Blastocyst biopsy with comprehensive chromosome screening and fresh embryo transfer significantly increases in vitro fertilization implantation and delivery rates: a randomized controlled trial

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Objective: To determine whether blastocyst biopsy and rapid quantitative real-time polymerase chain reaction (qPCR)–based comprehensive chromosome screening (CCS) improves in vitro fertilization (IVF) implantation and delivery rates.
Design: Randomized controlled trial.
Setting: Academic reproductive medicine center.
Patient(s): Infertile couples in whom the female partner (or oocyte donor) is between the ages of 21 and 42 years who are attempting conception through IVF.
Intervention(s): Embryonic aneuploidy screening.
Main Outcome Measure(s): Sustained implantation and delivery rates.
Result(s): We transferred 134 blastocysts to 72 patients in the study (CCS) group and 163 blastocysts to 83 patients in the routine care (control) group. Sustained implantation rates (probability that an embryo will implant and progress to delivery) were statistically significantly higher in the CCS group (89 of 134; 66.4%) compared with those from the control group (78 of 163; 47.9%). Delivery rates per cycle were also statistically significantly higher in the CCS group. Sixty one of 72 treatment cycles using CCS led to delivery (84.7%), and 56 of 83 (67.5%) control cycles ultimately delivered. Outcomes were excellent in both groups, but use of CCS clearly improved patient outcomes.
Conclusion(s): Blastocyst biopsy with rapid qPCR-based comprehensive chromosomal screening results in statistically significantly improved IVF outcomes, as evidenced by meaningful increases in sustained implantation and delivery rates.
Clinical Trial Registration Number: NCT01219283. (Fertil Steril® 2013;100:697–703. ©2013 by American Society for Reproductive Medicine.)
Key Words: IVF, preimplantation genetic screening, embryonic aneuploidy, comprehensive chromosomal screening, blastocyst

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Received February 21, 2013; revised and accepted April 18, 2013; published online June 1, 2013.
R.T.S. reports payment for lectures from EMD Serono, Merck USA, and Ferring. K.M.U. has nothing to disclose. E.J.F. reports a grant from Ferring Pharmaceuticals for gonadotropin injections and funding for all other study medications; Ferring had no other input regarding study design, data analysis, or manuscript preparation. K.H.H. has nothing to disclose. K.L.S. reports payment for lectures from EMD Serono, Merck USA, and Ferring. D.T. has nothing to disclose. X.T. has nothing to disclose. N.R.T. reports payment for lectures from ASRM, JSAR, Penn State University, Washington State University, Mayo Clinic, Applied Biosystems Inc., Texas ART Society, and AAB; payment for development of educational presentations from ASRM; and has a patent pending.
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Fertility and Sterility® Vol. 100, No. 3, September 2013 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2013.04.035

In spite of recent advances, only a small minority of embryos that are designated to be of sufficient quality to be transferred to patients actually implant and progress to delivery. The most recent data from the Centers for Disease Control and Prevention registry indicate that less than 19% of transferred embryos progress to delivery of a viable infant (1). In spite of apparently normal temporal and morphologic development, most embryos do not implant, and many of those that do subsequently arrest and miscarry early in pregnancy.

Sustained implantation rates are modest in younger patients and then decline steadily as women progress through their reproductive life. Paralleling this decline in implantation rates is an increase in aneuploidy detected in the products of conception after clinical spontaneous abortion as well as in ongoing pregnancies (2–4). For this reason, embryonic aneuploidy is a primary consideration when evaluating screening strategies for embryonic reproductive competence.

The initial paradigms to screen embryos for aneuploidy used fluorescence in-situ hybridization (FISH). Although the technique is theoretically sound, the clinical results were disappointing, with every randomized controlled trial failing to demonstrate benefit and some actually finding harm (5, 6). Many reasons, including the time of the embryo biopsy, may have contributed to the clinical failure of FISH-based aneuploidy screening, but recent data from more sophisticated technologies such as single-nucleotide polymorphism (SNP) microarrays (7) and strategies based on rapid quantitative real-time polymerase chain reaction (qPCR) (8) have been encouraging. In fact, for the first time ever with embryonic aneuploidy screening the predictive values of a positive and a negative result have been measured directly (9). This nonselection study, still unique for any technology used for embryonic aneuploidy screening, validates that test results may be highly predictive of the reproductive potential of the embryo.

