In vitro fertilization with single euploid blastocyst transfer: a randomized controlled trial

Eric J. Forman, M.D.,[a,b] Kathleen H. Hong, M.D.,[a,b] Kathleen M. Ferry, B.Sc.,[a] Xin Tao, M.Sc.,[a] Deanne Taylor, Ph.D.,[a] Brynn Levy, Ph.D.,[a,c] Nathan R. Treff, Ph.D.,[a,b] and Richard T. Scott Jr., M.D.[a,b]

[a] Reproductive Medicine Associates of New Jersey, Department of Reproductive Endocrinology, Basking Ridge, New Jersey; [b] UMDNJ-Robert Wood Johnson Medical School, Department of Obstetrics, Gynecology & Reproductive Sciences, New Brunswick, New Jersey; and [c] Department of Pathology, Columbia University, College of Physicians and Surgeons, New York, New York

Objective: To determine whether performing comprehensive chromosome screening (CCS) and transferring a single euploid blastocyst can result in an ongoing pregnancy rate that is equivalent to transferring two untested blastocysts while reducing the risk of multiple gestation.
Design: Randomized, noninferiority trial.
Setting: Academic center for reproductive medicine.
Patient(s): Infertile couples (n = 205) with a female partner less than 43 years old having a serum anti-Müllerian hormone level ≥1.2 ng/mL and day 3 FSH <12 IU/L.
Intervention(s): Randomization occurred when at least two blastocysts were suitable for trophectoderm biopsy. The study group (n = 89) had all viable blastocysts biopsied for real-time, polymerase chain reaction–based CCS and single euploid blastocyst transfer. The control group (n = 86) had their two best-quality, untested blastocysts transferred.
Main Outcome Measure(s): The ongoing pregnancy rate to ≥24 weeks (primary outcome) and the multiple gestation rate.
Result(s): The ongoing pregnancy rate per randomized patient after the first ET was similar between groups (60.7% after single euploid blastocyst transfer vs. 65.1% after untested two-blastocyst transfer; relative risk [RR], 0.9; 95% confidence interval [CI], 0.7–1.2). A difference of greater than 20% in favor of two-blastocyst transfer was excluded. The risk of multiple gestation was reduced after single euploid blastocyst transfer (53.4% to 0%), and patients were nearly twice as likely to have an ongoing singleton pregnancy (60.7% vs. 33.7%; RR, 1.8; 95% CI, 1.3–2.5).
Conclusion(s): In women ≤42 years old, transferring a single euploid blastocyst results in ongoing pregnancy rates that are the same as transferring two untested blastocysts while dramatically reducing the risk of twins.
Clinical Trial Registration Number: NCT01408433. (Fertil Steril® 2013;100:100–7. ©2013 by American Society for Reproductive Medicine.)
Key Words: Single embryo transfer, eSET, preimplantation genetic screening, comprehensive chromosome screening, aneuploidy

Discuss: You can discuss this article with its authors and with other ASRM members at http://fertstertforum.com/formanej-ivf-aneuploidy-single-embryo-transfer/

Use your smartphone to scan this QR code and connect to the discussion forum for this article now.*

* Download a free QR code scanner by searching for "QR scanner" in your smartphone's app store or app marketplace.

Received January 2, 2013; revised January 30, 2013; accepted February 25, 2013; published online March 30, 2013.
E.J.F. reports a grant from Ferring Pharmaceuticals, which provided gonadotropin injections and funding for all other study medications. K.H.H. has nothing to disclose. K.M.F. has nothing to disclose. X.T. has nothing to disclose. D.T. has nothing to disclose. B.L. reports a grant from the National Institute of Child Health and Human Development; consultancies with RMA Genetics, Nater, and Celula; payment for lectures by Affymetrix and Agilent; stock in Natera; and travel expenses paid by Affymetrix and Agilent. N.R.T. reports payment for lectures by American Society for Reproductive Medicine (ASRM), Japanese Society for Assisted Reproduction, Penn State University, Washington State University, Mayo Clinic, Applied Biosystems, Inc., Texas ART Society, and American Asscoiation of Bioanalysts; payment for development of educational presentations by ASRM; and patents pending. R.T.S. reports a grant from Ferring Pharmaceuticals, which provided gonadotropin injections and funding for all other study medications; that he is on the advisory boards of EMD Serono and Ferring Pharmaceuticals; and that he received payment for lectures and travel expenses from Merck.
Funding for all study medications provided by Ferring Pharmaceuticals.
Reprint requests: Eric J. Forman, M.D., Reproductive Medicine Associates of New Jersey, 140 Allen Road, Basking Ridge, New Jersey 07920 (E-mail: eforman@rmanj.com).

Fertility and Sterility® Vol. 100, No. 1, July 2013 0015-0282

http://dx.doi.org/10.1016/j.fertnstert.2013.02.056

Infertile couples undergoing an IVF cycle face enormous emotional, physical, and financial burdens. In most cases they have struggled with infertility for more than a year and failed to conceive with lesser means. Since the majority of IVF cycles in the United States are uninsured, patients have an incentive to maximize their chance of delivery in a given cycle to reduce the financial burden they must shoulder (1). When seeking care, patients often compare clinics based on their reported delivery rates per cycle. These

incentives have resulted in a preference among patients and clinicians to transfer multiple embryos, increasing the risk of twin and higher order pregnancies, which are at higher risk of maternal and neonatal complications than singleton pregnancies (2, 3). The societal cost of caring for preterm neonates resulting from multiple pregnancies far outweighs the couple's savings from preventing failed cycles (4, 5). Thus, as currently practiced, the infertile couple often chooses a transfer strategy that minimizes its own financial burden while assuming increased medical risk and adding to overall health care costs.

Multiple gestations is almost entirely preventable with the transfer of a single embryo after each IVF cycle. However, despite recommendations to consider elective single ET (eSET) for young, good-prognosis patients (6, 7), this technique is used in only 4% of cycles and 47% of all babies born as a result of IVF performed in the United States are part of a multiple birth (8).

The reluctance to accept eSET likely stems from the diminished chance for a delivery when only a single embryo is transferred (9, 10), reflecting the inadequacy of current methods of embryo selection. Embryonic aneuploidy (chromosomal imbalance) is the leading cause of implantation failure and miscarriage after IVF and increases dramatically with increasing maternal age (11–13). With eSET, the entire cycle outcome is dependent on the fate of the selected embryo; if an aneuploid embryo is selected, there is essentially no chance for delivery of a healthy newborn.

