. Few reports exist of embryo transport from and back to a central site where both oocyte retrieval and embryo transfer are performed in the same facility and embryo culture and genetic testing is done elsewhere. To our knowledge, this is the first report of long distance embryo transport by air

Table I. Summary

	Patient #1	Patient #2		Patient #3	Patient #4
		Cycle #1 ^a	Cycle #2		
Age	30	38	38	41	33
Total days of FSH	10	9	10	10	11
Total amps of FSH	53	48	60	60	59
Oocytes retrieved	13	5	4	10	5
Oocytes injected	7	2	3	8	4
Fertilized oocytes (2PN)/percent	4/57.1%	2/100%	2/66.6%	7/87.5%	2/50.0%
Genetically normal embryos/percent per 2PN	1/25.0%	0/0%	2/100%	1/14.3%	0/0%
Embryos transferred	1	0	2	1	0
B-hCG	Positive	N/A	Positive	Positive	N/A
Delivery	Yes	N/A	Yes	Yes	N/A

^aData illustrates patient #2 cycle #1 where all 2PN embryos were frozen then thawed to undergo PGD with same patient cycle #2 embryos.

for biopsy and genetic analysis with successful culture to Day 5 resulting in ongoing pregnancies or delivery in three of four cases (Table I).

In order to transport embryos to another facility for genetic testing and return those embryos for transfer, extended embryo culture is essential. Multicell embryo replacement on Day 3 would most certainly pose problems due to time constraints where embryo biopsy, genetic analysis, and the return of the embryos to the original laboratory would have to be done on the same day. With extended culture to Day 5 and even Day 6, fertility centers are able to avoid time constraints when transporting embryos from a central site, performing genetic analysis at an alternate site, and returning them to the original site for embryo transfer in a reasonable time frame. In some instances reanalysis was necessary to confirm a diagnosis requiring rehybridization of the initial metaphase spread and further evaluation to infer a result. Day 5 embryo transfer allowed sufficient time for reevaluating test results where in some cases final diagnosis was forwarded by facsimile to the Center for Assisted Reproduction on Day 5.

In addition to the cost of the IVF cycle and laboratory fees for genetic analysis the patients in these case studies were responsible for reasonable and customary fees. These fees were associated with a technician accompanying the transport of the embryos to and from each facility. Cost included air and ground transportation, hotel, and meals. A consultation with each patient was scheduled with the laboratory prior to initiation of gonadotropins to discuss all of the patients estimated financial obligations associated with PGD. After discussion of additional expenses involving PGD several advantages were apparent.

Primarily, eliminating patient travel ultimately creates a less stressful IVF cycle for the patient and allows the patient and their spouse to remain in town and undergo cycle management at the clinic of their choice, with a physician that they feel most comfortable overseeing their treatment.

In conclusion, these case studies of four patients demonstrate the ability to transport embryos twice during a single IVF cycle over long distances; transport to a clinic for the purpose of genetic testing then return the embryos to the original clinic for Day 5 embryo transfer. Transport does not appear to have an adverse effect on embryo development and implantation and presents an attractive option to patients requiring preimplantation genetic analysis to achieve viable offspring and to infertility clinics that do not have the genetic testing capabilities on-site.

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