A nonselection study is the only way to accurately determine the predictive values of a comprehensive chromosome screening (CCS) test for embryonic aneuploidy, but it is not sufficient by itself to determine whether that test has clinical value. The test will only find value when, in a meaningful number of cases, embryologists discard their traditional ranking criteria and select an embryo that normally would not have been chosen for transfer. Stated simply, if one always picks the same embryo, then there is no change in treatment and no possible change in outcome. Perhaps an embryologist consistently selects the euploid embryo if there is a viable one in the cohort. Even if the screening test increases the chance for selecting a euploid embryo for transfer, an embryologist may have selected an embryo that is suboptimal based on other established and meaningful criteria. The blended effect of both of those actions—enhanced selection for euploidy and reduced selection for morphologic-evaluation-based potential—will ultimately determine the magnitude of the benefit. That net impact may only be determined by a randomized, controlled trial. Our study, using an assay that has already achieved several stages of validation, examined this final stage to demonstrate whether clinical benefit results from application of aneuploidy screening and to determine the specific magnitude of that benefit.

MATERIALS AND METHODS

Population

Couples with a female partner between the ages of 21 and 42 years who were attempting conception through IVF and had no more than one prior failed IVF retrieval (multiple failed transfers from cryopreserved embryos were not disqualifying) were screened for participation in the study. In the case of oocyte donation, the couples were classified by the age of the oocyte donor. Additional selection criteria included a normal endometrial cavity, a basal follicle-stimulating hormone (FSH) level of 15 IU/L or less, a basal follicle count of eight or more, available ejaculated sperm from the male partner, and a willingness to limit the transfer order to a maximum of two embryos, independent of their age. All patients completed all screening and treatment at the Reproductive Medicine Associates of New Jersey clinical/embryology facility, and all genetic testing was performed in the genetics research laboratory located in the same facility.

Patients randomized to the CCS group were considered to be the study group; those who had only routine care were considered the control group. Initial study recruitment began in October of 2009, and the last delivery follow-up information was obtained in December 2012.

Experimental Design

Patients who met the study criteria were offered participation in the study. There were no restrictions or requirements regarding superovulation protocol. All patients underwent oocyte retrieval per routine and had intracytoplasmic sperm injection (ICSI) for insemination because of the possibility of genetic testing of embryos. All embryos were cultured to the blastocyst stage, which is standard in this program. On the afternoon of day 5, patients with two or more blastocysts were randomized to either the study or control group. The allocation ratio was 1:1. Embryos in the control group were transferred on day 5 of culture; embryos in the study group were transferred on day 6.

The blastocysts of the patients randomized to the study group underwent trophectoderm biopsy with qPCR-based CCS (10). Embryos were graded late in the afternoon of day 5 of development, and the trophectoderm biopsy was accomplished as previously described elsewhere (11). All embryos in both the study and control groups underwent laser hatching on day 3 of in vitro development. The laser was then used to biopsy the cells herniating through that opening on the afternoon of day 5. The biopsy was placed in lysis buffer and submitted for immediate analysis. Biopsies were typically available for processing before 10:00 PM on day 5 and were processed that night. Embryos were classified as euploid or aneuploid based on those screening results. A maximum of two predicted euploid embryos were selected for transfer the next day (day 6) as per routine. Luteal support and clinical management after transfer were completely as per routine.

A major design issue when planning the study was the decision regarding when to transfer the control embryos. The standard of care within the program at the time of the study was to perform blastocyst transfer on the afternoon of day 5 of development. The patients in the control group simply had routine morphologic-based embryo selection and subsequent transfer on the afternoon of day 5 per the routine clinical paradigm used within the practice. The study group was biopsied in the afternoon or evening of day 5, the CCS analysis was performed overnight and the next morning, and these embryos were subsequently transferred on day 6. Clinical management was otherwise identical between the control and study groups.

