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® 2009; ■: ■–■. ©2009 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,

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

TABLE 1

Patient information and outcome of treatment cycles using aneuploidy screening.

Patient no.	Maternal age	Notes on previous reproductive history	No. of blastocysts tested	No. of blastocysts with a diagnostic result	No. of abnormal blastocysts	No. of embryos transferred	Positive pregnancy test	Number of fetal hearts detected
1	30	SAB × 4	2	2	1	1	Yes	1
2	31	—	4	4	2	2	Yes	1
3	32	IF × 3	9	8	3	2	Yes	1
4	33	SAB × 2	3	3	1	2	Yes	1
5	33	IF × 4	5	5	2	2	Yes	2
6	33	SAB × 6	11	11	8	2	Yes	2
7	34	SAB × 3	7	7	2	3	Yes	3
8	34	IF × 10	5	5	2	2	No	0
9	34	IF × 1	8	7	3	2	Yes	2
10	34	IF × 2	4	4	4	0	No	No transfer
11	34	SAB × 1, IF × 2	9	9	5	2	Yes	2
12	34	—	7	7	2	2	Yes	2
13	35	—	5	3	0	3	Yes	3
14	36	SAB × 3	9	7	3	2	Yes	2
15	36	IF × 4	4	4	0	4	Yes	3
16	37	IF × 2	8	8	6	2	Yes	1
17	37	SAB × 3	9	9	4	2	Yes	1
18	37	—	9	8	2	2	Yes	2
19	37	SAB × 1	4	4	2	2	Yes	1
20	37	IF × 6	5	5	3	2	Yes	2
21	37	IF × 6	10	10	7	3	Yes	2
22	38	IF × 2	6	6	4	2	No	0
23	38	SAB × 1	6	6	3	2	Yes	2
24	38	IF × 3	6	6	2	2	Yes	2
25	39	SAB × 2	11	11	4	2	Yes	1
26	39	IF × 4	3	3	1	1	Yes	1
27	40	—	8	8	4	2	Yes	2
28	40	—	6	6	4	2	Yes	1
29	40	IF × 1	6	6	4	2	Yes	1
30	40	IF × 2	4	4	2	2	Yes	2
31	40	—	10	6	2	3	Yes	3
32	40	—	15	15	10	2	No	0
33	40	—	7	6	3	2	Yes	1
34	41	SAB × 3	8	7	6	1	Yes	1
35	41	—	5	4	2	2	No	0
36	41	IF × 6	5	4	3	1	Yes	1
37	41	—	5	5	3	2	No	0
38	41	IF × 1	4	4	2	2	Yes	1
39	41	—	9	9	6	2	Yes	2
40	42	SAB × 3	4	3	0	3	Yes	2
41	42	IF × 6	2	2	1	1	No	0
42	42	IF × 4	3	3	1	2	Yes	2
43	42	—	7	7	4	3	Yes	2
44	42	SAB × 2	7	6	4	2	Yes	1
45	43	SAB × 6	3	2	1	1	No	0
Total	Mean: 37.7		Mean per cycle: 6.4	Diagnostic efficiency: 93.7%	Blastocyst aneuploidy rate: 51.3%	Mean blastocysts transferred per cycle: 2.0	Pregnancy rate per cycle: 82.2%; per transfer: 84.1%	Implantation rate per embryos transferred: 68.9% (62/90)

Note: IF = implantation failure; SAB = spontaneous abortion.

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move toward single embryo transfer, reliable methods for identifying the embryo(s) with the greatest potential for producing a child become increasingly important.

Chromosome abnormality has a major impact on embryo viability and is one of the principal causes of failed IVF attempts; yet in most cases such anomalies are invisible to morphologic analysis.

TABLE 2

Comparison of patient characteristics and treatment cycle outcome for patients with aneuploidy screening and a set of contemporary cycles from the same clinic.