As currently practiced, the embryo chosen for transfer is selected based on temporal and morphologic grading criteria related to its appearance under the microscope. Embryo grading has significant inter- and intraobserver variability and has not been shown to correlate with chromosomal status (14–16). Extending embryo culture to the blastocyst stage was shown to improve outcomes from eSET (17), but morphologically normal blastocysts retain a significant risk of aneuploidy (18, 19).

Another selection strategy would be to culture all embryos to the blastocyst stage, perform a trophectoderm biopsy, use an assay to rapidly analyze the copy number status of each chromosome, and then transfer a single euploid blastocyst (20). This strategy was shown to improve singleton delivery rates in a retrospective study (18) and a small prospective study in young, good-prognosis patients (21).

The goal of the current trial was to determine whether infertile couples would have as good a chance for an ongoing pregnancy after transfer of a single euploid blastocyst as they would with the transfer of two untested blastocysts. In addition, we hypothesized they would have a significantly reduced risk of twins after single euploid blastocyst transfer. Although previous studies have shown similar delivery rates when two separate single ETs are performed (22, 23), the ability to achieve equivalent outcomes with one transfer has the potential to make single ET a more acceptable option regardless of the patient's age (24).

MATERIALS AND METHODS

Patient Population

The Blastocyst Euploid Selective Transfer (BEST) Trial was offered to women with an indication for IVF who were $\leq$42 years old at the start of their IVF cycle. They were eligible to participate if they had at most one prior failed IVF cycle, a normal endometrial cavity, and normal ovarian reserve as determined by a serum anti-Müllerian hormone level of $\geq$1.2 ng/mL and a day 3 FSH level of $<$12 IU/L. Couples with severe male factor infertility requiring surgical sperm extraction were excluded as were women with anovulatory polycystic ovarian syndrome or a body mass index $>$30 kg/m^2. To be eligible for randomization, there had to be at least two expanded blastocysts suitable for transfer or cryopreservation by day 6 of embryo development.

Study Design

The protocol was approved by the Copernicus Group Independent Review Board (Durham, NC) and registered with clinicaltrials.gov (NCT01408433) before patient enrollment. All embryology procedures were performed at Reproductive Medicine Associates of New Jersey, and patients gave consent between August 2011 and June 2012.

The IVF cycle was performed per practice routine. Multifollicular ovarian stimulation was achieved with highly purified urinary gonadotropins: urofollitropin (Bravelle, Ferring Pharmaceuticals) and menotropins (Menopur, Ferring Pharmaceuticals). Final oocyte maturation was induced with 5,000 to 10,000 IU of hCG when at least two follicles reached 18 mm in maximal diameter. Patients determined to be at risk for ovarian hyperstimulation syndrome by their physician received 5,000 IU of hCG. Transvaginal oocyte retrieval was performed 36 hours after hCG administration. Fertilization was achieved with intracytoplasmic sperm injection in all cases owing to the possibility of preimplantation genetic testing. Normally fertilized zygotes were cultured in cleavage media in a low-oxygen tension environment. On day 3 of embryo development, all cleaved embryos underwent laser-assisted hatching of the zona pellucida to facilitate possible trophectoderm biopsy. All embryos were then placed in extended culture media regardless of the size or quality of the cohort.

Randomization occurred when at least two embryos had developed sufficiently to allow for blastocyst-stage biopsy. A random number function was used to create blocks of 10 with patients assigned to the two groups in a 1:1 allocation. In the study group, all viable blastocysts would undergo trophectoderm biopsy with rapid, real-time polymerase chain reaction (PCR) based comprehensive chromosome screening (CCS) and transfer of the single, best-quality euploid blastocyst. The control group would receive traditional, morphology-based transfer of two untested blastocysts. The randomization was stratified by age group ($<$35, 35–37, 38–40, 41–42 years) and whether fresh or frozen ET was to be performed. Allocation concealment was achieved using sequentially numbered, opaque, sealed envelopes. The study was not blinded—patients were informed at the time of transfer or cryopreservation whether they were to receive one or two blastocysts and the results of chromosome screening if it had been performed.

Embryos were graded on day 5 according to the Gardner criteria by assessing degree of expansion and the morphology of the inner cell mass and trophectoderm (25). Patients were

eligible for a fresh ET if there were two or more expanded blastocysts (expansion stage ≥3) by the afternoon of day 5 and they had no contraindications to receiving a transfer. All fresh transfers were performed in the morning of day 6, which is standard procedure for the practice.

Patients with fewer than two expanded blastocysts on day 5 were not eligible for fresh transfer owing to embryo-endometrial dyssychrony (26–28). Their embryos were reassessed and they were randomized if there were at least two expanded blastocysts by the afternoon of day 6. All viable blastocysts were cryopreserved by vitrification for use in a future frozen ET cycle. Patients with only one expanded blastocyst were not randomized and received a nonelective single blastocyst transfer in the fresh cycle if the embryo was expanded on day 5 or in a frozen transfer if it did not expand until day 6.

Some patients were not eligible for fresh ET owing to risk of ovarian hyperstimulation syndrome, abnormal endometrial proliferation (<7 mm thickness on ultrasound), premature P elevation (≥2 ng/mL before administration of hCG), or detection of hydrosalpinges that communicated with the endometrial cavity. These patients were randomized on day 6 to the two previously described groups in the frozen transfer arm of the study.

For patients randomized to fresh single euploid blastocyst transfer, all expanded blastocysts underwent laser-assisted trophectoderm biopsy late in the afternoon of day 5. The biopsy was processed for CCS by placing it in an alkaline lysis buffer and performing real-time PCR as described elsewhere (20). A copy number assignment was made for each chromosome using a modification of the comparative threshold cycle method (29). A predicted karyotype was assigned to each blastocyst by a certified cytogeneticist (Supplemental Fig. 1). Embryos with failed amplification or results that were nondiagnostic according to previously established thresholds were subject to repeat biopsy and analysis. The single, morphologically best euploid blastocyst was selected for ultrasound-guided transfer on the morning of day 6. All other euploid blastocysts were vitrified individually. Additional blastocysts that expanded by the afternoon of day 6 underwent trophectoderm biopsy and were vitrified.