The randomization table used for this study was generated using a computer-based random number generator. A separate randomization table was created for each of four maternal age groups (<35 years, 35–37 years, 38–40 years, and 41–42 years) and those using oocyte donation. Block randomization was used for each randomization such that 5 out of every 10 patients would be designated as study patients and the other 5 as controls. The use of block randomization was important so that each age group was represented in approximately equal proportions for the study and the control groups. This was particularly important because the study was not powered to allow meaningful analysis of the outcomes within each age group. Although randomization alone would eventually provide an equal distribution of patients allocated to the study and control groups for each age group, that may not always occur when the numbers for any given age group might be small. Block randomization assures that parity of distribution is attained within in each block for each age group and avoids this potential source of bias.

The principal investigator prepared the initial randomization. Based on the randomization table, a single sheet of paper was printed out for each case indicating whether that number was a study patient or a control. These were folded and placed into sequentially numbered opaque sealed envelopes. Once a patient qualified for the study, the envelope was opened in the laboratory, and the patient was designated to go into the study or control group.

The study was approved by the institutional review board, and all patients gave informed consent. This randomized controlled trial was registered at www.clinicaltrials.gov with ID designation NCT01219283.

Assays

The trophectoderm biopsies underwent qPCR-based screening as previously described elsewhere (10). The samples were analyzed on the day that the biopsy was attained, and the results were immediately available to the embryologists and clinicians.

Data Analysis

The primary end point of the study was the sustained implantation rate—that is, the probability that an embryo once transferred would progress to delivery. The secondary end points included the clinical implantation rate and the various pregnancy rates including chemical, clinical, and delivery rates. Comparisons were made using contingency table analyses.

To ensure that the randomization had created equivalent patient populations, age, number of oocytes retrieved, number of high quality blastocysts (expansion score of 3 to 6 and an inner cell mass score of A or B) (11), and number of embryos transferred were all compared. Student's *t*-tests were used as appropriate. An alpha error of 0.05 was considered significant. The study was powered to detect a 20% difference in sustained implantation rates with a power of 80%, assuming a baseline implantation rate of 40% in the control group using a 1:1 randomization ratio. To achieve this, a minimum of 107 embryos were required to be transferred in each group. The anticipated number of embryos transferred per patient was two, and patients had to have at least two blastocysts to be randomized. Therefore, the control group would always have two embryos available. Within the CCS group, it was possible that some patients would have either zero or one euploid embryo for transfer. However, given that these patients had good prognoses and based on the prevalence of embryonic aneuploidy in prior studies, the decision was made to power the study assuming that two would be available in the CCS group as well. Because there was a possibility that some patients in the study group would only have a single euploid embryo for transfer, the planned sample size was for a total of 150 patients randomized.

RESULTS

A total of 288 patients applied for consideration for participation in the study. Of these, 155 satisfied the study inclusion and exclusion criteria as described. The most common disqualifying factor was evidence of limited ovarian reserve. All 155 patients were randomized on the afternoon of day 5 of embryo development. The CCS group had 72 patients, and the control had 83 patients (Table 1). There were three

TABLE 1

The demographics of the comprehensive chromosomal screening (CCS) and control groups were equivalent as were basic parameters of follicular stimulation during their treatment cycles.

Characteristic	CCS study group	Nonintervention (control group)
Patients (n)	72	83
Age (y)	32.2 ± 0.5	32.4 ± 0.5
Undergoing oocyte donation (n)	3	5
Mature follicles on day of hCG (≥14 mm)	11.4 ± 1.1	12.4 ± 1.8
Peak E_2 level on day of hCG (pg/mL)	2,432 ± 88	2,648 ± 92
Number coasted, n (%)	0 (0)	0 (0)
Proportion of cases using ICSI (%)	100	100
Clinical diagnoses, n (%)		
Male factor	38 (52.8)	40 (48.2)
Tubal factor	9 (12.5)	11 (13.3)
PCOS	8 (11.1)	10 (12.0)
Endometriosis	6 (8.3)	7 (8.4)
Idiopathic	11 (15.3)	15 (18.1)

Note: Data are expressed as mean and standard error where appropriate. There were no significant differences between the groups for any of these indices.

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TABLE 2

Embryology laboratory data for the comprehensive chromosomal screening (CCS) and control groups.

