Successful Elective Single Blastocyst Transfer in a Patient With Prior Repetitive High-Order Multiple Gestations

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Multiple gestations remain one of the leading causes of morbidity related to infertility therapy. In the realm of assisted reproductive technologies, multiple gestations can be significantly limited by the reduction in the number of embryos transferred. Significant concern remains that a reduction in the number of embryos transferred may appreciably lower overall chances for pregnancy. Promising new developments are unfolding that may permit improved detection of a single human embryo with high implantation potential. One such development is the use of sequential culture media to allow prolonged culture of embryos to the blastocyst stage. We report a case in which sequential culture and elective transfer of one blastocyst was successfully used in a patient with a profoundly poor obstetrical history because of the complications of high-order multiple gestations.

KEY WORDS: Blastocyst transfer; in vitro fertilization; multiple gestations; single embryo transfer.

INTRODUCTION

One of the most difficult challenges in the field of assisted reproductive technologies (ART) is to find ways to reduce the risks of multiple gestations while maintaining reasonable treatment efficacy. In the 1997 national summary describing the outcomes of all ART cycles initiated in the United States, 39% of total births were multiple births, compared with less than 3% expected in the general population (1). High-order multiple births comprised 7.0% of total ART births.

Multiple gestations are associated with substantial increases in both maternal and neonatal morbidity and mortality. Maternal risks include an increase in pregnancy-induced hypertension, dilutional anemia, preterm labor, operative delivery, and postpartum hemorrhage. Maternal mortality rates have

been estimated to be approximately threefold higher for multiple gestations as compared to singleton births (2). Both prematurity and intrauterine growth retardation are the most important factors that determine neonatal morbidity and mortality. Significant prematurity (<32 gestational weeks) occurs in 1.2, 5.1, and 23.6% of fetuses born from singleton, twin, and triplet gestations, respectively (3). Very low birth weight (<1500 g) similarly occurs in 1.6, 5.1, and 27.0% of fetuses born in these respective categories. As a result, even twin gestations are associated with an increased risk of neonatal death (approximately fivefold more than singletons) (4). Appreciable numbers of surviving multiple neonates experience long-term consequences of prematurity or low birth weight, including cerebral palsy and learning disabilities.

Since maternal and neonatal risks might be even higher for patients who have exhibited a poor obstetrical history, these patients require careful planning and consideration when ART therapy is required. In this report, an elective single embryo transfer was undertaken in a high-risk patient utilizing sequential culture media and selection of the highest quality embryo at

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the blastocyst stage. This resulted in a successful singleton gestation and delivery of a healthy term infant.

CASE REPORT

A 29-year-old woman, gravida 4, para 1-2-1-1, presented with a 12-year history of secondary infertility. She had conceived spontaneously in 1987 and proceeded to a term delivery of a female infant weighing 3010 g. Following delivery, she began to experience irregular menstrual cycles. She sought infertility treatment after attempting to conceive for 4 years. Initially, she was noted to fail to respond to clomiphene citrate. She was then treated with gonadotropin therapy and conceived during her third cycle of treatment. This pregnancy was complicated by an ongoing quadruplet gestation. Despite placement of a cervical cerclage following an episode of cervical dilatation, she proceeded to deliver during the 5th month of gestation. None of the fetuses survived. Following this, she again conceived with gonadotropin therapy. This latter pregnancy was complicated by severe ovarian hyperstimulation syndrome (requiring paracentesis) and an ongoing septuplet gestation. She experienced a spontaneous reduction of one fetus and selective reduction of two additional fetuses. Despite an elective repeat cervical cerclage, four previable infants were again delivered at 6 months gestation.

The patient then discontinued infertility treatment efforts for several years. She then experienced a spontaneous conception which resulted in a first trimester spontaneous abortion. Several months after this pregnancy loss, she presented for consideration of further infertility therapy.