	Contemporary comparison group (n = 113)	Comprehensive chromosome screening group (n = 45)
Average maternal age (yrs)	37.1	37.7
Average no. of previous failed IVF treatments	1.24	2.42
Day 3 FSH (IU)	7.6	7.3
Average no. of oocytes retrieved per cycle	19.4	18.6
Average no. of blastocysts transferred per cycle	2.7 (299 transfrred in 113 cycles)	2.0 (90 transferred in 45 cycles)
Biochemical pregnancy (positive pregnancy test) per cycle[a]	84.0% (95/113)	82.2% (37/45)[a]
Implantation rate (proportion of transferred embryos producing a fetal sac)	46.5% (139/299)	72.2% (65/90)[b]
Implantation rate (proportion of transferred embryos producing a fetus with heartbeat)	44.8% (134/299)	68.9% (62/90)[c]

[a] Only one patient had no euploid embryos available for transfer. The pregnancy rate per cycle with an embryo transfer was 84.1% (37/44).
[b] $P<.0001$ (chi-squared test with Yates correction).
[c] $P<.0001$ (chi-squared test with Yates correction).

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To avoid the transfer of embryos harboring fatal chromosome errors, many laboratories now perform PGS for various indications. Typically, PGS involves the biopsy of a single cell from embryos at the 6–10-cell stage (3 days after fertilization), followed by chromosomal analysis and preferential transfer of euploid embryos. Although several authors have reported improvements in IVF outcome after PGS (6–12), a number of recent studies, focused on patients of advanced maternal age, have failed to demonstrate improvements in IVF outcome, casting doubt upon the efficacy of chromosome screening (13–15).

Despite the controversy surrounding the use of PGS, there is universal agreement that chromosome abnormality is common in oocytes and embryos produced during IVF. Additionally, there is consensus that aneuploidy, at least that originating during meiosis, is almost always lethal to the developing embryo. Why then has PGS been unsuccessful in some studies? There are biologic and technical explanations for this apparent contradiction.

In terms of biology, the main problem is chromosomal mosaicism. The central premise of PGS is that the single cell biopsied is representative of the rest of the embryo, yet studies have shown that approximately 20%–40% of human embryos are composed of a mixture of normal and aneuploid cells, so-called chromosomal mosaics (21–24). This fact poses a potential problem for diagnostics based on the sampling of a single cell. In the present study, the risk of

FIGURE 1

Blastocyst biopsy. On day 3, a small "channel" in the zona pellucida approximately 5 μm wide was created using a laser (Hamilton Thorne). Embryos were then transferred to blastocyst-stage medium and incubated for a further 48 hours (day 5 of development). Day 5 and 6 blastocysts graded as grade 3 or higher (19) with herniating trophectoderm cells were identified for biopsy (**A**). Using an inverted Nikon microscope with Narishige manipulators and injectors, the herniating trophectoderm cells protruding through the day 3 hole in the zona pellucida were aspirated into a 30 μm biopsy tool (**B**), and the laser was fired at the constricted area of cells to gently separate them from the rest of the blastocyst (**C**).