	CCS study group	Nonintervention (control group)	*P* value
Oocytes recovered	17.2 ± 0.9	17.1 ± 0.9	.95
Mature oocytes	13.1 ± 0.9	12.8 ± 1.0	.82
Blastocysts	8.0 ± 0.7	7.9 ± 0.7	.92
High quality blastocysts (of sufficient quality to be biopsied)	7.1 ± 0.5	6.2 ± 0.4	.64
Embryos transferred (n)	1.86	2.0	.0004
Embryos cryopreserved (n)	2.6 ± 0.3	3.4 ± 0.3	.19

Note: Data are expressed as mean with associated standard error.

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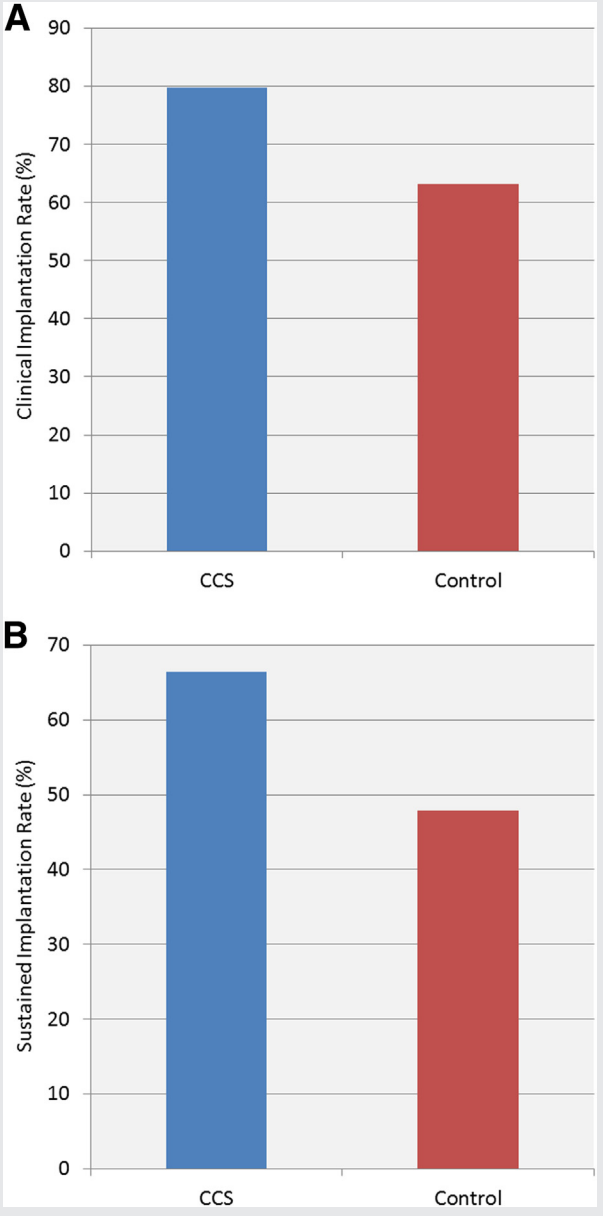
FIGURE 1



Outcomes per embryo transferred. (A) Clinical implantation rates are statistically significantly higher in embryos that have undergone comprehensive chromosome screening (CCS) (*P*=.01). (B) Embryos selected after CCS are also statistically significantly more likely to progress to delivery than unscreened embryos from the control group (*P*=.01).

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oocyte donation recipients in the CCS group and five in the control group. The difference in the relative sizes of the study groups represents the fact that block randomization was performed across five different age groups so that overall parity in size was not maintained. This was necessary to ensure a uniform distribution of subjects and controls within each age group. Once randomized, all patients completed the study. No patients were excluded from the analysis.

The CCS and control groups were equivalent as evidenced by maternal age (32.2 ± 0.5 vs. 32.4 ± 0.5 years), number of oocytes recovered (17.2 ± 0.9 vs. 17.1 ± 0.9), number of prior IVF attempts (0.30 ± 0.03 vs. 0.32 ± 0.03 cycles), total number of embryos in extended culture (9.8 ± 0.4 vs. 10.1 ± 0.5), and the number of supernumerary embryos cryopreserved (2.6 ± 0.3 vs. 3.4 ± 0.3) (Table 2). There was a statistically significant difference in the number of blastocysts transferred (1.8 ± 0.04 vs. 2 ± 0.0; $P<.0001$). This reflects the fact that 10 patients had only a single euploid blastocyst available for transfer but all patients in the control group had two embryos transferred.