Her past medical history was also notable for a 12-year history of refractory pseudotumor cerebri. Acetazolamide therapy and repeated lumbar punctures were unsuccessful in controlling this condition. She required repeated cerebrospinal fluid shunts (three lumboperitoneal and one ventriculoperitoneal shunt). Despite bilateral optic nerve sheath fenestration procedures, she had significant bilateral optic atrophy and very limited vision. In addition, she developed a mild Arnold-Chiari Type 1 malformation and a left sixth cranial nerve palsy.

Following careful consultation, including a detailed discussion of treatment options and potential risks, the patient elected to have in vitro fertilization with the specific intent of a single embryo transfer. Her treatment protocol consisted of a dual

suppression downregulation protocol consisting of 25 days of an oral contraceptive (Ortho Novum 1/35; Ortho-McNeil Pharmaceutical, Raritan, NJ) overlapped by 5 days of daily 1.0 mg subcutaneous injections of leuprolide acetate (Lupron; TAP Pharmaceuticals, Deerfield, IL). Leuprolide acetate therapy was continued at a dosage of 0.5 mg subcutaneously daily after confirmation of appropriate downregulation. Recombinant FSH (Follistim; Organon, West Orange, NJ) was started at an initial dose of 150 IU by subcutaneous injection daily and was reduced to 75 IU daily during the final 2 days of stimulation. Human chorionic gonadotropin (10,000 units) (Profasi; Serono Laboratories, Norwell, MA) was administered by intramuscular injection on stimulation Day 9 at which time serum estradiol concentration was 2201 pg/mL.

Transvaginal oocyte retrieval resulted in recovery of 15 oocytes. Twelve oocytes were mature (metaphase-II), and three oocytes were of intermediate maturity (metaphase-I). The 12 mature oocytes were coincubated for 3 h with a sperm concentration of 0.5×10^6 /mL. The oocytes were then transferred to 50 μ L droplets of human tubal fluid supplemented with 10% Synthetic Serum Substitute (Irvine Scientific; Santa Clara, CA) under mineral oil overlay. Approximately 18 h postinsemination, 10 oocytes revealing normal fertilization were transferred in groups to 100 μ L droplets of G 1.2 media (IVF Science Scandinavia, Gothenburg, Sweden) under mineral oil. On Day 3, the cleavage stage embryos were then transferred to 100 μ L droplets of G 2.2 media (IVF Science Scandinavia, Gothenburg, Sweden) under mineral oil. On Day 5, a single high-quality blastocyst was chosen for transfer, and four other blastocysts of sufficient quality were cryopreserved. The blastocyst chosen for transfer had the highest possible quality grade (1.0) used by our laboratory and was characterized by a large fully expanded blastocoele cavity with no vacuolization, trophoctoderm characterized by >50 regularly shaped cells, a distinct inner cell mass, and a thinning zona pellucida. A transcervical embryo transfer was performed atraumatically with a Marrs catheter (Cook Ob/Gyn, Spencer, IN).

Daily intramuscular injections of 50 mg of progesterone-in-oil were used for luteal support. Eleven days posttransfer, a serum β -hCG determination was positive. Three weeks later, a viable singleton intrauterine pregnancy was confirmed on ultrasound. At 14 weeks gestation, the patient had a cervical cerclage placed using 5 mm Mersilene tape

(Ethicon, Somerville, NJ). Her prenatal course was otherwise uneventful.

At 37 weeks gestation, she experienced a spontaneous rupture of amniotic fluid. The cervical cerclage was then removed and labor rapidly ensued. A healthy female infant, weighing 2850 g, was born by spontaneous vaginal delivery. There were no intrapartum or postpartum complications.

DISCUSSION

The iatrogenic generation of multiple gestations has complicated nearly all infertility treatments, including the assisted reproductive technologies. Since the birth of the first baby conceived through in vitro fertilization techniques in 1978, it became apparent that the implantation efficiency of transferred human concepti was relatively low (generally between 10 and 20%). This influenced the general practice of transfer of multiple embryos to improve the overall chances of pregnancy, albeit with the consequent appreciable risks of multiple gestations.