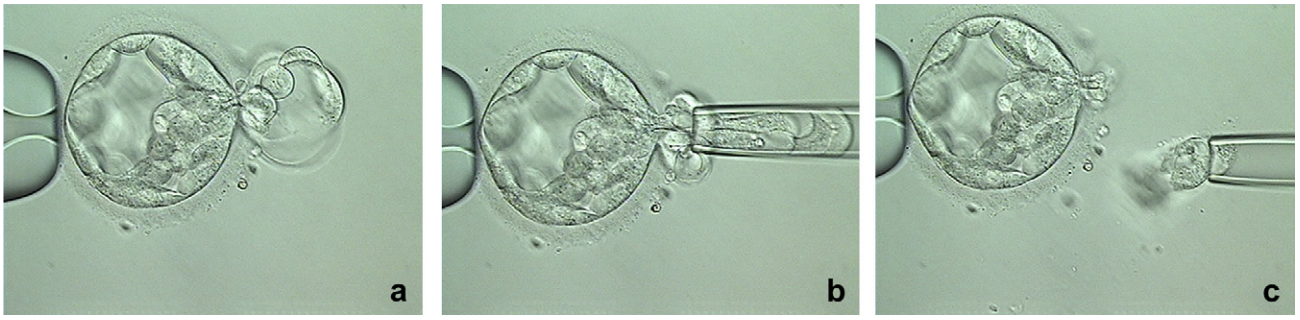

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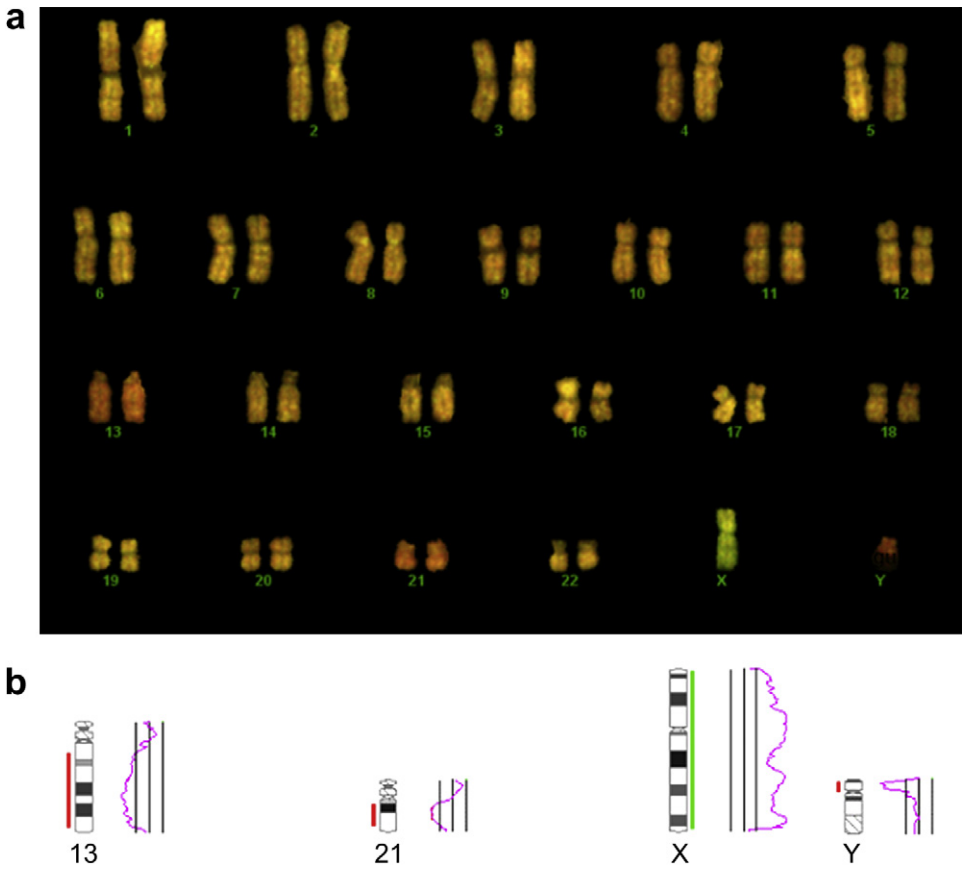
FIGURE 2

Comparative genomic hybridization (CGH) analysis of a blastocyst-stage embryo. (**A**) Normal male metaphase chromosomes used for CGH. If there are a normal number of chromosomes in the sample, the red (normal male) and green (embryo) DNAs hybridize equally, resulting in a 1:1 ratio of red to green fluorescence along the length of each chromosome. However, if any chromosomes are present in excess (e.g., if the embryo has a trisomy) green DNA out-competes red for hybridization to the affected chromosome, resulting in a shift in the red:green ratio in favor of green coloration. Conversely, chromosome loss results in a shift toward the red. In this example, chromosomes 13 and 21 display an excess of red fluorescence, indicating monosomy. Additionally, the X-chromosome appears green and the Y-chromosome red, indicating that the embryo is female. The predicted karyotype of this embryo is therefore 44,XX,−13,−21. (**B**) Red:green fluorescence ratios for chromosomes 13, 21, X, and Y. The central black line represents a 1:1 ratio, the line on the left is considered to be the threshold for chromosome loss, and the line on the right represents the threshold for chromosome gain (relative to the normal male reference DNA).