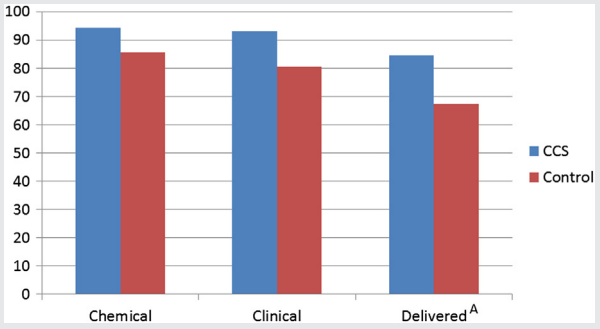
The patients who were randomized to the CCS group had a mean of 7.1 blastocysts biopsied per case. Of the 483 blastocysts evaluated, 338 were euploid, 138 were aneuploid, and 7 embryos were considered indeterminate after a nonconcordant CCS result. These proportions (28.6% aneuploid rate) are consistent with the prevalence of aneuploidy typically seen in these age groups.

The primary end point used in the study related to implantation. This was evaluated in two ways—the clinical implantation rate (probability an embryo would form a gestational sac) and the sustained implantation rate (probability an embryo would deliver). We transferred 134 embryos to 72 patients in the CCS group and 163 blastocysts to 83 patients in the control group. Ten patients in the CCS group had only a single euploid blastocyst available for transfer. No patient had zero embryos available for transfer.

The clinical implantation rates were statistically significantly higher in the CCS group (107 of 134; 79.8%) versus the control group (103 of 163; 63.2%) (RR 1.26; 95% CI, 1.04–1.39; $P=.002$) (Fig. 1A). Similarly, there was a statistically significant increase in the proportion of CCS-screened embryos that progressed to delivery (89 of 134; 66.4%) compared with those from the control group (78 of 163; 47.9%) (RR 1.39; 95% CI, 1.07–1.60; $P=.001$) (Fig. 1B).

The secondary end points for this randomized trial, which remain highly clinically relevant, were the per cycle pregnancy rates. There was no difference in the initial (biochemical) pregnancy rate. Sixty-eight of 72 patients (94.4%) in the CCS group had a positive pregnancy test versus 71 of 83 (85.5%) in the control group ($P=.07$). The clinical pregnancy rates were statistically significantly higher in the

FIGURE 2



Outcome per treatment cycle: Delivery rates are statistically significantly increased in treatment cycles in which embryos undergo comprehensive chromosome screening (P=.03). The initial chemical and clinical pregnancy rates were not different.

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CCS group. The CCS group achieved a clinical pregnancy in 67 (93.1%) of 72 cycles while the control group developed a clinical pregnancy in 71 (80.7%) of 83 cycles (RR 1.15; 95% CI, 1.03–1.43; P=.03) (Fig. 2).

Delivery rates were statistically significantly higher in the CCS group (RR 1.26; 95% CI, 1.06–1.53; P=.01). Sixty-one (84.7%) of 72 treatment cycles using CCS led to delivery while 56 (67.5%) of 83 control cycles ultimately delivered. The outcomes were excellent in both groups, but use of CCS clearly improved patient outcomes (Fig. 2).

As expected, implantation and delivery rates declined with increasing maternal age in the control group and were constant in the CCS group consistent with prior reports (9). This study was not powered to study the impact of age on outcome in either group.

DISCUSSION

This study provides class I evidence (12) that aneuploidy screening using qPCR-based CCS meaningfully improves implantation and delivery rates. These data clearly demonstrate that embryo selection is meaningfully enhanced when CCS is employed. The study was initially powered to evaluate implantation rates as this technology evaluates embryos (not patients), and this provides the most direct end point for those embryos that were screened with CCS relative to those that were not. This end point is also independent of transfer order and may be most applicable as the proportion of single-embryo transfer continues to increase. It further shows that significant improvements in delivery rates per transfer can be achieved even when selecting among morphologically normal embryos. These conclusions are limited to morphologically normal embryos as only embryos considered morphologically adequate were considered for inclusion in the study.

