Clinical application of comprehensive chromosomal screening at the blastocyst stage

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Objective: To evaluate a new strategy for comprehensive chromosome screening at the blastocyst stage.
Design: Clinical research study.
Setting: An IVF clinic and a specialist preimplantation genetic diagnosis laboratory.
Patient(s): Forty-five infertile couples participated in the study. The mean maternal age was 37.7 years, and most couples had at least one previous unsuccessful IVF treatment cycle (mean 2.4).
Intervention(s): This study used a novel chromosome screening approach, combining biopsy of several trophectoderm cells on day 5 after fertilization and detailed analysis of all 24 types of chromosome using comparative genomic hybridization.
Main Outcome Measure(s): Proportion of embryos yielding a diagnostic result, aneuploidy rate, implantation rate, and pregnancy rate.
Result(s): A diagnosis was obtained from 93.7% of embryos tested. The aneuploidy rate was 51.3%. The probability of an individual transferred embryo forming a pregnancy reaching the third trimester/birth was 68.9%, an implantation rate 50% higher than contemporary cycles from the same clinic. The pregnancy rate was 82.2%.
Conclusion(s): The comprehensive chromosome screening method described overcomes many of the problems that limited earlier aneuploidy screening techniques and may finally allow preimplantation genetic screening to achieve the benefits predicted by theory. The high embryo implantation rate achieved is particularly encouraging and, if confirmed in subsequent studies, will be of great significance for IVF clinics attempting to reduce the number of embryos transferred or to implement single embryo transfer. (Fertil Steril® 2010;94:1700–6. ©2010 by American Society for Reproductive Medicine.)

Key Words: Microarray, chromosome, aneuploidy, preimplantation genetic diagnosis, preimplantation genetic screening, single embryo transfer, implantation, in vitro fertilization

In vitro fertilization (IVF) treatments typically involve the production of multiple embryos. However, the viability of individual embryos is highly variable. Even among a cohort of sibling embryos competence can vary greatly. The challenge for IVF clinics is to correctly identify the most viable embryos and prioritize them for transfer to the uterus. Currently, the decision of which embryo(s) to transfer is made on the basis of morphologic assessments conducted in the IVF laboratory. Unfortunately, such examinations do not provide reliable information concerning chromosomal copy number, one of the most important aspects of embryo viability. Abnormalities of chromosome copy number (aneuploidies) are common in human oocytes and embryos and increase markedly with advancing maternal age; by the age of 40 years it is not unusual for the proportion of aneuploid oocytes to exceed 50% (1, 2).

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Embryos produced from chromosomally abnormal oocytes display aneuploidy in all of their cells, and it is widely accepted that such embryos have little potential for forming a viable pregnancy. The lethality of meiotic aneuploidy is highlighted by the fact that the majority (>60%) of first-trimester spontaneous abortions have an abnormal number of chromosomes (3, 4). It is likely that the rapid increase in oocyte aneuploidy seen with advancing age is one of the principal causes of the equally rapid age-related decline in IVF success rates.

The inability of conventional methods of embryo evaluation to detect aneuploidy has led to the proposal that IVF-generated embryos should undergo chromosomal screening (5). In theory, the identification and transfer of euploid embryos during IVF cycles should lead to increased pregnancy rates and decreased risks of spontaneous abortion and chromosomal syndromes such as Down syndrome (6–12). Given the high frequency and lethality of chromosome abnormalities, the hypothesis underlying preimplantation genetic screening (PGS) seems to be reasonable. However, recent studies aimed at assessing efficacy of PGS for patients of advanced maternal age have failed to show any improvement in IVF outcome using traditional chromosome screening methods (13–15). There are many possible reasons why these clinical studies failed to deliver the expected improvements in IVF outcome. Disappointing results may have been a consequence of an incomplete understanding of important aspects of embryo biology, such as chromosomal mosaicism. Alternatively, it has been argued that poor embryo biopsy technique,

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coupled with inadequate cytogenetic methods, may have led to impaired embryo viability and reduced diagnostic accuracy, eliminating any potential benefit of screening (16).

Here we report clinical application of a novel aneuploidy screening strategy. The method is applied to embryos at the blastocyst stage, 2–3 days later than traditional methods of PGS. Additionally, the procedure involves screening of the entire chromosome complement, rather than the limited chromosome assessment typically used for the purpose of PGS. We suggest that this approach may overcome the technical difficulties that beset earlier PGS studies, allowing preimplantation aneuploidy screening to finally achieve the clinical potential predicted by theory.

METHODS

Patients

Chromosome screening was offered to infertile patients of advanced maternal age (>35 years) and/or with a history of unsuccessful IVF treatments or previous spontaneous abortion (Table 1). The study was conducted after receiving Institutional Review Board approval. Patients were provided with counseling, and signed consents were obtained. A total of 45 patients underwent comparative genomic hybridization (CGH) screening. Additionally, 113 patients undergoing blastocyst transfer in the same center during the same time period were assessed for comparative purposes. The group was well matched for all relevant clinical parameters, including maternal age, day 3 FSH level, day of transfer (all cycles involved blastocyst transfers), number of oocytes retrieved, fertilization rate, number of blastocysts produced, and number of previous unsuccessful IVF attempts (Table 2).