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misdiagnosis due to mosaicism was greatly reduced by performing embryo biopsy at the blastocyst stage, 2 days later than traditional PGS methods. At this point, several cells can be safely sampled from the trophectoderm, providing a more representative overview of the chromosomal status of the embryo. Our previous preclinical work has confirmed that the cells sampled during blastocyst biopsy are highly representative of the remainder of the embryo (25).

In terms of technical issues (16), the main problem is the biopsy technique used to sample genetic material from the embryo. There is good evidence that the removal of a single cell from a 6–10-cell embryo may lead to a small reduction in viability (26). The negative impact of biopsy is likely to be much greater if the biopsy is poorly performed, potentially eliminating any benefit obtained by embryo screening. Excessive biopsy damage appears to have played a role in at least one recent PGS study that found no benefit of chromosome screening (27, 28). The blastocyst biopsy used in the present study is thought to be less detrimental to embryo viability than day 3 biopsy. This hypothesis is supported by the 100% survival and high implantation rates observed, as well as by earlier studies (29). With little or no impact of biopsy at the blastocyst stage, aneuploidy screening can be carried out safely, without any penalty to the embryo regarding reduced viability.

The effectiveness of chromosomal screening methods also depends on the ability to accurately distinguish euploid embryos from those affected by aneuploidy. Almost all previous PGS studies have been based upon the use of fluorescence in situ hybridization (FISH). Although FISH has allowed accurate screening of restricted numbers of chromosomes, the method is limited in that less than one-half of the chromosomes can be enumerated in each biopsied cell. Data from the present study, as well as our previous preclinical work using comprehensive screening methods, indicates that even the best FISH-based methods fail to detect ~20% of abnormal embryos, and for others more than one-half of the abnormal embryos may go undetected (30). The use of CGH allows all of the

chromosomes to be evaluated, thus revealing nearly 100% of aneuploid embryos. Additionally, the CGH method provides the advantage of avoiding the technically challenging process of cell fixation on a microscope slide.

If screening is to improve IVF outcomes, it is vital that results are obtained from the vast majority of embryos tested. The present study displayed a high diagnostic efficiency, with comprehensive aneuploidy screening results obtained from 93.7% of embryos. This is similar to the best success rates achieved using FISH (11) and far exceeds the diagnostic efficiency reported by some laboratories. For example, one high-profile study that found no benefit of PGS using FISH, failed to obtain any diagnosis from one-fifth of all embryos tested, severely impairing the identification of euploid embryos (14).

The present study was intended to be a preliminary clinical evaluation, providing proof of principle for a new strategy. As such, there was no attempt to randomize patients or to create a flawless control group. Nonetheless, it was interesting, and possibly indicative, to compare results obtained after CGH screening of blastocysts with contemporary cycles occurring in the same clinic during the same time period. The comparison cycles were exceptionally well matched for all key clinical parameters. Blastocyst-stage embryos from the cycles used for comparison were prioritized for transfer on the basis of traditional morphologic analysis, whereas those from the study group were selected primarily on the basis of euploidy. Ongoing pregnancy rates per oocyte retrieval were slightly higher for cycles involving chromosome screening (75.6% vs. 69.0%). Although this increase was not statistically significant, it is noteworthy that fewer embryos were transferred in cycles involving CGH (mean 2.0 transferred in cycles with aneuploidy screening vs. 2.7 in non-CGH cycles). The patients in the CGH group and those in the comparison group were all capable of producing blastocysts and can therefore be considered to be of good prognosis from an embryologic perspective. However, it should also be remembered that most patients were of advanced maternal age and/or had a history of failed IVF attempts or spontaneous abortions. A 75.6% pregnancy rate per cycle for this patient group is therefore very encouraging. Furthermore, all of the pregnancies reported here were at the time of writing in the third trimester or had delivered, and thus it is very likely that the birth rate after CGH will also be ~75% per cycle.

