Comprehensive chromosome screening alters traditional morphology-based embryo selection: a prospective study of 100 consecutive cycles of planned fresh euploid blastocyst transfer

Eric J. Forman, M.D.,a,b Kathleen M. Upham, B.S.,a Michael Cheng, B.S.,a Tian Zhao, B.S.,a Kathleen H. Hong, M.D.,a,b 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; and b University of Medicine and Dentistry of New Jersey–Robert Wood Johnson Medical School, Department of Obstetrics, Gynecology & Reproductive Sciences, New Brunswick, New Jersey

Objective: To determine how often trophectoderm biopsy and rapid, real-time quantitative polymerase chain reaction (PCR)-based comprehensive chromosome screening (CCS) alters clinical management by resulting in the transfer of a different embryo than would have been chosen by traditional day 5 morphology-based criteria.

Design: Prospective.

Setting: Academic center for reproductive medicine.

Patient(s): Infertile couples (n = 100; mean age 35 ± 4 years) with at least two blastocysts suitable for biopsy on day 5.

Intervention(s): Prior to trophectoderm biopsy for CCS the embryologist identified which embryo would have been selected for traditional day 5 elective single ET.

Main Outcome Measure(s): The risk of aneuploidy in the embryos that would have been selected on day 5 was calculated and compared with the aneuploidy rate of the cohort of all embryos that underwent CCS testing. The aneuploidy risk was compared between age groups.

Result(s): After quantitative PCR-based CCS, 22% (95% confidence interval 15%–31%) of the embryos selected by day 5 morphology were aneuploid, which was lower than the 32% aneuploidy rate of the cohort. Patients ≥35 years had a higher risk of an aneuploid blastocyst being selected by morphology than those <35 years old (31% vs. 14%). Among patients who had selection altered by CCS, 74% (14/19) delivered, including 77% (10/13) after elective single ET. Most patients (77%) had an additional euploid blastocyst vitrified for future use.

Conclusion(s): The CCS results alter embryo selection due to the presence of aneuploidy in embryos with optimal day 5 morphology. Excellent outcomes were obtained when CCS-based selection was different than morphology-based selection. [Fertil Steril® 2013;100:718–24. ©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/formanje-comprehensive-chromosome-screening-embryo-selection/

Received February 26, 2013; revised April 25, 2013; accepted April 26, 2013; published online May 30, 2013.

E.J.F. has nothing to disclose. K.M.U. has nothing to disclose. M.C. has nothing to disclose. T.Z. has nothing to disclose. K.H.H. has nothing to disclose. N.R.T. reports payment for lectures from the American Society for Reproductive Medicine (ASRM), Japanese Society for Assisted Reproduction, Penn State University, Washington State University, Mayo Clinic, Applied Biosystems Inc., Texas Assisted Reproductive Technologies Society, and American Association of Bioanalysts; payment for development of educational material from ASRM; and patents pending. R.T.S. is a member of the advisory boards of EMD Serono and Ferring Pharmaceuticals, and has received payment for lectures and travel expenses from Merck.

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).
Preimplantation genetic screening has been proposed primarily as a method to improve embryo selection for patients with a poor prognosis for IVF success as a result of previous implantation failures or advanced age (1). On the contrary, for good prognosis patients, increased utilization of blastocyst stage, elective single ET (eSET), without aneuploidy screening, has been promoted to reduce the risk of multiple gestation and its resulting sequelae (2). Although prior studies using fluorescence in situ hybridization did not improve eSET selection (3, 4), more accurate comprehensive chromosome screening (CCS) methods have demonstrated benefit (5, 6, 7). The degree to which CCS impacts clinical management by altering embryo selection for transfer and subsequent cryopreservation, even among high-quality blastocysts, has not previously been quantified.