For patients randomized to the control group, two blastocysts were selected by traditional morphologic grading criteria for transfer on the morning of day 6. All other viable blastocysts were vitrified in pairs. No embryos underwent trophectoderm biopsy.

In both groups, the luteal phase was supported with 100 mg of P (Endometrin, Ferring Pharmaceuticals) inserted vaginally 3 times daily until 8 weeks of gestation.

Patients randomized in the frozen transfer arm were immediately prepared for a synthetic cycle by starting oral E_2 supplementation on the third day of their menses. When the endometrial thickness reached at least 7 mm after at least 1 week of E_2 supplementation, IM P was initiated at a dose of 50 mg daily. Frozen ET was performed 5 days after starting P. Supplemental E_2 was continued until 8 weeks of gestation and P was continued until 10 weeks of gestation.

Pregnancy monitoring was per practice routine with patients receiving their first serum pregnancy test approximately 8 days after ET. If the initial hCG level was ≥5 IU/L, it was repeated 48 hours later. Those with normally rising levels had a transvaginal ultrasound performed 1 week later and weekly thereafter. Patients were discharged to an obstetrician at 9 weeks of gestation.

Calculation of Sample Size

Given that prior trials demonstrated single ET to be inferior to double ET, this study was designed as a noninferiority trial with a margin of 20% assuming a baseline ongoing pregnancy rate of 60% in each group. To be 80% certain that the upper limit of a one-sided 95% confidence interval (CI) would exclude a difference in favor of the two-blastocyst transfer control group by more than 20%, 75 patients were required in each group. Since it was estimated that approximately 25% of patients would not be randomized owing to insufficient blastocyst development, 200 cycles were planned.

Statistical Analysis

An ongoing pregnancy was a pregnancy that reached a viable gestational age of ≥24 weeks.

A clinical miscarriage was the loss of a pregnancy after visualization of a gestational sac. The sustained implantation rate was the number of gestational sacs with fetal cardiac activity at the time of discharge to obstetrical care divided by the number of embryos transferred. A multiple pregnancy had more than one fetus with cardiac activity at the time of discharge to obstetrical care.

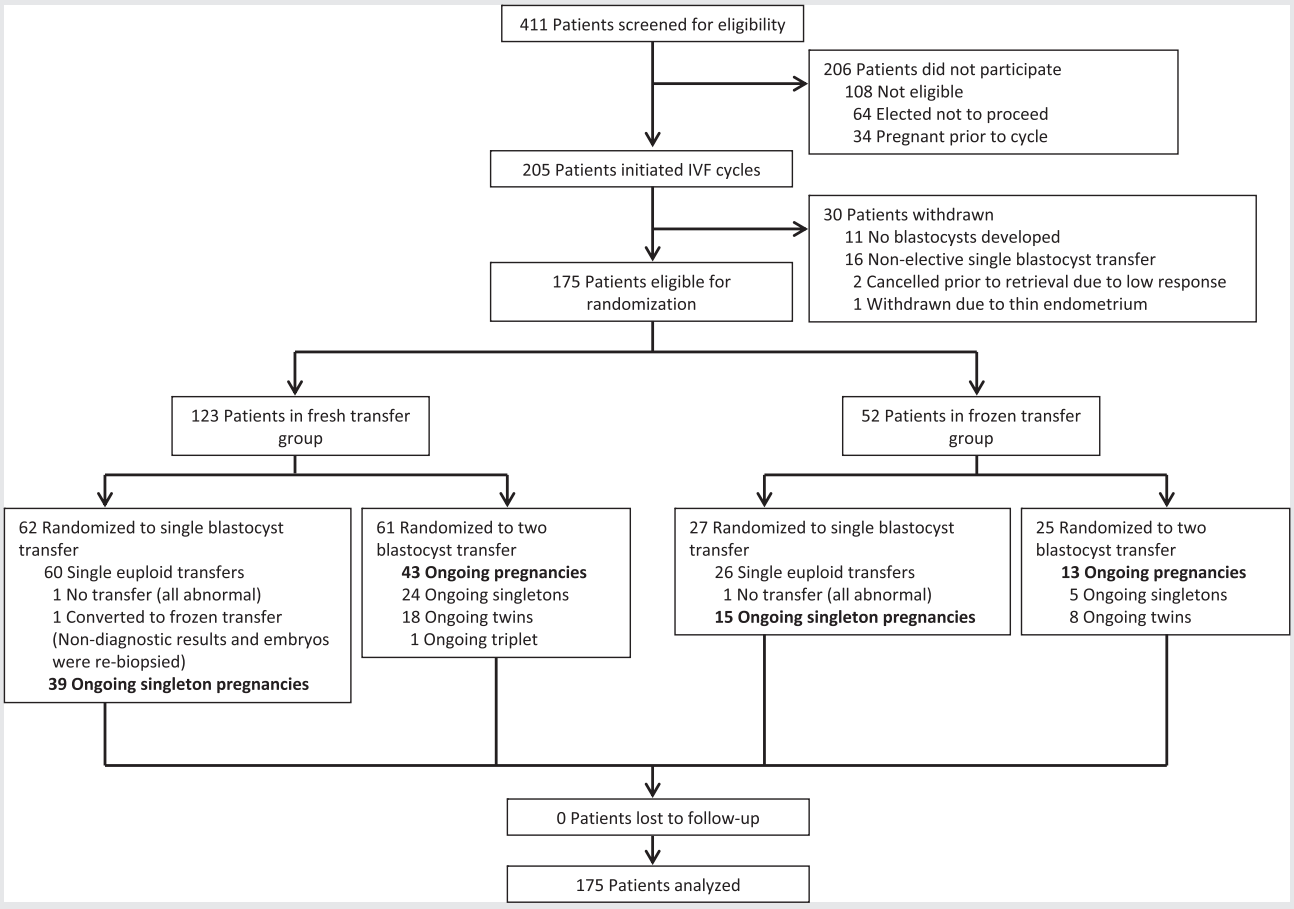
The primary outcome was the rate of ongoing pregnancy per patient randomized. The outcome of only the first transfer was included. Secondary outcomes included the multiple pregnancy rate, sustained implantation rate, and clinical miscarriage rate.