This study represents the third of a three-phase strategy to validate the use of CCS. The first was to demonstrate using known euploid and aneuploid status cell lines that the diagnostic techniques were accurate and reliable (10). The second phase focused on calculation of the positive and negative predictive value of the test (9). This critical phase of the validation process was never performed for the application of FISH in the clinical setting, which has likely contributed to the confusion regarding the clinical utility of that technology.

Phase 2 involved calculation of the positive and negative predictive values of the test. It is not possible to know the value of a diagnostic test or to counsel patients adequately without directly evaluating both the positive and negative predictive values. This is among the most fundamental tenants of clinical statistics. Furthermore, just because one predictive value is good does not mean the other meets a reasonable clinical standard. In the end, the predictive values must be measured directly with actual clinical data, or the test must be thoroughly standardized against another test that has met this rigorous standard. Particularly in a clinical setting such as aneuploidy screening where embryos with abnormal screening results are discarded, it is critical to have direct measurement that demonstrates the predictive value of an abnormal result.

The third phase is a randomized controlled trial showing actual improvements in outcome. Even precise calculation of predictive values is insufficient for discerning whether application of a clinical test provides an actual clinical benefit. The data in this study clearly demonstrate such benefit. It indicates that selection is meaningfully altered in a sufficiently high percentage of the cases to increase the probability of delivery.

Caution should be used when interpreting the fact that the CCS and control groups had an equivalent number of supernumerary blastocysts cryopreserved for use in future cycles. The comparison is significantly underpowered. Additionally, the embryologists were not blinded to the CCS results at the time they were making decisions about whether a given embryo had sufficient quality to be vitrified. Although no formal policy is in place within the laboratory, it is widely held that embryos of marginal morphologic quality are more likely to be cryopreserved if the embryologist knows that they are euploid. With greater statistical power, it is probable that the increased selection provided by CCS before cryopreservation would ultimately result in enough embryos being excluded secondary to aneuploidy that fewer embryos would be cryopreserved.

The high implantation rates demonstrated in these good-prognosis patients suggests that single-embryo transfer may be becoming more practical. Elective single-embryo transfer has long been advocated, but the reality is that the clinical results have been disappointing (13, 14). In fact, in patients with two or more morphologically acceptable embryos, it is mathematically impossible for a single-embryo transfer to produce the same clinical pregnancy rates as two embryo transfers. Although many retrospective studies might take exception to this fact, the reality is that it is a true mathematical certainty that two embryos will result in a higher pregnancy rate than a single-embryo transfer in an adequately powered study. That is why per-cycle delivery rates were lower in all six randomized, controlled trials performed to date and also why the meta-analysis confirmed the finding of significantly diminished outcomes per transfer (14).

The numeric advantage of transferring two embryos comes with a substantial clinical burden—multiple gestations (15). As implantation rates increase beyond 50%, the theoretical difference in ongoing pregnancy rates between single-embryo and two-embryo transfer actually gets progressively smaller, but the twin risk continues to rise dramatically. Given the increased neonatal and maternal morbidity from twins, the best strategy is clearly to optimize implantation rates to the greatest extent possible and proceed with single-embryo transfer. This strategy, employed with good effect in young women, has been difficult to apply to older patients because their implantation rates are lower (16). Forman et al. (17) has recently demonstrated in a randomized, controlled trial that the routine application of CCS allows women through age 42 to enjoy the same delivery rates with a single-embryo transfer that are attained with the transfer of two unscreened embryos. Significantly, good outcomes were maintained with single-embryo transfer after CCS with no multiple gestations. Use of CCS does not require single-embryo transfer, but this technology—or others, should they become adequately validated—have the potential to maintain or even improve outcomes while nearly eliminating multiple gestations.

