

Live birth outcome with trophectoderm biopsy, blastocyst vitrification, and single-nucleotide polymorphism microarray–based comprehensive chromosome screening in infertile patients

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The combination of trophectoderm biopsy, blastocyst vitrification, and single-nucleotide polymorphism microarray–based technology for comprehensive chromosome screening results in high implantation and live birth rates that could contribute to the practical application of single embryo transfer for infertility patients. (*Fertil Steril*® 2011;96:638–40. ©2011 by American Society for Reproductive Medicine.)

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Embryo assessment is a crucial component to the success of in vitro fertilization (IVF). Current selection methods are based on detailed embryo morphology with the highest implantation rates observed with the use of optimal morphologic characteristics (1, 2). Although this information contributes to the prediction of reproductive competence and is relatively successful in improving pregnancy rates and reducing multiple gestations, morphology is not absolute, with >70% of embryos created in vitro failing to implant. This failure is likely due to both the absence of developmentally competent embryos as well as our inability to select the most viable embryo in the cohort.

Chromosome aneuploidies are responsible for a portion of the observed reproductive waste. Cytogenetic studies of human oocytes, spontaneous miscarriages, and live births have highlighted the substantial prevalence of chromosome aneuploidies in human reproduction (3, 4). A direct association between chromosome aneuploidies and maternal age has been well documented (4). This led to the development of preimplantation genetic diagnosis (PGD) for aneuploidy screening to determine the chromosome constitution of embryos before transfer. Until recently, PGD for aneuploidy screening was based on 9–10 chromosome fluorescent in situ hybridization (FISH) assays. Although the general maternal age–related trends in chromosome aneuploidies were confirmed, clinical results from cleavage-stage embryos and single-cell FISH were disappointing, with randomized controlled trials reporting no clinical benefit (5–9).

The failure of PGD for aneuploidy screening to improve outcomes has been attributed to many factors, including the time point of biopsy, the intrinsic single-cell FISH error rate, and the fact that FISH is unable to screen for the whole complement of 23 pairs of human chromosomes. With the development of comprehensive chromosome

screening platforms, the promise of aneuploidy screening is becoming a reality in assisted reproduction technologies (ART) (10–13). More recently, a reliable and rapid single cell single-nucleotide polymorphism (SNP)–based microarray for aneuploidy screening of all chromosomes was developed and validated (13). During the same time period, procedures for trophectoderm biopsy and blastocyst vitrification were also optimized, allowing for their clinical application (14). The purpose of the present study was to clinically evaluate blastocyst comprehensive chromosome screening (CCS) with vitrification and the use of a validated SNP microarray–based technology.

Infertile couples presenting for infertility treatment at the Colorado Center for Reproductive Medicine were candidates for enrollment in this Institutional Review Board–approved study. Inclusion criteria included patients with advanced maternal age (≥ 38 years), recurrent pregnancy loss (≥ 2), or recurrent implantation failure (≥ 2 cycles).

All embryos were cultured in sequential media to the blastocyst stage. On day 3 of embryonic development a 5–10- μm channel was opened in the zona pellucida with a series of five pulses at 200 microseconds each at 100% power (Hamilton-Thorne Research). A trophectoderm (TE) biopsy was performed on day 5 or 6 of embryonic development relative to blastocyst development. Herniating TE cells were aspirated into a biopsy pipette and detached from the blastocyst by firing several pulses at the area of constriction. The aggregate of TE cells was placed intact into a polymerase chain reaction tube after several washes through hypotonic solution.

Whole-genome amplification and SNP-based microarray analysis was performed on biopsied TE cells at Reproductive Medicine Associates of New Jersey as previously described (13). Briefly, DNA produced using the WGA4 GenomePlex Whole Genome Amplification Kit (Sigma Aldrich) was then evaluated on the NspI Genchip Mapping 262K microarray as recommended by the supplier (Affymetrix). Copy number assignments were made using the CNAT 4.0 algorithm (Affymetrix) to assign aneuploidy status to each embryo.

Biopsied blastocysts were vitrified shortly after TE biopsy with the use of the Cryotop method and a dimethylsulfoxide/ethylene

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glycol protocol (15). Embryo selection for transfer was based first on the CCS diagnosis, with only euploid blastocysts having the correct number of chromosomes transferred in a subsequent natural frozen embryo transfer (FET) cycle. If there were several euploid blastocysts identified after CCS, morphology was the secondary selection tool.