The ongoing pregnancy rates were compared by calculating 95% CIs of the relative risk and risk difference using OpenEpi Version 2.3.1. The rates of multiple pregnancy, implantation, and clinical miscarriage were compared using a χ^2 distribution; $P<.05$ was considered statistically significant. A t test or Mann-Whitney U–test was used for demographic data where appropriate. Comparisons were made using both intention-to-treat and per-protocol analyses. In the per-protocol analysis, patients were excluded if they did not adhere to clinical recommendations for luteal phase support or had their frozen ET cycle cancelled owing to abnormal endometrial development. Descriptive statistics are reported with mean ± SD and range.

RESULTS

A total of 205 patient initiated gonadotropin stimulation with 175 randomized and 30 withdrawn before randomization (Fig. 1). Of the 175 randomized patients, 76 (43.4%) were <35 years old, 54 (30.9%) were 35–37 years old, 34 (19.4%) were 38–40 years old, and 11 (6.3%) were 41–42 years old. Sixty-nine percent of ETs were performed in the fresh cycle. One patient did not have a fresh transfer owing to nondiagnostic CCS results; her blastocysts were rebiopsied and cryopreserved. Two patients (both 40 years old) did not have

FIGURE 1



Flow chart showing enrollment and status of study cycles.

Forman. Blastocyst Euploid Selective Transfer (BEST) Trial. Fertil Steril 2013.

a transfer as their blastocysts were all aneuploid. The demographics of the patients in each group were similar (Table 1).

Intention-to-Treat Analysis

CCS results. The 89 patients randomized to single euploid blastocyst transfer had a total of 521 blastocysts of sufficient quality to biopsy. After the initial biopsy, 2% of embryos did not yield a diagnostic result. Each embryo was rebiopsied and a reliable result was obtained. The overall rate of aneuploidy was 31% (162/521). As expected, there was an association between increasing aneuploidy and maternal age ($r = 0.53$, $P<.001$). Sixteen aneuploid blastocysts with the ability to result in viable pregnancies (four trisomy 13; five trisomy 18; six trisomy 21; one 45,X; one 47,XXY; and one 47,XYY) were detected and excluded from transfer, perhaps preventing anomalous pregnancies.

Clinical outcomes. The clinical performance in the IVF cycle was similar between groups (Table 2; Fig. 2). The ongoing pregnancy rate after each patient's first transfer, whether fresh or frozen, was 60.7% (54/89) after single euploid blastocyst transfer and 65.1% (56/86) after untested two-blastocyst transfer. The 95% CI of the difference in ongoing pregnancy rate was −18.7% to 9.9%, indicating that single euploid blastocyst transfer is not inferior to two-blastocyst transfer. The ongoing pregnancy rates after single euploid blastocyst transfer and untested two-blastocyst transfer were similar in fresh transfers (63.9% [39/61] vs. 70.5% [43/61]; $P=.4$) and frozen transfers (53.6% [15/28] vs. 52.0% [13/25]; $P=.9$).

The multiple pregnancy rate at the time of discharge to obstetrical care was significantly higher in the two-blastocyst transfer group, (53.4% [31/58] to 0% [0/57]; $P<.001$). Two patients with twin pregnancies delivered at a nonviable gestational age. One patient in the two-blastocyst transfer group had a preterm triplet delivery owing to monozygotic twinning of one embryo. There were no monozygotic twins after single euploid blastocyst transfer.

Importantly, patients who received single euploid blastocyst transfer were nearly twice as likely to have an ongoing singleton pregnancy as those with two-blastocyst transfer (60.7% [54/89] vs. 33.7% [29/86]; relative risk, 1.8; 95% CI, 1.3–2.5; $P<.001$).

There was a trend toward a higher sustained implantation rate after single euploid blastocyst transfer (63.2% [55/87] vs.

TABLE 1

Characteristics of the patients.

Characteristic	Single euploid blastocyst transfer (n = 89)	Double blastocyst transfer (n = 86)	*P* value
Age at oocyte retrieval, y			
Mean ± SD	35.1 ± 3.9	34.5 ± 4.7	.5
Range	25.1–41.4	22.9–42.6	
Body mass index, kg/m^2			
Mean ± SD	23.5 ± 3.5	23.8 ± 3.2	.4
Range	17.6–30.2	18.0–30.8	
Anti-Müllerian hormone level, ng/mL			
Mean ± SD	3.5 ± 2.4	4.0 ± 3.5	.2
Range	1.2–18.0	1.2–22.0	
Day 3 FSH, IU/L			
Mean ± SD	6.9 ± 1.8	6.6 ± 1.7	.2
Range	3.2–11.6	2.8–10.8	
Primary cause of infertility, n (%)			
Male factor	25 (28.1)	22 (25.6)	.9
Unexplained	24 (27.0)	24 (27.9)	
Ovulatory dysfunction	12 (13.5)	17 (19.8)	
Tubal factor	11 (12.4)	13 (15.1)	
Endometriosis	6 (6.7)	4 (4.7)	
Other	11 (12.4)	6 (7.0)	
History of previous pregnancies, n (%)	47 (52.8)	42 (48.8)	.6
Live birth	23 (25.8)	22 (25.6)	
Clinical miscarriage	25 (28.1)	14 (16.3)	
Termination of pregnancy	11 (12.4)	9 (10.5)	
History of prior treatment with IVF, n (%)	18 (20.2)	16 (18.6)	.8
Live birth	8 (9.0)	4 (4.7)	

Forman. Blastocyst Euploid Selective Transfer (BEST) Trial. Fertil Steril 2013.