Although pregnancy rates were impressive, the implantation rate (i.e., the probability of an embryo selected for transfer forming a clinical pregnancy) was even more striking. The implantation rate is the most important statistic to note when considering single embryo transfer, because in such cases implantation rate and pregnancy rate are equivalent. Our data suggest that single embryo transfer after CGH screening would yield pregnancy rates of ~69% per blastocyst transfer compared with ~45% in cycles without screening, a 1.5-fold increase in the probability of an embryo selected for transfer producing a child ($P<.0001$). It is probable that implantation and pregnancy rates would be higher still in younger patients or those without a history of failed IVF attempts.

The high implantation rate observed during this study may be indicative of improved embryo selection due to aneuploidy screening. However, an interesting alternative is that implantation rates may have benefited from embryo transfers taking place 1–2 months after the original stimulated cycle. There have been several reports suggesting decreased uterine receptivity in stimulated cycles (31, 32). To overcome this problem, some researchers have advocated routine cryopreservation of all embryos, with thaw and transfer in a subsequent cycle. We suspect that the apparent increase in implantation rate seen during the present study is attributable to a combination of optimized uterine receptivity and improved embryo selection. It would be useful if future randomized controlled trials could be designed such that the relative contribution of these two factors to any improvements in implantation could be delineated.

Although chromosome analysis at the blastocyst stage appears to overcome several significant difficulties facing PGS, a recent trial, conducted by a group that has pioneered the use of blastocyst biopsy for preimplantation genetic diagnosis, yielded disappointing results (33). The study in question involved trophectoderm biopsy followed by FISH and found no improvement in IVF outcome. There are two likely reasons for these disappointing results. First, only five chromosomes were screened, leaving almost four-fifths of the chromosome complement untested, weakening the selective power of the screening (30). Second, embryos were diagnosed as aneuploid and excluded from transfer even if only one of the biopsied cells gave an abnormal number of FISH signals. Many blastocysts are chromosomally normal in the vast majority of their cells, but contain occasional aneuploid cells. Although unproven at this time, it is highly likely that the presence of small numbers of aneuploid cells is of little or no clinical consequence. The exclusion from transfer of potentially viable embryos in this case probably contributed to a reduction in pregnancy rates.

The CGH study described in the present paper treated the entire trophectoderm biopsy as a single entity rather than as individual cells. The diagnosis thus reflects an average result from all of the cells biopsied, with aneuploidies only detected if they represent approximately one-third or more of the biopsied cells. While such an approach may rarely lead to a diagnostic error, we suggest that in most cases it helps to avoid inappropriate diagnosis caused by clinically irrelevant mosaicism. The high pregnancy and implantation rates recorded in this study suggest that few, if any, clinically significant errors occurred during the present series.

The exceptional implantation rate achieved during this study suggests that the blastocyst-CGH strategy would be particularly advantageous when used in conjunction with single embryo transfer. Indeed, the transfer of more than one embryo in most cycles during the present study led to a high rate of multiple pregnancy (57% of gestations involved more than one fetus). It is clear that with such high implantation rates, the number of embryos transferred per cycle needs to be reduced.

It has often been argued that the transfer of more than one embryo is advisable for patients of advanced maternal age or those with a history of multiple unsuccessful IVF treatments. In the present study, a 73% implantation rate was achieved for the 11 patients that had three or more previous failed IVF treatments (mean 5.1). The implantation rate for the 19 patients with a maternal age of $\geq$40 years (mean 41 years) was 59.5%. These data suggest that single embryo transfer should still be considered for such patients, at least for those capable of producing chromosomally normal blastocysts.