Like all technologies, CCS has limits that must be considered. Application of CCS should be done with blastocyst biopsy. Blastomere biopsy at the cleavage stage has been shown to adversely impact implantation rates (18). That was not an issue in this study because the diagnostic laboratory is located in the same building. The rapid time-line of qPCR (4 hours) allowed the patients in this study to have blastocyst biopsies late on day 5 and still have a fresh transfer on day 6. In most centers, the trophectoderm biopsies will need to be sent to a reference laboratory for analysis, which will necessitate cryopreservation. A recent study has addressed this issue and demonstrated that biopsied embryos may be safely vitrified and that excellent implantation rates are maintained (7). Clearly it is not necessary for every program to have a genetics laboratory in their facility—much more important than speed will be the use of a carefully validated assay.

Another limit of this study is that these patients all had good prognoses, as evidenced by the normal markers of ovarian reserve. They were good responders with large oocyte yields and an abundance of embryos to evaluate. Further prospective studies will be required to validate the best way to apply CCS in women who are low responders or who have other abnormal markers of ovarian reserve. Clinical experience at our center with qPCR-based CCS (unpublished data) and at another center using the related microarray platforms (19) has demonstrated that implantation rates are maintained among euploid embryos from women with abnormal markers of ovarian reserve. As with all embryo assessment techniques, clinicians and patients should focus on the fact that the technique is diagnostic. It attains its therapeutic advantage by enhancing current morphologic embryo selection criteria. Patients who cannot produce embryos or whose embryos uniformly arrest will not benefit from this technique. The application of CCS cannot make an embryo with little or no reproductive potential better.

A final limit to consider relates to the nature of the experimental design. The paradigm for the study group was straightforward: biopsy of the blastocysts once they are fully expanded, which is typically in the late afternoon or evening of day 5; analysis of the samples overnight and the following morning; and transfer on day 6. In contrast, there were two options for transferring the control group embryos. First and perhaps most intuitive, the embryos could be transferred on day 6 at the same time as the study embryos. This isolates the effect of the CCS to the maximum extent. The other alternative was to transfer the embryos per routine on the afternoon of day 5. The latter paradigm cannot separate the impact of enhanced selection gained by aneuploidy screening from any other factor that might alter outcomes by extending in vitro culture to day 6, such as enhanced morphologic embryo selection (among the euploid embryos) and adverse or beneficial changes in endometrial receptivity. However, it does provide an accurate assessment of the overall impact of implementing the technique relative to routine treatment.

The former paradigm, delaying transfer of the control group until day 6, provides the greatest insight into the impact of the actual aneuploidy screening but does not necessarily provide the most comprehensive assessment of the impact of using the technique. For example, what if CCS aids embryo selection but waiting until day 6 is harmful? Transferring both groups on day 6 would demonstrate the superiority of CCS in that setting but would not really demonstrate that it is better than routine care, as there may be a detrimental effect to waiting to day 6 for transfer that exceeds the beneficial effect of CCS screening. For these reasons, this study was performed as a comparison of routine care (day-5 transfer for the control group) versus all that is necessary to complete CCS (biopsy on day 5, CCS, and transfer on day 6).

The most optimal design to provide the greatest information would have been a three-arm study comparing the following: CCS with day-6 transfer; day-6 transfers without CCS; and routine care with transfer on the afternoon of day 5. This approach would have provided the most insight as to the factors that resulted in the enhanced outcomes seen in the CCS group.

The possibility remains that some of the improvement in clinical outcomes seen in the CCS group are attributable to more than just the assessment of the ploidy status of the embryos. Altered selection or changes in endometrial receptivity may also have contributed. Nevertheless, this study does provide class I data demonstrating that the overall impact of applying this paradigm for CCS on blastocysts is superior to routine blastocyst transfer on day 5.

This study completes the initial validation of CCS using this technology. Full exploration of how best to apply the technology awaits further randomized trials in a wide variety of clinical settings. Although the high predictive values, enhanced implantation and delivery rates, and encouraging data regarding improved outcomes with single-embryo transfer are exciting, further studies and broad based clinical

application will be required to fully define the role of CCS in the continuum of clinical care (17).

Acknowledgments: Research support and follow up provided by Monica Benson, B.S.N., and Stephanie Sgambati, M.S., J.D.

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