51.7% [89/172]; $P=.08$). Among patients ≥35 years old, who are not routinely offered eSET, the sustained implantation rate was higher in the single euploid blastocyst transfer group (58.3% [28/48] vs. 39.8% [39/98]; $P=.03$). This improvement in the implantation rate likely relates to the higher probability of the CCS result overriding traditional embryo morphology

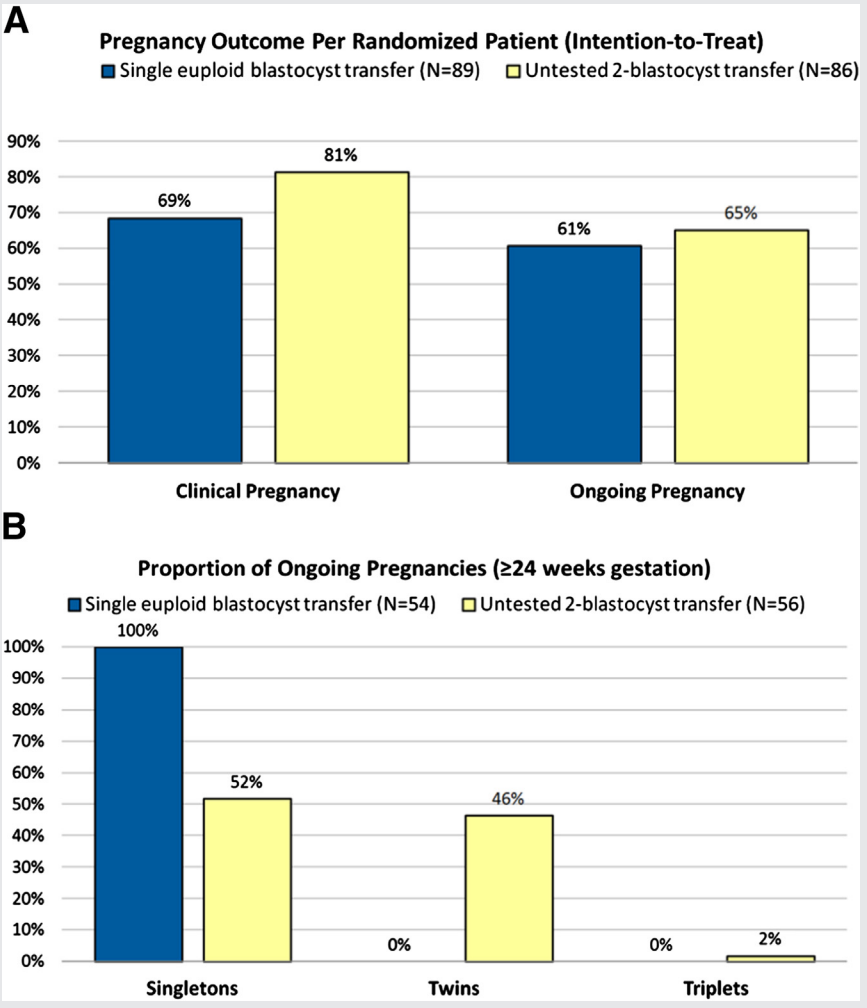
TABLE 2

Outcomes according to treatment group (intention-to-treat analysis).

Outcome	Single euploid blastocyst transfer (n = 89)	Double blastocyst transfer (n = 86)	*P* value
Total dose of gonadotropins, ampules			
Mean ± SD	37.8 ± 12.9	37.0 ± 13.6	.7
Range	15.5–72	14.5–81	
E_2, pg/mL, at surge			
Mean ± SD	2,437 ± 1,212	2,540 ± 1,236	.6
Range	513–6,267	605–6,000	
Retrieved oocytes			
Mean ± SD	16.9 ± 8.4	15.7 ± 7.1	.6
Range	5–45	3–42	
Fertilized oocytes (two pronuclei)			
Mean ± SD	11.1 ± 5.9	10.8 ± 5.7	.9
Range	4–30	3–33	
High-quality blastocysts			
Mean ± SD	5.8 ± 3.6	5.3 ± 3.0	.5
Range	2–22	2–18	
Vitrified blastocysts			
Mean ± SD	3.7 ± 2.9	3.9 ± 2.8	.5
Range	0–17	0–16	
Vitrified euploid blastocysts			
Mean ± SD	3.2 ± 2.8	NA	
Range	0–17	NA	
Patients who received fresh ET (%)	60 (67)	61 (71)	.6
Patients who received frozen embryo transfer due to			
Embryo-endometrial dyssynchrony	22	20	
Ovarian hyperstimulation syndrome risk	5	3	
Hydrosalpinx	0	2	
Nondiagnostic result of embryo biopsy	1	0	

Forman. Blastocyst Euploid Selective Transfer (BEST) Trial. Fertil Steril 2013.

FIGURE 2



Equivalent ongoing pregnancy rates with significantly fewer multiples after single euploid blastocyst transfer. (A) In the intention-to-treat analysis, the ongoing pregnancy rate (≥24 weeks gestation) after single euploid blastocyst transfer was not inferior to the rate after transferring two untested blastocysts. (B) In the single euploid blastocyst transfer group, all ongoing pregnancies were singletons. After accounting for second trimester losses and vanishing twins, multiples accounted for 48% of the ongoing pregnancies after untested two-blastocyst transfer.

Forman. Blastocyst Euploid Selective Transfer (BEST) Trial. Fertil Steril 2013.

for selection. The clinical miscarriage rate was lower after single euploid blastocyst transfer, but the difference was not significant (11.5% [7/61] vs. 20.0% [14/70]; $P=.2$).

Although patients in the single euploid blastocyst transfer group had aneuploid blastocysts discarded, they also had fewer embryos transferred. The overall effect resulted in an equivalent number of supernumerary vitrified blastocysts in each group after their first transfer ($P=.3$); however, patients in the study group had the added assurance that their vitrified blastocysts were all euploid. After their first single euploid blastocyst transfer, 81% of patients had at least one additional euploid blastocyst cryopreserved for possible future transfer.

Per-Protocol Analysis

Among patients who followed the study protocol (85 in each group), the ongoing pregnancy rate was 63.5% (54/85) after single euploid blastocyst transfer and 64.7% (55/85) after two-blastocyst transfer. The 95% CI of the difference in ongoing pregnancy rates was −14.8% to +14.1%. The sustained implantation rate was significantly higher in the single euploid blastocyst transfer group (66.3% [55/83] vs. 51.2% [87/170]; $P=.02$).