The blastocyst-CGH approach shows enormous promise, but comes in the wake of trials suggesting that chromosome screening is of no advantage to patients undergoing IVF. The methods reported here differ in many important respects to earlier PGS studies and overcome many of the difficulties that beset earlier diagnostic approaches. However, we acknowledge that some skepticism over the findings is inevitable. It is clear that the establishment of a multicenter randomized trial to confirm the efficacy of this technology must now follow.

REFERENCES

1. Kuliev A, Verlinsky Y. Meiotic and mitotic nondisjunction: lessons from preimplantation genetic diagnosis. Hum Reprod Update 2004;10:401–7.
2. Pellestor F, Andréo B, Anahory T, Hamamah S. The occurrence of aneuploidy in human: lessons from the cytogenetic studies of human oocytes. Eur J Med Genet 2006;49:103–16.
3. Boué J, Boué A, Lazar P. Retrospective and prospective epidemiological studies of 1500 karyotyped spontaneous human abortions. Teratology 1975;12:11–26.
4. Menasha J, Levy B, Hirschhorn K, Kardon NB. Incidence and spectrum of chromosome abnormalities in spontaneous abortions: new insights from a 12-year study. Genet Med 2005;7:251–63.
5. Munné S, Lee A, Rosenwaks Z, Grifo J, Cohen J. Diagnosis of major chromosome aneuploidies in human preimplantation embryos. Hum Reprod 1993;8: 2185–91.
6. Gianaroli L, Magli MC, Ferraretti AP, Fiorentino A, Garrisi J, Munné S. Preimplantation genetic diagnosis increases the implantation rate in human in vitro fertilization by avoiding the transfer of chromosomally abnormal embryos. Fertil Steril 1997;68: 1128–31.
7. Munné S, Magli C, Bahçe M, Fung J, Legator M, Morrison L, et al. Preimplantation diagnosis of the aneuploidies most commonly found in spontaneous abortions and live births: XY, 13, 14, 15, 16, 18, 21, 22. Prenat Diagn 1998;18:1459–66.
8. Munné S, Magli C, Cohen J, Morton P, Sadowy S, Gianaroli L, et al. Positive outcome after preimplantation diagnosis of aneuploidy in human embryos. Hum Reprod 1999;14:2191–9.
9. Munné S, Sandalinas M, Escudero T, Velilla E, Walmsley R, Sadowy S, et al. Improved implantation after preimplantation genetic diagnosis of aneuploidy. Reprod Biomed Online 2003;7:91–7.
10. Munné S, Fischer J, Warner A, Chen S, Zouves C, Cohen J, Referring Centers PGD Group. Preimplantation genetic diagnosis significantly reduces pregnancy loss in infertile couples: a multicenter study. Fertil Steril 2006;85:326–32.
11. Colls P, Escudero T, Cekleniak N, Sadowy S, Cohen J, Munné S. Increased efficiency of preimplantation genetic diagnosis for infertility using "no result rescue." Fertil Steril 2007;88:53–61.
12. Garrisi GJ, Colls P, Ferry KM, Zheng X, Garrisi MG, Munné S. Effect of infertility, maternal age and number of previous miscarriages on the outcome of preimplantation genetic diagnosis for idiopathic recurrent pregnancy loss. Fertil Steril 2009;92:288–95.
13. Staessen C, Platteau P, Van Assche E, Michiels A, Tournaye H, Camus M, et al. Comparison of blastocyst transfer with or without preimplantation genetic diagnosis for aneuploidy screening in couples with advanced maternal age: a prospective randomized controlled trial. Hum Reprod 2004;19: 2849–58.
14. Mastenbroek S, Twisk M, van Echten-Arends J, Sikkema-Raddatz B, Korevaar JC, Verhoeve HR, et al. In vitro fertilization with preimplantation genetic screening. N Engl J Med 2007;357:9–17.