In Vitro Fertilization, Embryo Culture, and Blastocyst Biopsy

Standard methods of controlled ovarian stimulation were used. Intracytoplasmic sperm injection (ICSI) was performed in all cases. Embryos with two pronuclei were transferred to individual 30 μL drops of cleavage-stage medium (Sage or Vitrolife) in a 30 mm Falcon culture dish, overlaid with 3 mL Ovoil (Vitrolife) and cultured for 48 hours at37°C in 5% O_2 and 6% CO_2. On day 3 of embryonic development, the zona pellucida was breached using a laser (Hamilton Thorne) and all embryos were transferred to individual 30 μL drops of blastocyst medium (Sage or Vitrolife) and incubated for a further 48 hours at 37°C in 5% O_2 and 6% CO_2. On the morning of day 5, blastocyst development was assessed (17). Expanding and expanded blastocysts underwent biopsy of herniating trophectoderm cells using a laser (Fig. 1). Cavitating morulas and early blastocysts were transferred to a fresh individual 30 μL drop of blastocyst medium (Sage or Vitrolife) and biopsy was attempted 24 hours later.

Comparative Genomic Hybridization

Each trophectoderm biopsy was washed in sterile phosphate-buffered saline and transferred to a microcentrifuge tube. To generate the ~1 μg of DNA required for CGH analysis, the biopsied cells were lysed and the entire genome amplified using degenerate oligonucleotide–primed polymerase chain reaction (18). Amplified DNA was labeled with a green fluorescent molecule (Spectrum Green–dUTP; Abbott) via nick translation. Similarly, DNA from a chromosomally normal individual was amplified and labeled with red fluorescence (Spectrum Red–dUTP; Abbott). The green (embryo) and red (normal reference) DNAs were mixed together and simultaneously applied to normal male metaphase chromosomes on a microscope slide as we have previously described (18). The two DNA samples hybridize to complementary sequences on the chromosomes, coating them with red and green DNA fragments. The ratio of green to red fluorescence observed for each chromosome is indicative of copy number (Fig. 2).

Vitrification

Because the approach described here requires biopsy on day 5 or 6 followed by a CGH procedure taking ~72 hours, cryopreservation was necessary. For this purpose, biopsied blastocysts were vitrified using the Cryotop method (19).

Blastocyst Transfer

Endometrial preparation consisted of oral contraceptive pill for 14 days with daily Lupron (10 IU; TAP Pharmeceuticals) 5 days before the last pill. On day 3 of menstrual bleeding transdermal E_2 patches (0.1 mg; Vivelle; Novartis) were administered every other day at increasing doses for 14 days. After 14 days, patients began micronized progesterone (200 mg daily; Prometrium; Solvay). Blastocyst transfers were performed on the sixth day of progesterone administration.

Before transfer, blastocysts were warmed at 37°C in thawing solution of 1 mol/L sucrose for 60 seconds, followed by a dilution solution of 0.5 mol/L sucrose for 3 minutes and washing with no sucrose for 5 minutes. Transfer was performed 3–4 hours after warming, as described previously (20).

RESULTS

The data described here were derived from all 45 patients that participated in the study, without any omissions or exclusions. The 45 patients produced a total of 287 blastocyst-stage embryos. Biopsy was successful for 100% of embryos, yielding 3–10 cells (mean 5) for subsequent CGH analysis. A total of 269 blastocysts were successfully assessed using CGH (93.7% diagnostic efficiency). Of these, 138 (51.3%) were diagnosed as aneuploid. Ninety of the normal embryos were subsequently thawed and transferred to the uterus. Embryo survival after biopsy, vitrification, and thaw was excellent: 90/90 (100%).

Measurement of hCG 9 days after embryo transfer, revealed a pregnancy rate per oocyte retrieval in the CGH group of 82.2% (Table 2). This compared well with the matched contemporary cycles (84% pregnancy rate), despite the fact that ~25% fewer embryos were transferred in cycles involving chromosome screening. The proportion of transferred embryos that successfully implanted was evaluated by ultrasound 6.5 weeks after transfer, revealing that 72.2% (65/90) of embryos transferred after aneuploidy screening produced a fetal sac. In comparison, clinically similar, contemporary cycles without screening were associated with an implantation rate of 46.5% (139/299). Later ultrasound screening to assess fetal heartbeat provided similar results. For the CGH group, 68.9% of embryos transferred produced a fetus, whereas for the clinically matched contemporary cycles the figure was 44.8% (Table 2). Live birth rates per cycle were 75.6% in the CGH group, compared with 69.0% in contemporary matched cycles.

Data from our ongoing clinical work indicates that many of the observations reported here will be reciprocated in even larger patient populations. At the time of writing, we had already analyzed embryos from a further 30 cycles. These patients had not yet had their embryos transferred and consequently no clinical data were yet available. However, we were able to confirm that the aneuploidy rate is essentially identical to that observed in the first 45 cycles reported here and that CGH had continued to yield diagnostic results for ~95% of embryos tested. Importantly, almost all patients have at least one embryo eligible for transfer. Thus far, only 4% of patients have had no transfer owing to all embryos diagnosed as abnormal.

DISCUSSION

One of the greatest challenges currently facing IVF practitioners is how to reduce the risk of multiple pregnancy, while maintaining (or improving) pregnancy rates. The transfer of fewer embryos is effective at reducing multiple pregnancies but increases the probability that no viable embryo will be transferred, potentially decreasing the pregnancy rate per embryo transfer. As attempts are made to