As eSET is currently practiced, one embryo is selected by temporal and morphological criteria from multiple blastocysts deemed suitable for transfer on day 5 of development (8). With rapid CCS, however, blastocysts undergo trophectoderm biopsy on day 5 with real-time, quantitative polymerase chain reaction (qPCR)-based CCS performed. The best quality embryo from among the euploid blastocysts is then selected for transfer the following day (Figure 1). If the embryo selected for fresh transfer after CCS is always the same embryo that would have been selected on day 5 (i.e., if the best quality embryo is always euploid), then CCS would not have the opportunity to improve outcomes.

The current study seeks to determine how often CCS would alter selection for eSET and thus provide an estimate of the degree to which CCS may improve fresh eSET outcomes. To evaluate this prospectively, an embryologist identified the embryo that would have been selected for traditional day 5 eSET in 100 consecutive cases. Trophectoderm biopsy and qPCR-based CCS were performed and it was determined how often the previously selected embryo would have a predicted aneuploid karyotype, yielding an estimate for how often CCS alters clinical care in different age groups.

**MATERIALS AND METHODS**

**Population**

The study population consisted of 100 consecutive patients planning to undergo rapid CCS with trophectoderm biopsy on day 5 and fresh transfer on day 6. All cycles were performed at Reproductive Medicine Associates of New Jersey between September 2011 and March 2012. To be eligible, patients had to have at least two blastocysts suitable for trophectoderm biopsy by late in the afternoon of day 5. During the study period CCS was offered to all patients undergoing IVF. It was recommended for patients of advanced reproductive age, those with recurrent pregnancy loss, a prior aneuploid ongoing pregnancy, or a prior failed IVF cycle. This was a noninterventional, observational study of consecutive ETs. All patients participated through an institutional review board-approved protocol. In addition, some patients having transfers during this time period were participating in an institutional review board-approved randomized controlled trial investigating CCS to optimize single ET (ClinicalTrials.gov identifier NCT01408433). Their participation in that trial did not impact this observational study.

Patients whose embryos arrested before the blastocyst stage or could not be biopsied until day 6 were not included in the analysis. Patients who had a contraindication to fresh transfer (risk of ovarian hyperstimulation syndrome [OHSS], premature P elevation, abnormal endometrial proliferation) were excluded. Patients who were planning to cryopreserve all of their blastocysts for a future frozen ET were also excluded.

Patients were treated per routine with an IVF protocol selected by their treating physician. All stimulations included LH activity in the form of purified menotropins (Menopur; Ferring Pharmaceuticals) or low-dose hCG. Final oocyte maturation was induced when at least two follicles reached a maximal diameter of $\geq 18$ mm and oocyte retrieval was performed 36 hours later. All mature oocytes underwent intracytoplasmic sperm injection (ICSI), even in cases with normal semen parameters, to prevent genetic contamination during CCS. All cleaved embryos underwent laser-assisted hatching of the zona pellucida (ZP) on day 3 to facilitate trophectoderm biopsy on day 5. Per practice routine, all embryos were placed in extended culture regardless of the size or quality of the cohort. Embryos were graded on day 5.

**FIGURE 1**

Real-time, quantitative polymerase chain reaction (PCR)-based comprehensive chromosome screening results after day 5 trophectoderm biopsy of four blastocysts from a 31-year-old patient with a history of recurrent pregnancy loss. Had a day 5 elective single ET been performed, the top embryo would have been selected based on morphological criteria. After comprehensive chromosome screening results were obtained, that embryo was found to be aneuploid (47,XY,+21). On the morning of day 6, the embryo with the karyotype prediction of 46,XY was transferred, although its morphology grade was 4BB, compared with 6AA for the 47,XY,+21 embryo. The patient delivered a healthy boy at 39 weeks, 4 days gestation.

and an embryologist experienced in trophectoderm biopsy determined whether they were suitable for day 5 trophectoderm biopsy. To be biopsied, blastocysts had to reach at least an expansion stage of 3, have a distinct inner cell mass (ICM), and have an adequately cellular trophectoderm (9). Embryos that were not suitable for biopsy by the afternoon of day 5 were considered not to be developing synchronously with endometrial receptivity as they have been shown to have lower implantation rates in fresh ETs (10, 11). These embryos are therefore reassessed on day 6 for possible trophectoderm biopsy and vitrification.

