Clinically recognizable error rate after the transfer of comprehensive chromosomal screened euploid embryos is low

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Objective: To determine the clinically recognizable error rate with the use of quantitative polymerase chain reaction (qPCR)–based comprehensive chromosomal screening (CCS).
Design: Retrospective study.
Setting: Multiple fertility centers.
Patient(s): All patients receiving euploid designated embryos.
Intervention(s): Trophectoderm biopsy for CCS.
Main Outcome Measure(s): Evaluation of the pregnancy outcomes following the transfer of qPCR-designated euploid embryos. Calculation of the clinically recognizable error rate.
Result(s): A total of 3,168 transfers led to 2,354 pregnancies (74.3%). Of 4,794 CCS euploid embryos transferred, 2,976 gestational sacs developed, reflecting a clinical implantation rate of 62.1%. In the cases where a miscarriage occurred and products of conception were available for analysis, ten were ultimately found to be aneuploid. Seven were identified in the products of conception following clinical losses and three in ongoing pregnancies. The clinically recognizable error rate per embryo designated as euploid was 0.21% (95% confidence interval [CI] 0.10–0.37). The clinically recognizable error rate per transfer was 0.32% (95% CI 0.16–0.56). The clinically recognizable error rate per ongoing pregnancy was 0.13% (95% CI 0.03–0.37). Three products of conception from aneuploid losses were available to the molecular laboratory for detailed examination, and all of them demonstrated fetal mosaicism.
Conclusion(s): The clinically recognizable error rate with qPCR-based CCS is real but quite low. Although evaluated in only a limited number of specimens, mosaicism appears to play a prominent role in misdiagnoses. Mosaic errors present a genuine limit to the effectiveness of aneuploidy screening, because they are not attributable to technical issues in the embryology or analytic laboratories. (Fertil Steril® 2014;102:1613–8. ©2014 by American Society for Reproductive Medicine.)
Key Words: Aneuploidy, preimplantation genetic screening, misdiagnosis, trophectoderm biopsy, quantitative PCR

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Embryonic aneuploidy screening has been used with success in assisted reproduction to improve overall pregnancy outcomes. The magnitude of improvement has been demonstrated by class I data that have shown the transfer of chromosomally normal embryos screened by comprehensive chromosomal screening (CCS) to significantly increase implantation

and delivery rates compared with unscreened embryos (1–3). By selecting only euploid embryos for transfer, investigators have also reported a reduced risk of clinical pregnancy loss (4). Perhaps most importantly, elective single-embryo transfer with CCS-screened embryos provides delivery rates per transfer equivalent to multiembryo transfer (5). The dramatic reduction in polyzygotic multiple gestation meaningfully enhances obstetrical and neonatal outcomes for patients who conceive with the use of these technologies (6).

Although clinical results have been excellent, the reality is that no screening paradigm is perfect. Embryonic aneuploidy screening with CCS is subject to both biologic and technical errors (7). Inevitably, that means that some patients will develop aneuploid gestations even after undergoing CCS during their IVF treatment cycle. A biologic error is any misdiagnosis that results from a complexity within the embryo rather than an error of test function. As such, biologic errors are limitations of the test rather than errors of test function. For example, these tests require normalization of each chromosome within a specimen against the other chromosomes within that same specimen (8, 9). This corrects for variation in the number of the cells in the biopsy, the loading volume when the biopsy is placed in the reaction tube, and the variability in the fidelity of the amplification itself. Therefore, haploidy, triploidy, and tetraploidy are not currently predictable.

Perhaps most important is the broader impact of embryonic mosaicism (10, 11). There are two clinically relevant types of mosaicism, that within the embryo and that within the biopsy sample. When mosaicism exists elsewhere within the embryo, an accurately processed and evaluated biopsy may correctly be designated as euploid while some portion of the embryo is aneuploid and may result in an abnormal clinical gestation. However, when mosaicism exists within the biopsy sample it can be detected with the use of single-nucleotide polymorphism (SNP) microarray when $\geq$40% of the cells are mosaic and with the use of array comparative genome hybridization (aCGH) when $\geq$50% are aneuploid (12, 13).

Technical errors might be attributed to specimen processing and handling, amplification fidelity, and a variety of factors affecting the informatics used to calculate the final result. Any one or more of these factors, alone or in combination with the biologic factors, may compromise the predictive value of the test and lead to the transfer of an embryo that results in an aneuploid gestation.

Data evaluating the predictive value of a normal result from quantitative polymerase chain reaction (qPCR) and SNP microarray are available, and within those studies no embryo that screened as euploid implanted and progressed into a clinical aneuploid pregnancy (1, 2, 4). Similarly, data are available evaluating the predictive value of a normal result from 204 day-3 aCGH cycles; 13 miscarriages were observed, three with evaluable products of conception, and no misdiagnoses were identified although maternal contamination was not excluded (14). The published misdiagnosis rate with aCGH is 1.9% when comparing outcomes to fluorescence in situ hybridization (FISH) results, which is problematic given the substantial error rate associated with FISH (15). In a comparative study, SNP microarray reanalysis indicated a significantly higher error rate with aCGH (7%) compared with qPCR (0%) (16). Although this is reassuring that the clinically recognizable error rate is low with all techniques, the reality is that these studies were not powered to provide a comprehensive evaluation of the false-normal rate of embryonic aneuploidy screening.

Unfortunately, clinical experience has shown that clinical misdiagnoses do occur and that aneuploid gestations have rarely developed after transferring embryos that were screened as euploid (7). These pregnancies represent adverse outcomes for patients. In those cases where the pregnancies arrest in early development and miscarry, the patients suffer the physical and emotional consequences of pregnancy loss and lose valuable time from their efforts to conceive and deliver a healthy gestation. Development into an ongoing aneuploid gestation has even more complex and potentially longstanding consequences for these patients.

Only very large clinical experiences would be sufficiently powered to estimate how often these potentially serious adverse outcomes result. The present study sought to review a large multicentered clinical experience to determine how often clinically detectable aneuploid gestations develop after transferring embryos designated as euploid with the use of CCS.

MATERIALS AND METHODS

Experimental Design

All centers using CCS in conjunction with Reproductive Medicine Associates Genetics were queried regarding the outcomes of the cycles in which screened embryos were transferred. All transfer cycles from the participating centers were reviewed to determine the following: 1) the total number of transfers; 2) the total number of embryos transferred; 3) the number of transfers where a clinical pregnancy was established; 4) the number of transfers where no pregnancies occurred; and 5) the number of transfers where evidence of aneuploid gestation was found. In the event where an aneuploid gestation was identified, the pregnancy was further categorized as having resulted in either a clinical loss or an ongoing/delivered gestation.

Owing to the multicenter and retrospective nature of the study design, not all pregnancies that resulted in a loss had fetal cellular material obtained for examination: ~50% of patients with a clinically recognized loss had an evaluation with tissue for diagnosis, and ~90% of those underwent cytogenetic analysis; therefore, no conclusion can be drawn from cases that did not undergo this procedure. In the case of a misdiagnosis, the study center was alerted and all other tested specimens were assumed to be of normal karyotype. Clearly, this methodology is not comprehensive, because some clinical pregnancies were lost but did not undergo dilation and curettage and thus had no tissue available for cytogenetic analysis. It is unknown if these losses were euploid or aneuploid. Regarding pregnancies that delivered, it seems very unlikely that an aneuploid gestation would remain unrecognized by the couple or the clinicians caring for the baby.