15. Hardarson T, Hanson C, Lundin K, Hillensjö T, Nilsson L, Stevic J, et al. Preimplantation genetic screening in women of advanced maternal age caused a decrease in clinical pregnancy rate: a randomized controlled trial. Hum Reprod 2008;23:2806–12.
16. Munné S, Wells D, Cohen J. Technology requirements for preimplantation genetic diagnosis to improve art outcome. Fertil Steril. Published online April 29, 2009 [Epub ahead of print].
17. Gardner DK, Schoolcraft WB. Culture and transfer of human blastocysts. Curr Opin Obstet Gynecol 1999;11:307–11.
18. Wells D, Escudero T, Levy B, Hirschhorn K, Delhanty JD, Munné S. First clinical application of comparative genomic hybridization and polar body testing for preimplantation genetic diagnosis of aneuploidy. Fertil Steril 2002;78:543–9.
19. Kuwayama M. Highly efficient vitrification for cryopreservation of human oocytes and embryos: the Cryotop method. Theriogenology 2007;67:73–80.
20. Schoolcraft WB, Surrey ES, Gardner DK. Embryo transfer: techniques and variables affecting success. Fertil Steril 2001;76:863–70.
21. Wells D, Delhanty JD. Comprehensive chromosomal analysis of human preimplantation embryos using whole genome amplification and single cell comparative genomic hybridization. Mol Hum Reprod 2000;6:1055–62.
22. Voullaire L, Slater H, Williamson R, Wilton L. Chromosome analysis of blastomeres from human embryos by using comparative genomic hybridization. Hum Genet 2000;106:210–7.
23. Vanneste E, Voet T, Le Caignec C, Ampe M, Konings P, Melotte C, et al. Chromosome instability is common in human cleavage-stage embryos. Nat Med 2009;15:577–83.
24. Munné S, Chen S, Colls P, Garrisi J, Zheng X, Cekleniak N, et al. Maternal age, morphology, development and chromosome abnormalities in over 6000 cleavage-stage embryos. Reprod Biomed Online 2007;14:628–34.
25. Fragouli E, Lenzi M, Ross R, Katz-Jaffe M, Schoolcraft WB, Wells D. Comprehensive molecular cytogenetic analysis of the human blastocyst stage. Hum Reprod 2008;23:2596–608.
26. Cohen J, Wells D, Munné S. Removal of 2 cells from cleavage stage embryos is likely to reduce the efficacy of chromosomal tests that are used to enhance implantation rates. Fertil Steril 2007;87: 496–503.
27. Cohen J, Grifo JA. Multicentre trial of preimplantation genetic screening reported in the *New England Journal of Medicine*: an in-depth look at the findings. Reprod Biomed Online 2007;15:365–6.
28. Munné S, Cohen J, Simpson JL. In vitro fertilization with preimplantation genetic screening. N Engl J Med 2007;357:1769–70.
29. McArthur SJ, Leigh D, Marshall JT, Gee AJ, De Boer KA, Jansen RP. Blastocyst trophectoderm biopsy and preimplantation genetic diagnosis for familial monogenic disorders and chromosomal translocations. Prenat Diagn 2008;28:434–42.
30. Munné S, Fragouli E, Colls P, Katz M, Wells D. Comprehensive chromosome screening indicates that an appropriately designed 12-chromosome FISH test would detect 91% of aneuploid blastocysts. RBM Online. In press.
31. Ho PC. New frontiers of assisted reproductive technology (Chien Tien Hsu Memorial Lecture 2007). J Obstet Gynaecol Res 2009;35:1–8.
32. Shih W, Rushford DD, Bourne H, Garrett C, McBain JC, Healy DL, Baker HW. Factors affecting low birthweight after assisted reproduction technology: difference between transfer of fresh and cryopreserved embryos suggests an adverse effect of oocyte collection. Hum Reprod 2008;23: 1644–53.
33. Jansen RP, Bowman MC, de Boer KA, Leigh DA, Lieberman DB, McArthur SJ. What next for preimplantation genetic screening (PGS)? Experience with blastocyst biopsy and testing for aneuploidy. Hum Reprod 2008;23:1476–8.