**Embryo Selection**

The biopsy was performed with an infrared diode laser by removing approximately five trophectoderm cells that had herniated through the previously created breech in the ZP. At the time of embryo biopsy, in addition to performing a standard morphological assessment, the embryologist identified which blastocyst would have been selected for a day 5, fresh single blastocyst transfer. The identity of the specific embryo selected was sent to the principal investigator. The selected embryo was otherwise treated as per routine.

The biopsies were processed for qPCR-based CCS as previously described (12). In brief, multiplex amplification of 96 loci (four for each chromosome) was performed and a method of relative quantitation was applied to predict the copy number status of each chromosome. This methodology was designed to specifically identify whole chromosome, not segmental, aneuploidy and was validated in preclinical (12) and clinical studies (13, 14). A karyotype prediction was made for each embryo by a certified cytogeneticist. The laboratory technicians who processed the biopsy and the genetics team that analyzed the qPCR data were not aware of which embryo had been selected.

Transfers all occurred on the morning of day 6, before 6 complete days (144 hours) had elapsed from the time of oocyte retrieval, as is standard for all fresh ETs at this center. Patients received a transfer of one or two euploid blastocysts with the best morphology. The transfer decision was not impacted by the previous day’s selection. Transfer order was determined by the patient and her physician.

**Statistical Analysis**

The aneuploidy rate of the embryos that would have been selected for a traditional day 5 single blastocyst transfer was calculated and compared with the aneuploidy rate of the overall cohort of blastocysts. Age group-specific comparisons were also made by comparing the aneuploidy rate of the selected blastocyst in patients <35 years old with those ≥ 35 years old. Comparisons were made using a $\chi^2$ distribution with a $P$ value of .05 considered significant. The number of patients needed to treat (NNT) to prevent the transfer of an aneuploid blastocyst was calculated as the inverse of the aneuploidy rate of the selected day 5 blastocyst. For example, if there was a 50% aneuploidy risk, the NNT would be 2 (1/0.5) (i.e., two patients would have to use qPCR-based CCS and eSET to prevent the transfer of one aneuploid blastocyst).

The impact of CCS on eSET delivery rates was estimated using previously published implantation rates of predicted aneuploid blastocysts (13) and the overall implantation rate of euploid blastocysts in the current cohort. Based on these estimates, the NNT was calculated and represents the number of patients who would have to use CCS-based selection to achieve an additional singleton delivery from eSET.

**RESULTS**

A consecutive series of 100 patients was evaluated. The demographics of the patients are presented in Table 1. Overall, 22% (22/100; 95% confidence interval 15%–31%) of the blastocysts that would have been selected for by traditional day 5 morphological criteria were aneuploid and, therefore, were not transferred. This was lower than the overall 32% (192/597) aneuploidy rate of the entire cohort of biopsied blastocysts ($P = .04$; Table 2). The different types of aneuploidy detected in the best quality day 5 blastocyst are detailed in Table 3. The traditionally selected blastocyst among patients <35 years old had an aneuploidy rate of 14% (7/51; 95% confidence interval 6%–25%), which was lower than the aneuploidy rate of 31% (15/49; 95% confidence interval 19%–45%) in the traditionally selected blastocyst from patients ≥35 years old (relative risk 0.4, 95% confidence interval 0.2–1.0; $P = .04$).

**Clinical Outcomes**

A total of 96 fresh transfers were performed. Two patients did not have a transfer as all of their blastocysts were aneuploid based on CCS test results. Both of these patients had a poor IVF prognosis: a 42-year-old with three aneuploid blastocysts and a 41-year-old with an elevated day 3 FSH of 18.8 IU/L and five aneuploid blastocysts. One patient did not have a transfer as initial CCS results were nondiagnostic (nonconcurrent) and the four blastocysts were rebiopsied on day 6 and vitrified. One patient had one euploid blastocyst and elected to undergo another fresh cycle in an attempt to obtain additional blastocysts for transfer.