DISCUSSION

The BEST Trial has demonstrated that altering embryo selection by choosing a single, chromosomally normal blastocyst for transfer results in ongoing pregnancy rates that are not inferior to those attained when transferring two blastocysts with an unknown chromosomal complement. Given the reduction in the proportion of multiple pregnancies, the long-term outcomes from this treatment paradigm are likely to be safer, resulting in fewer maternal and neonatal complications. Twins have a significantly higher risk of costly neonatal intensive care unit stays, resulting in estimates that twin

deliveries cost up to 5 times more than singleton deliveries (30). By reducing the risk of preterm deliveries, the BEST treatment strategy is likely to be cost effective, even accounting for the additional cost of embryo biopsy and CCS. Importantly, while single ET has been promoted for good-prognosis patients, even patients ≥40 years old are at a significantly increased risk of multiple birth when more than one embryo is transferred (31). The current trial demonstrates that it is feasible to consider women up to age 43 for single ET, provided they can produce a euploid blastocyst.

The concept of embryo selection has recently been criticized in favor of sequentially transferring individual unscreened, vitrified embryos (32). However, the results of the BEST Trial show that by enhancing embryo selection for the first transfer, excellent ongoing pregnancy rates can be achieved across age groups, while nearly eliminating the risk of multiple gestation. A prior retrospective study showed that fewer patients had clinical miscarriages requiring surgical intervention after single euploid blastocyst transfer (18). The ability to prevent aneuploid pregnancies allows many patients to avoid the physical and emotional burden of miscarriage and is arguably a more patient-friendly approach. Consistent with prior studies, aneuploidy is not the sole cause of miscarriage, and this selection strategy cannot completely eliminate miscarriages.

Although there is concern that a strategy of blastocyst culture may result in higher cycle cancellation rates (33), the current study demonstrates that, using even a modest ovarian reserve requirement, only 5.4% percent of patients did not produce a transferable blastocyst. Furthermore, 86.2% of patients had at least two blastocysts and were randomized, including 76.3% (45/59) who were ≥38 years old. Only two patients were cancelled due to aneuploidy screening. Although young patients with diminished ovarian reserve were not included, they too are likely to benefit from a reduced risk of multiple gestation should they produce two euploid blastocysts in a given cycle.

Earlier attempts to improve IVF outcomes with aneuploidy screening did not show benefit in prospective trials (34). Those studies were conducted using fluorescence in situ hybridization (FISH), a technology whose accuracy was not properly validated in embryos. Research using more robust technologies such as single nucleotide polymorphism microarrays demonstrated that FISH overdiagnoses aneuploidy in some embryos and underdiagnoses aneuploidy for chromosomes that are not probed (35, 36). The stage of embryo biopsy may have also hampered prior attempts to apply aneuploidy screening clinically. Recent evidence suggests that blastocyst-stage trophectoderm biopsy, which involves removal of extraembryonic cells destined to form the placenta and membranes, may be safer and more accurate than cleavage-stage blastomere biopsy (37, 38). The screening technology used in this trial underwent extensive validation to document its accuracy in preclinical trials (20) and to demonstrate that embryos diagnosed as aneuploid possess negligible reproductive potential (11).

Many infertile couples initially report a desire to have twins (39). At first glance, this is understandable given their long struggle with childlessness. However, multiple gestation has inherent risks that all patients want to avoid, including preeclampsia, obstetrical hemorrhage, neonatal intensive care unit stays, and increased neonatal and maternal mortality. The obstetrical complications often lead to recommended bed rest and lost time from work, and there may be an increased risk of divorce among parents of twins (40). After receiving proper counseling about the risks of twins, many patients prefer singleton over twin pregnancies (39).

A recent meta-analysis showed that slightly more singletons were born after double ET (29.5%) than after single ET (25.9%) when the same selection criteria were used in both groups (9). To increase the number of singleton deliveries, which are considered the ideal outcome of IVF (41), an enhanced method of embryo selection that results in higher implantation rates is required. The results of the BEST Trial demonstrate that with a validated method for assessing the chromosomal status of blastocysts, singleton delivery rates can be improved without compromising overall success rates. This treatment paradigm has the potential to improve the efficacy and safety of assisted reproductive technologies with resulting cost savings by preventing twins and higher order multiples. In so doing, the infertile couple's interest in maximizing success rates and minimizing the burden of treatment would dovetail with the need to improve safety and minimize overall health care costs.

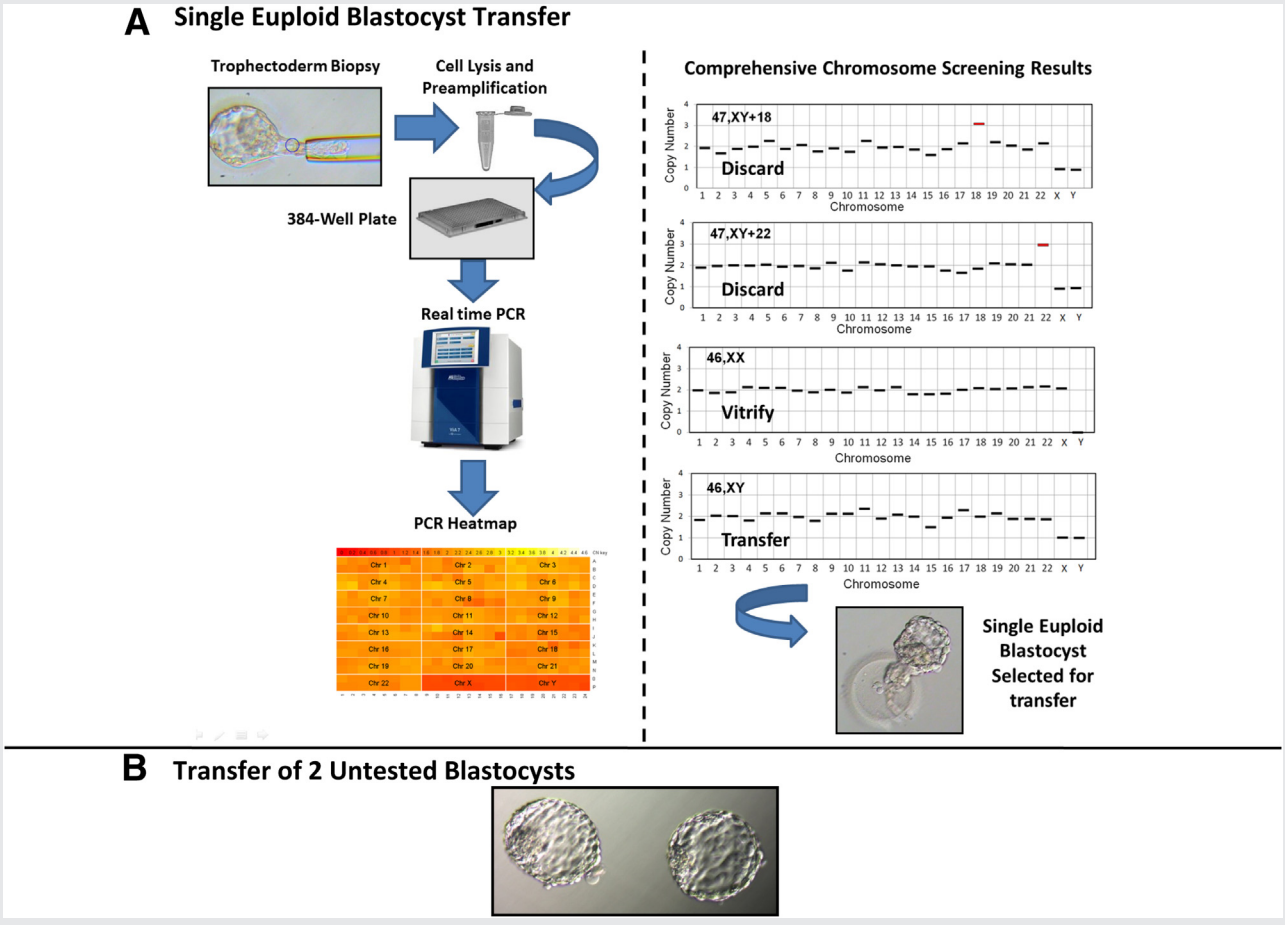
Acknowledgments: All medications used in this trial were provided with the assistance of a grant from Ferring Pharmaceuticals. Ferring had no role in the design of the study, data collection, or preparation of the manuscript. The authors would like to acknowledge Monica Benson, Stephanie Sgambati, and the Clinical Research Team at Reproductive Medicine Associates of New Jersey for recruiting patients and coordinating their cycles. The authors also acknowledge Heather Garnsey and the team of RMA Genetics for coordinating CCS data.