New developments have focused on methods of improved embryo selection, to limit the necessity of transferring multiple embryos. One approach has been to carefully evaluate morphologic features of the pronuclear stage oocyte at 16–18 h postinsemination. Scott and Smith developed a system of cumulative embryo score based on early first embryonic cleavage by 24–26 h postinsemination as well as morphologic features of the pronuclear oocyte (5). Patients with the most favorable embryos as determined by the highest mean cumulative scores demonstrated a 28% embryonic implantation rate. Tesarik and Greco developed a similar scheme based on morphologic features of the pronuclear oocyte (6). From studying pronuclear features present in cycles that resulted in 100% implantation cycles, they defined the normal range of pronuclear variability. In this report, clinical pregnancy was achieved in 50% of treatment cycles in which at least one pattern 0 embryo was transferred, but only in 9% of cycles in which pattern 1–5 embryos were transferred.

A popular method of embryo selection in assisted reproduction clinics worldwide has focused on cleavage stage morphologic characteristics, most commonly on the 3rd day of culture. Unfavorable features suggesting lowered implantation potential at this stage include slow rates of embryonic cleavage, high proportions of cellular fragmentation, and the presence of multinucleated blastomeres. Many early

trials of cleavage-stage embryo selection resulted in only modest improvements in implantation rates. Certain investigators, however, have recently described improved results. Van Royen *et al.* reported that the absence of multinucleated blastomeres, four or five blastomeres on Day 2, seven or more cells on Day 3, and $\leq 20\%$ anucleate fragments were features of a top quality embryo (7). In this series, transfers of two top quality embryos resulted in 63% ongoing pregnancy rates and 49% implantation rates. Similarly, Tiitinen *et al.* reported a 39.8% implantation rate obtained with four-cell embryos on Day 2 with $<10\%$ fragmentation rates (8). Using these methods, a randomized trial has been performed involving either single or double embryo transfers for patients who met eligibility criteria. This trial resulted in an encouraging ongoing pregnancy rate in the elective single embryo transfer group (38.5%) (9).

A further method to select embryos of high implantation potential is the use of extended in vitro culture. The lack of appropriate culture media and the generally poor efficiency of human conceptuses progressing to advanced stages (i.e., morula or blastocyst) previously discouraged most clinics from attempting to extend in vitro culture. Recently, however, new culture media has become available that has been specifically designed with the presumed changing needs of the developing human embryo. In a randomized trial of selected patients who met certain stimulation characteristics, Gardner *et al.* reported that the implantation rate of blastocysts (50.5%) was significantly higher compared to cleavage stage embryos transferred on Day 3 (30.1%) utilizing a sequential extended culture approach (10). Some of the reasons proposed for the high efficiency of blastocyst transfer include better embryonic–endometrial synchronization, better nutritional support for the cleavage stage embryo, and assessment of embryos after the activation of the embryonic genome. Subsequently, transfer of two blastocysts has been noted to result in similar pregnancy rates, although with reduced multiple gestation rates compared with three blastocyst transfers (11). Currently, there is very little available information on the elective use of single blastocyst transfer using new extended culture methods. In a recent review, it is stated that “to date no published data on elective transfer of one blastocyst are available although this is obviously only a matter of time” (12).