The delivery rate was 65% per patient and 68% per transfer. The implantation rate (number of fetal heart beats divided by total number of embryos transferred) was 63% (79/126). Among patients who had single euploid blastocyst transfer, the delivery rate was 64% (42/66) and all were singletons. Among those who had two euploid blastocysts transferred, the delivery rate was 77% (23/30) including 10 twins and 1 triplet due to monozygotic twinning of an...

<table>
<thead>
<tr>
<th>Characteristics of study population.</th>
<th>Mean ± SD (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>35 ± 4 (22–44)</td>
</tr>
<tr>
<td>Maximal day 3 FSH (IU/L)</td>
<td>7.0 ± 2.3 (1.5–18.8)</td>
</tr>
<tr>
<td>Antimüllerian hormone (ng/mL)</td>
<td>3.3 ± 2.1 (0.3–8.6)</td>
</tr>
<tr>
<td>No. of oocytes retrieved</td>
<td>18 ± 9 (5–56)</td>
</tr>
<tr>
<td>No. of blastocysts biopsied</td>
<td>6 ± 4 (2–21)</td>
</tr>
<tr>
<td>No. of euploid blastocysts</td>
<td>4 ± 3 (0–17)</td>
</tr>
</tbody>
</table>

Another triplet pregnancy miscarried at 12 weeks gestation. Overall, the clinical miscarriage rate was 11% (8/73).

### Impact of Changing Selection with CCS

The 22 patients whose traditionally selected day 5 blastocyst was aneuploid had a mean age of 37 years. Nineteen of these patients had a fresh transfer of a different, CCS-selected, euploid blastocyst with a delivery rate of 74% (14/19). The delivery rate after single euploid blastocyst transfer in this group was 77% (10/13), indicating that excellent outcomes can be maintained with eSET even when the best quality embryo by traditional morphological criteria is selected against due to aneuploidy. There were three blastocysts with the ability to result in viable gestations (two with predicted trisomy 21 and one with predicted 47,XXY) that would have been selected by traditional day 5 morphology-based criteria.

When performing eSET in this population, the number NNT to prevent transfer of a predicted aneuploid blastocyst was 5. For patients <35 years old, the NNT was 7; for those ≥35 years old, the NNT was 3.

A previous nonselection study demonstrated that embryos predicted to be aneuploid had a delivery rate of 4% (13). In the current study population, euploid blastocysts had an implantation rate of 63%. Thus, with traditional morphology-based day 5 single blastocyst transfer, the estimated expected delivery rate would be 50% (1 delivery among 22 predicted aneuploid and 49 deliveries among 78 predicted euploid). After CCS, the expected delivery rate would be 62% (62 deliveries among 98 predicted euploid and 2 with no transfer). Thus, a relatively low aneuploidy rate of 22% among selected blastocysts may translate into a

### TABLE 2

<table>
<thead>
<tr>
<th>Maternal age, y</th>
<th>Biopsied blastocysts</th>
<th>Euploid blastocysts</th>
<th>CCS predicted karyotype of selected embryo</th>
<th>Morphology of transferred euploid embryo 1</th>
<th>Morphology of transferred euploid embryo 2</th>
<th>NNT to prevent transfer of predicted aneuploid blastocyst</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>22% (22/100)</td>
<td>32% (192/597)</td>
<td>Relative risk (95% confidence interval)</td>
<td>P value</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;35 years-old</td>
<td>14% (7/51)</td>
<td>25% (83/336)</td>
<td></td>
<td>.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥35 years-old</td>
<td>31% (15/49)</td>
<td>42% (109/261)</td>
<td></td>
<td>.08</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: eSET = elective single ET.