The following results represent the first 100 FETs performed with only warmed euploid blastocysts diagnosed by SNP-based microarray analysis. These 100 transfers represented 130 cycles from 127 patients (three patients had a repeated cycle) who presented with advanced maternal age ($n = 38$), repeated miscarriage ($n = 39$), or repeated implantation failure ($n = 50$). The mean maternal age of recruited patients was 37.8 years at the time of oocyte retrieval. The majority (86.1%) of the women presented with normal ovarian reserve (based on day 3 FSH, antimullerian hormone, and antral follicle count), and the majority (90.2%) of their male partners showed no indications of male factor infertility (based on sperm concentration and motility; Table 1). An average 17.3 ± 8.1 oocytes were collected per retrieval, which resulted in a mean per patient of 5.9 ± 3.5 good-quality blastocysts (grade $\geq 3BB$) available for biopsy (Table 1). Five cycles (3.9%) did not produce any blastocysts on day 5 or 6 of embryonic development.

After SNP-based microarray analysis, 4.5% of biopsied TE cells produced “no result” and 47.4% blastocysts (356/751) were diagnosed as euploid and were therefore available for transfer (Table 1). Overall, the majority of CCS patients (80%) who had blastocysts biopsied produced euploid blastocysts eligible for transfer. Examination of CCS patients who produced 100% aneuploid blastocysts ($n = 25$) revealed a significantly greater mean maternal age (40.2 ± 3.2 years) than CCS patients with at least one euploid blastocyst (37.2 ± 3.2 years; $P < .0001$). All chromosomes were represented with losses and gains in these 361 aneuploid blastocysts. The smaller chromosomes, specifically chromosomes 15 (11.9%), 16 (17.7%), 19 (13.6%), 21 (14.9%), and 22 (17.4%), were most predominantly represented.

The biochemical pregnancy rate (positive β -hCG) for these initial 100 FETs performed with only warmed euploid blastocysts diagnosed by SNP-based microarray analysis was recorded at 87% (87/100). The clinical pregnancy rate with fetal heart tone for this series of patients was 73% (73/100). Of the 178 euploid blastocysts transferred (mean per FET 1.78), 119 (66.9%) implanted (sac), 115 (64.6%) with fetal heart tone. Two pregnancy losses occurred (2.7%) owing to uterine rupture and cardiac malformations. The remaining 71 ongoing clinical pregnancies resulted in 113 euploid babies born (71% live birth rate), with one infant diagnosed with a heart murmur, one with a hernia, and one who underwent heart surgery at birth. The live birth rate was also calculated as a percentage of all 130 CCS oocyte retrievals at 55.9% (Table 1). Regarding pregnancy history, two patients were diagnosed with gestational diabetes, and all premature deliveries (<37 weeks' gestation) involved multiple gestations.

The Colorado Center for Reproductive Medicine has published similar clinical outcomes using a reliable metaphase comparative genomic hybridization protocol as the CCS platform in conjunction with TE biopsy and blastocyst vitrification (14). Nonetheless, a SNP microarray-based technology, as reported in the present study, has many additional advantages as a CCS platform, including high throughput capacity, rapid turnaround, automation, and objective interpretation (13). Further genetic information that can be provided by an SNP microarray-based analysis includes inherited genetic variations, parental origin of aneuploidy, uniparental disomy, loss

TABLE 1

A. Patient (n = 127) clinical and cycle (n = 130) information.	
Maternal age (y)	37.8 (range 30–42)
Day 3 FSH	7.39 \pm 2.2
Antimullerian hormone	2.98 \pm 2.6
Antral follicle count	17.3 \pm 8.1
No. of oocytes retrieved	19.1 \pm 8.3
No. of oocytes fertilized by ICSI	12.8 \pm 5.5
Sperm motility	52.3%
Sperm concentration	86.9 million/mL
Good blastocyst development (grade $\geq 3BB$)	38%
No. of blastocysts biopsied and vitrified	5.9 \pm 3.5
B. Patient comprehensive chromosome screening (CCS) and clinical outcome.	
CCS results (n = 125 cycles)	
No result	4.5%
All aneuploid cycle	20%
Euploid blastocysts	47.4% (356/751)
Outcome results (n = 100 fresh frozen embryo transfers)	
Blastocyst survival after warming	96.8% (179/185)
Mean no. of euploid blastocysts transferred	1.78
Biochemical pregnancy	87% (87/100)
Clinical pregnancy (fetal heart tone)	73% (73/100)
Missed abortion	2.7% (2/73)
Implantation rate (fetal heart tone)	64.6% (115/178)
Euploid babies born	113 = 71% live birth rate per transfer = 55.9% live birth rate per oocyte retrieval
<i>Schoolcraft. Comprehensive chromosome screening. Fertil Steril 2011.</i>	

of heterozygosity to confirm monosomy, and DNA fingerprinting for contamination and/or embryo tracking (13).

In conclusion, the combination of TE biopsy, blastocyst vitrification, and SNP microarray-based CCS technology resulted in high implantation rates and low miscarriage rates for infertility patients and could contribute to the realization of the expected benefit of aneuploidy screening in ART. Currently, this platform is being examined for further clinical significance in an Institutional Review Board-approved randomized control trial. If validated, this platform could offer infertility couples who are indicated for aneuploidy screening the practical application of single embryo transfer and successful singleton live birth.

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