Single blastocyst transfer offered numerous advantages for our patient. She was a known adequate responder to gonadotropin therapy. She was therefore predicted to produce a sufficient number of oocytes in

order to make extended culture and blastocyst transfer feasible. One uncertainty with this approach is that the number of fertilized oocytes which progress to the blastocyst stage is quite variable. Patients need to be prepared for the possibility of having no embryos available for transfer on Day 5 or 6. Not only did this patient have several embryos progress to the blastocyst stage, several blastocysts were available allowing selection of a single blastocyst with optimal morphologic features. Investigators have reported a high correlation with favorable blastocyst morphologic features and implantation (13). Since this patient had a profoundly poor obstetrical history (loss of 11 consecutive previable fetuses), it was felt that even a twin gestation might place her at significant risk. A single blastocyst transfer nearly eliminated this risk, although the low possibility of monozygotic twinning remained. Indeed, some investigators have reported that the risk of monozygotic twinning might even be increased with extended culture and blastocyst transfer (14).

In conclusion, we report the successful use of extended culture and single blastocyst transfer in a patient with a profoundly poor obstetrical history resulting in a singleton gestation and healthy term infant. These methods may therefore allow safe and effective treatment of similar high-risk infertile patients desiring pregnancy.

REFERENCES

1. Society for Assisted Reproductive Technology and American Society for Reproductive Medicine: Assisted reproductive technology in the United States: 1997 results generated from the American Society for Reproductive Medicine/Society for Assisted Reproductive Technology Registry. *Fertil Steril* 2000;74:641-653
2. Senat MV, Ancel PY, Bouvier-Colle MH, Bréart G: How does multiple pregnancy affect maternal mortality and morbidity? *Clin Obstet Gynecol* 1998;41:79-83
3. The ESHRE Capri Workshop Group: Multiple gestation pregnancy. *Hum Reprod* 2000;15:1856-1864
4. Schieve LA, Peterson HB, Meikle SF, Jeng G, Danel I, Burnett NM, Wilcox LS: Live-birth rates and multiple-birth risks using in vitro fertilization. *JAMA* 1999;282:1832-1838
5. Scott LA, Smith S: The successful use of pronuclear embryo transfers the day following oocyte retrieval. *Hum Reprod* 1998;13:1003-1013
6. Greco E, Tesarik J: The probability of abnormal preimplantation development can be predicted by a single static observation on pronuclear stage morphology. *Hum Reprod* 1999;14:1318-1323
7. Van Royen E, Mangelschots K, De Neubourg D, Valkenburg M, Van de Meerssche M, Ryckaert G, Eestermans W, Gerris J: Characterization of a top quality embryo, a step towards single-embryo transfer. *Hum Reprod* 1999;14:2345-2349
8. Tiitinen A, Halttunen M, Härkki P, Vuoristo P, Hyden-Granskog: Elective single embryo transfer: The value of cryopreservation. *Hum Reprod* 2001;16:1140-1144
9. Gerris J, De Neubourg D, Mangelschots K, Van Royen E, Van de Meerssche M, Valkenburg M: Prevention of twin pregnancy after in-vitro fertilization or intracytoplasmic sperm injection based on strict embryo criteria: A prospective randomized clinical trial. *Hum Reprod* 1999;14:2581-2587
10. Gardner DK, Schoolcraft WB, Wagley L, Schlenker T, Stevens J, Hesla J: A prospective randomized trial of blastocyst culture and transfer in in-vitro fertilization. *Hum Reprod* 1998;13:3434-3440
11. Milki AA, Fisch JD, Behr B: Two-blastocyst transfer has similar pregnancy rates and a decreased multiple gestation rate compared with three-blastocyst transfer. *Fertil Steril* 1999;72:225-228
12. ESHRE Campus Course Report: Prevention of twin pregnancies after IVF/ICSI by single embryo transfer. *Hum Reprod* 2001;16:790-800
13. Gardner DK, Lane M, Stevens J, Schlenker T, Schoolcraft WB: Blastocyst score affects implantation and pregnancy outcome: Towards a single blastocyst transfer. *Fertil Steril* 2000;73:1155-1158
14. Behr B, Fisch JD, Racowsky C, Miller K, Pool TB, Milki AA: Blastocyst-ET and monozygotic twinning. *J Assist Reprod Genet* 2000;17:349-351