### TABLE 3

<table>
<thead>
<tr>
<th>Maternal age, y</th>
<th>Biopsied blastocysts</th>
<th>Euploid blastocysts</th>
<th>CCS predicted karyotype of selected embryo</th>
<th>Morphology of transferred euploid embryo 1</th>
<th>Morphology of transferred euploid embryo 2</th>
<th>Transfer outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>28.8a</td>
<td>12</td>
<td>5BB</td>
<td>47,XX,+7</td>
<td>6BB</td>
<td>5BB</td>
<td>Not pregnant</td>
</tr>
<tr>
<td>31.6b</td>
<td>4</td>
<td>5BB</td>
<td>47,XY,+21</td>
<td>6AA</td>
<td>6BB</td>
<td>Term singleton</td>
</tr>
<tr>
<td>32.2</td>
<td>12</td>
<td>5BB</td>
<td>47,XY,+22</td>
<td>6AA</td>
<td>6BA</td>
<td>Preterm twins</td>
</tr>
<tr>
<td>33.3</td>
<td>6</td>
<td>5BB</td>
<td>47,XY,+16</td>
<td>6AA</td>
<td>6BA</td>
<td>Biochemical loss</td>
</tr>
<tr>
<td>34.9</td>
<td>4</td>
<td>5BB</td>
<td>45,XY,+16</td>
<td>6AA</td>
<td>6BA</td>
<td>Term singleton</td>
</tr>
<tr>
<td>34.5</td>
<td>2</td>
<td>6AA</td>
<td>45,XY,+16</td>
<td>6AA</td>
<td>6BA</td>
<td>Term twins</td>
</tr>
<tr>
<td>36.4</td>
<td>2</td>
<td>6BB</td>
<td>45,XY,+16</td>
<td>6BA</td>
<td>6BB</td>
<td>Term singleton</td>
</tr>
<tr>
<td>37.1</td>
<td>3</td>
<td>4BB</td>
<td>45,XY,+4</td>
<td>6AA</td>
<td>6BA</td>
<td>Term singleton</td>
</tr>
<tr>
<td>37.3</td>
<td>5</td>
<td>4BB</td>
<td>45,XY,+4</td>
<td>6BB</td>
<td>6BB</td>
<td>Term singleton</td>
</tr>
<tr>
<td>37.4</td>
<td>4</td>
<td>5BB</td>
<td>45,XY,+16</td>
<td>6AA</td>
<td>6BA</td>
<td>Term singleton</td>
</tr>
<tr>
<td>37.7</td>
<td>11</td>
<td>7</td>
<td>45,XY,+16</td>
<td>6BA</td>
<td>6BB</td>
<td>Term singleton</td>
</tr>
<tr>
<td>37.8</td>
<td>7</td>
<td>6AA</td>
<td>46,XX,+5</td>
<td>6BB</td>
<td>6BB</td>
<td>Preterm singleton</td>
</tr>
<tr>
<td>37.9</td>
<td>8</td>
<td>6AA</td>
<td>45,XX,+2</td>
<td>6BB</td>
<td>6BB</td>
<td>Term singleton</td>
</tr>
<tr>
<td>38.8</td>
<td>4</td>
<td>4BB</td>
<td>47,XY</td>
<td>6CA</td>
<td>6BB</td>
<td>Not pregnant</td>
</tr>
<tr>
<td>39.3</td>
<td>6</td>
<td>4BB</td>
<td>47,XY,+21</td>
<td>6BA</td>
<td>6BA</td>
<td>Biochemical loss</td>
</tr>
<tr>
<td>39.5</td>
<td>5</td>
<td>4BB</td>
<td>5BA</td>
<td>44,XX,+13,+16</td>
<td>5BB</td>
<td>Not pregnant</td>
</tr>
<tr>
<td>40.8</td>
<td>8</td>
<td>4BB</td>
<td>45,XY,+2</td>
<td>6AA</td>
<td>6BA</td>
<td>Term singleton</td>
</tr>
<tr>
<td>40.9</td>
<td>5</td>
<td>6AA</td>
<td>47,XY,+15</td>
<td>6BB</td>
<td>6BB</td>
<td>Not transfer</td>
</tr>
<tr>
<td>41.1</td>
<td>4</td>
<td>4BB</td>
<td>49,XY,+15,+19</td>
<td>6BB</td>
<td>6BB</td>
<td>No transfer</td>
</tr>
<tr>
<td>42.7</td>
<td>3</td>
<td>4BB</td>
<td>45,XY,+22</td>
<td>6BB</td>
<td>6BB</td>
<td>No transfer</td>
</tr>
<tr>
<td>44.2</td>
<td>3</td>
<td>4BB</td>
<td>48,XX,+15,+16</td>
<td>6AA</td>
<td>6BA</td>
<td>Term singleton</td>
</tr>
</tbody>
</table>