REFERENCES

1. Stillman RJ, Richter KS, Banks NK, Graham JR. Elective single embryo transfer: a 6-year progressive implementation of 784 single blastocyst transfers and the influence of payment method on patient choice. Fertil Steril 2009; 92:1895–906.
2. American College of Obstetricians and Gynecologists Committee on Practice Bulletins-Obstetrics. ACOG Practice Bulletin #56: Multiple gestation: complicated twin, triplet, and high-order multifetal pregnancy. Obstet Gynecol 2004;104:869–83.
3. Reddy UM, Wapner RJ, Rebar RW, Tasca RJ. Infertility, assisted reproductive technology, and adverse pregnancy outcomes: executive summary of a National Institute of Child Health and Human Development workshop. Obstet Gynecol 2007;109:967–77.
4. Callahan TL, Hall JE, Ettner SL, Christiansen CL, Greene MF, Crowley WF Jr. The economic impact of multiple-gestation pregnancies and the contribution of assisted-reproduction techniques to their incidence. N Engl J Med 1994;331:244–9.
5. Collins J. Cost efficiency of reducing multiple births. Reproductive biomedicine online 2007;15(Suppl 3):35–9.
6. Practice Committee of the Society for Assisted Reproductive Technology and Practice Committee of the American Society for Reproductive Medicine. Guidelines on number of embryos transferred. Fertil Steril 2009;92:1518–9.
7. Practice Committee of Society for Assisted Reproductive Technology and Practice Committee of American Society for Reproductive Medicine. Elective single-embryo transfer. Fertil Steril 2012;97:835–42.