Note: CCS = comprehensive chromosome screening.

a Anonymous oocyte donation.

b qPCR results demonstrated for this patient in Figure 1.

clinically significant improvement in single blastocyst transfer delivery rates of 12% per cycle. Based on this estimate, the NNT to achieve an additional singleton delivery from eSET with CCS was 8.

Despite only cryopreserving euploid blastocysts for future frozen ETs, 77 patients had at least one supernumerary euploid blastocyst vitrified. Of those who had eSET, 51/66 (77%) had a euploid embryo cryopreserved.

**DISCUSSION**

This prospective study has demonstrated that in candidates for fresh day 5 eSET, performing CCS results in a change in embryo selection, when compared with traditional morphology-based selection, a substantial proportion of the time. The data from this study also suggest that this change may result in clinically meaningful improvement in the eSET success rate. It is not surprising that patients of advanced reproductive age, who have a higher underlying prevalence of embryonic aneuploidy, were more likely to have selection altered by the CCS result.

Despite the benefits of singleton versus multiple pregnancy, efforts to promote eSET have been inadequate as the less than 10% of ETs after IVF use this approach (15). The reluctance to accept eSET likely relates to its lower per transfer delivery rates when conventional methods of embryo selection are used (16). Preimplantation human embryos have a substantial risk of aneuploidy, regardless of maternal age, and if an aneuploid embryo is selected for eSET, the cycle is destined to fail. Although promising results have been demonstrated in early studies using CCS to avoid transferring aneuploid embryos (17), for CCS to improve the outcome after eSET, it must result in a change from traditional methods of selection. That is, the morphologically best embryo must sometimes be selected against due to aneuploidy. This prospective study demonstrates that blastocyst stage CCS changes embryo selection even among high-quality expanded blastocysts. Although eSET has primarily been promoted among young, good prognosis patients, all infertility patients could benefit from the reduced risk of multiple gestation, as long as overall outcomes are not compromised. A retrospective study and a small prospective study in a good prognosis population demonstrated that CCS may improve the outcome of single ET (5, 6). A randomized noninferiority trial demonstrated that eSET after CCS results in similar ongoing pregnancy rates as transferring two untested embryos in patients up to age 42 years with age-appropriate ovarian reserve (7). There are limited data on outcomes of eSET in an older population, but a recent study of 264 eSETs without CCS in women aged 40-44 years found a clinical miscarriage risk of 37.5% and live birth rate of 13.6% (18). Improvements in embryo selection are likely to be required to make eSET a viable option in this age group. The potential benefits of the improved selection offered by CCS are not restricted to patients of advanced age. Even in patients <35 years old, based on this study’s findings, one in seven eSETs with a high-quality blastocyst would be expected to have a negligible chance for delivery. Transferring an untested blastocyst may increase the risk for clinical miscarriage or an anomalous pregnancy (5).

The concept of eSET, especially in an older population, has been recently questioned given the relatively low risk of multiple gestation and the potential impact on overall success after IVF (19). This argument minimizes the increased obstetric risk in older patients and the worse obstetric outcome from twin pregnancies compared with two singleton pregnancies (20). The current study demonstrates that most couples who want to have two children will be able to have a second euploid blastocyst transfer after a successful cycle. The fact that their cryopreserved embryos are all individually vitrified euploid blastocysts makes it likely that future frozen transfers will be single ETs as well.

Although similar cumulative outcomes may be achieved by sequentially performing frozen single ETs of untested embryos, the practicality of this approach is in question. Most clinicians report deviating from American Society for Reproductive Medicine transfer guidelines after a failed IVF cycle (21). Patients experience increased depression and anxiety after IVF failure (22), adding pressure to transfer more embryos in the subsequent cycle. In reality, many patients are likely to opt for transfer of two embryos after failing with a fresh eSET.

To improve acceptance of eSET by patients and clinicians, there is a critical need to optimize the outcome of the first eSET. Improving embryo selection is an important step in that direction. A variety of noninvasive methods have been proposed to enhance selection for eSET including analysis of the transcriptome of cumulus cells (23, 24, 25), the embryonic secretome (26, 27), the metabolomic profile of spent culture media (28, 29), nutrient uptake and utilization (30), and time-lapse imaging parameters of early development (31, 32). However, none of these modalities has been shown to improve selection for eSET in a prospective clinical trial. Should benefit be documented from one of these approaches, it could be applied in parallel with an invasive approach as described in the present study and may further enhance selection among high-quality, euploid blastocysts.

Although the study population included a wide range of ages, all patients produced at least two high-quality blastocysts by day 5 and were true candidates for fresh eSET. Although patients whose embryos blastulated on day 6 did not receive fresh ETs, there is evidence that outcomes may be improved in these patients by vitrifying their blastocysts for a future frozen ET (33). Furthermore, there is emerging evidence that early blastocysts selected for day 5 eSET are significantly less likely to implant than expanded blastocysts (8). Because vitrified euploid blastocysts maintain high implantation rates (5, 17), further studies are required to determine whether vitrification and future frozen eSET is an optimal strategy and how often CCS would alter selection in these cases.

Although this study suggests that qPCR-based CCS may improve embryo selection and assist in optimizing eSET, this technology is currently available at a limited number of centers. There are other CCS platforms, such as single nucleotide polymorphism arrays, that have been extensively validated in preclinical studies (34) and the predictive value of an abnormal result has been determined using a.
nonselection trial [13]. The rapid nature of the qPCR technique used in the present study (relative to array-based methods) allows for transfers within the same cycle when embryos develop synchronously and blastulate by day 5. However, excellent outcomes may be achieved by vitrifying all tested blastocysts and sending biopsy samples to a reference laboratory [17]. Finally this approach may be cost effective when compared with array-based CCS methodologies as the added expense of whole genome amplification is not required.

It should be noted that this treatment approach does introduce additional laboratory procedures for some patients. For example, ICSI is recommended when genetic testing is performed to eliminate the risk of contamination from sperm or granulosa cells. In the current study, 68% of patients had a medical indication for ICSI. Although there is concern about increased risks of congenital malformations in babies conceived after ICSI [35], these may relate to the underlying male factor infertility rather than the procedure itself. Increased risk has not been documented in couples with normal semen parameters who use ICSI for preimplantation genetic screening rather than due to severe male factor infertility [36]. Assisted hatching is also required for all embryos being placed in extended culture as it facilitates the trophoectoderm biopsy procedure, which has been shown to be safer than cleavage stage biopsy [37]. Several prospective trials have demonstrated that assisted hatching is not harmful in an unselected infertile population [38]. Although there is concern that hatching may increase the risk of monozygotic twinning [39], centers with experience in extended culture and micromanipulation have not found this to be the case [40]. The low risk of monozygotic twinning in this study and other centers using assisted hatching for CCS [5,7] are reassuring, but continued collection of safety data will be important if this approach is to be widely implemented.

In summary, high-quality blastocysts selected by traditional day 5 morphology-based criteria have a substantial risk of aneuploidy across age groups. When selection is altered to prioritize euploidy versus morphology, patients achieve excellent outcomes with high implantation rates and low clinical miscarriage rates. Future research to identify noninvasive markers of reproductive potential may further enhance selection among euploid blastocysts.

REFERENCES