8. Sunderam S, Kissin DM, Flowers L, Anderson JE, Folger SG, Jamieson DJ, et al. Assisted reproductive technology surveillance—United States, 2009. MMWR Surveill Summ 2012;61:1–23.
9. Pandian Z, Bhattacharya S, Ozturk O, Serour G, Templeton A. Number of embryos for transfer following in-vitro fertilisation or intra-cytoplasmic sperm injection. Cochrane database of systematic reviews (Online) 2009; 2:CD003416.
10. Gelbaya TA, Tsoumpou I, Nardo LG. The likelihood of live birth and multiple birth after single versus double embryo transfer at the cleavage stage: a systematic review and meta-analysis. Fertil Steril 2010;94:936–45.
11. Scott RT Jr, Ferry K, Su J, Tao X, Scott K, Treff NR. Comprehensive chromosome screening is highly predictive of the reproductive potential of human embryos: a prospective, blinded, nonselection study. Fertil Steril 2012;97: 870–5.
12. Nasseri A, Mukherjee T, Grifo JA, Noyes N, Krey L, Copperman AB. Elevated day 3 serum follicle stimulating hormone and/or estradiol may predict fetal aneuploidy. Fertil Steril 1999;71:715–8.
13. Werner M, Reh A, Grifo J, Perle MA. Characteristics of chromosomal abnormalities diagnosed after spontaneous abortions in an infertile population. J Assist Reprod Genet 2012;29:817–20.
14. Baxter Bendus AE, Mayer JF, Shipley SK, Catherino WH. Interobserver and intraobserver variation in day 3 embryo grading. Fertil Steril 2006;86: 1608–15.
15. Paternot G, Devroe J, Debrock S, D'Hooghe TM, Spiessens C. Intra- and inter-observer analysis in the morphological assessment of early-stage embryos. Reprod Biol Endocrinol 2009;7:105.
16. Paternot G, Wetzels AM, Thonon F, Vansteenbrugge A, Willemen D, Devroe J, et al. Intra- and interobserver analysis in the morphological assessment of early stage embryos during an IVF procedure: a multicentre study. Reprod Biol Endocrinol 2011;9:127.
17. Papanikolaou EG, Camus M, Kolibianakis EM, Van Landuyt L, Van Steirteghem A, Devroey P. In vitro fertilization with single blastocyst-stage versus single cleavage-stage embryos. N Engl J Med 2006;354:1139–46.
18. Forman EJ, Tao X, Ferry KM, Taylor D, Treff NR, Scott RT Jr. Single embryo transfer with comprehensive chromosome screening results in improved ongoing pregnancy rates and decreased miscarriage rates. Hum Reprod 2012; 27:1217–22.
19. Fragouli E, Wells D. Aneuploidy in the human blastocyst. Cytogenet Genome Res 2011;133:149–59.
20. Treff NR, Tao X, Ferry KM, Su J, Taylor D, Scott RT Jr. Development and validation of an accurate quantitative real-time polymerase chain reaction-based assay for human blastocyst comprehensive chromosomal aneuploidy screening. Fertil Steril 2012;97:819–24.
21. Yang Z, Liu J, Collins GS, Salem SA, Liu X, Lyle SS, et al. Selection of single blastocysts for fresh transfer via standard morphology assessment alone and with array CGH for good prognosis IVF patients: results from a randomized pilot study. Mol Cytogenet 2012;5:24.
22. Thurin A, Hausken J, Hillensjo T, Jablonowska B, Pinborg A, Strandell A, et al. Elective single-embryo transfer versus double-embryo transfer in in vitro fertilization. N Engl J Med 2004;351:2392–402.
23. Lukassen HG, Braat DD, Wetzels AM, Zielhuis GA, Adang EM, Scheenjes E, et al. Two cycles with single embryo transfer versus one cycle with double embryo transfer: a randomized controlled trial. Hum Reprod 2005;20: 702–8.
24. Murray S, Shetty A, Rattray A, Taylor V, Bhattacharya S. A randomized comparison of alternative methods of information provision on the acceptability of elective single embryo transfer. Hum Reprod 2004;19:911–6.
25. Gardner DK, Schoolcraft WB. In vitro culture of human blastocysts. In: Jansen R, Mortimer D, editors. Towards reproductive certainty: infertility and genetics beyond. Carnforth: Parthenon Press; 1999:378–88.
26. Shapiro BS, Richter KS, Harris DC, Daneshmand ST. A comparison of day 5 and day 6 blastocyst transfers. Fertil Steril 2001;75:1126–30.
27. Barrenetxea G, Lopez de Larruzea A, Ganzabal T, Jimenez R, Carbonero K, Mandiola M. Blastocyst culture after repeated failure of cleavage-stage embryo transfers: a comparison of day 5 and day 6 transfers. Fertil Steril 2005; 83:49–53.
28. Richter KS, Shipley SK, McVearry I, Tucker MJ, Widra EA. Cryopreserved embryo transfers suggest that endometrial receptivity may contribute to reduced success rates of later developing embryos. Fertil Steril 2006;86: 862–6.
29. Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative CT method. NatProtocols 2008;3:1101–8.
30. Lukassen HG, Schonbeck Y, Adang EM, Braat DD, Zielhuis GA, Kremer JA. Cost analysis of singleton versus twin pregnancies after in vitro fertilization. Fertil Steril 2004;81:1240–6.
31. Lawlor DA, Nelson SM. Effect of age on decisions about the numbers of embryos to transfer in assisted conception: a prospective study. Lancet 2012; 379:521–7.
32. Mastenbroek S, van der Veen F, Aflatoonian A, Shapiro B, Bossuyt P, Repping S. Embryo selection in IVF. Hum Reprod 2011;26:964–6.
33. Glujovsky D, Blake D, Farquhar C, Bardach A. Cleavage stage versus blastocyst stage embryo transfer in assisted reproductive technology. Cochrane database of systematic reviews (Online) 2012;7:CD002118.
34. Fritz MA. Perspectives on the efficacy and indications for preimplantation genetic screening: where are we now? Hum Reprod 2008;23:2617–21.
35. Northrop LE, Treff NR, Levy B, Scott RT Jr. SNP microarray-based 24 chromosome aneuploidy screening demonstrates that cleavage-stage FISH poorly predicts aneuploidy in embryos that develop to morphologically normal blastocysts. Mol Hum Reprod 2010;16:590–600.
36. Treff NR, Levy B, Su J, Northrop LE, Tao X, Scott RT Jr. SNP microarray-based 24 chromosome aneuploidy screening is significantly more consistent than FISH. Mol Hum Reprod 2010;16:583–9.
37. McArthur SJ, Leigh D, Marshall JT, de Boer KA, Jansen RP. Pregnancies and live births after trophectoderm biopsy and preimplantation genetic testing of human blastocysts. Fertil Steril 2005;84:1628–36.
38. Kokkali G, Traeger-Synodinos J, Vrettou C, Stavrou D, Jones GM, Cram DS, et al. Blastocyst biopsy versus cleavage stage biopsy and blastocyst transfer for preimplantation genetic diagnosis of beta-thalassaemia: a pilot study. Hum Reprod 2007;22:1443–9.
39. Ryan GL, Sparks AE, Sipe CS, Syrop CH, Dokras A, Van Voorhis BJ. A mandatory single blastocyst transfer policy with educational campaign in a United States IVF program reduces multiple gestation rates without sacrificing pregnancy rates. Fertil Steril 2007;88:354–60.
40. Jena AB, Goldman DP, Joyce G. Association between the birth of twins and parental divorce. Obstet Gynecol 2011;117:892–7.
41. Land JA, Evers JL. Risks and complications in assisted reproduction techniques: report of an ESHRE consensus meeting. Hum Reprod 2003;18: 455–7.

SUPPLEMENTAL FIGURE 1



Single euploid blastocyst transfer after CCS versus transfer of two untested blastocysts. (A) For patients randomized to single euploid blastocyst transfer, trophectoderm biopsy was performed on day 5 (fresh transfer) or day 6 (frozen transfers) of in vitro development. The biopsy underwent an alkaline lysis and then preamplification of assays on each chromosome. The preamplification product was placed in a 384-well reaction plate, and real-time polymerase chain reaction was performed in quadruplicate using a Viia 7 System (Applied Biosystems). A heat map was generated using the cycle threshold method, and a predicted karyotype was assigned to each embryo. The morphologically best euploid blastocyst was selected for transfer. (B) For patients randomized to receive a transfer of two untested blastocysts, the blastocysts were selected by traditional morphologic grading criteria. These embryos were not biopsied or genetically screened.

Forman. Blastocyst Euploid Selective Transfer (BEST) Trial. Fertil Steril 2013.