Preimplantation genetic screening does not improve delivery rate in women under the age of 36 following single-embryo transfer

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BACKGROUND: Single-embryo transfer is a well-accepted strategy to avoid multiple pregnancies in an assisted reproductive technology (ART) programme. Besides the morphological quality and embryo kinetics up to the blastocyst stage, preimplantation genetic screening (PGS) of aneuploidy has been advocated as an adjuvant approach to select the embryo. METHODS: Couples with a female partner younger than 36 were randomly assigned to undergo transfer of a single blastocyst in a cycle with or without PGS using FISH for the chromosomes X, Y, 13, 16, 18, 21, 22. RESULTS: After the enrolment of 120 of the projected 447 patients in each group, study recruitment was terminated prematurely on the basis of futility. The observed live birth delivery rates after ART were 30.8 versus 30.8% per randomized patient, 34.6 versus 34.6% per cycle initiated, 37.8 versus 37.0% per aspirated cycle and 41.6 versus 43.5% per embryo transfer for the control versus the PGS group, respectively, with absolute between-group differences (95% CI; P value) of 0% (−11.7 to 11.7; P = 1.00), 0% (−12.7 to 12.7; P = 1.00), −0.8% (−14.2 to 12.7; P = 0.91) and 2.1% (−12.7 to 16.7; P = 0.79), respectively. Even in this younger age group, only 61% of the embryos had a normal diploid status. CONCLUSIONS: The absence of a beneficial treatment effect in this randomized clinical trial provides no arguments in favour of PGS to improve live birth delivery rate following single-embryo transfer in women under the age 36.

Clinical Trials.gov: NCT00670059.

Keywords: single-embryo transfer; aneuploidy screening; preimplantation genetic screening

Introduction

Globally, single-embryo transfer has been advocated as a strategy to avoid multiple pregnancies in assisted reproductive technology (ART) programmes (Vilska et al., 1999; Thurin et al., 2004; Van Landuyt et al., 2006). The Belgian government has instituted a policy whereby IVF is covered by health insurance under well-defined conditions. For women under the age of 36, single-embryo transfer is obligatory in the first treatment cycle and strongly advocated in the second cycle unless no top quality embryo is available for transfer.

One of the crucial steps in an ART programme, especially when a single-embryo transfer has to be performed, is the selection of the embryo with the highest implantation potential. Prolonged culture until the blastocyst stage allows improved selection of the best-quality embryo, resulting in significantly higher delivery rates compared with transfer on Day 3 (Pananikolaou et al., 2006). Besides morphological quality and embryo kinetics up to the blastocyst stage, adjunctive preimplantation genetic screening (PGS) for aneuploidy has been advocated as another approach by which to define the right embryo for transfer. PGS involves the removal of a single blastomere from a Day 3 embryo, to be used for genetic analysis of six to nine chromosome pairs using multi-probe fluorescence in situ hybridization (FISH).

The exclusion of aneuploid embryos for transfer may not only improve implantation rate, but also reduce abortion rates and avoid the birth of children with numerical chromosomal abnormalities after ART (Gianaroli et al., 1999; Munné et al., 1999, 2003). In different studies, the prevalence of chromosomal abnormalities in embryos of advanced maternal age patients is found to vary from 40 to 70%, depending on the number of chromosomes being investigated (Munné et al., 1998; Gianaroli et al., 1999; Pellicer et al., 1999; Kahraman et al., 2000; Staessen et al., 2004). In previous randomized
controlled studies (RCTs) performed (Staessen et al., 2004; Stevens et al., 2004; Mastenbrook et al., 2007) in couples with advanced maternal age no arguments were found in favour of PGS to improve clinical outcome per initiated cycle. More recent observational studies have revealed a high incidence of aneuploidy, also in embryos of young women undergoing IVF (Munné et al., 2004; Baart et al., 2006).

This study aims to establish whether in a population of IVF patients receiving a single blastocyst transfer PGS enhances embryo selection beyond any improvement provided by transfer at the blastocyst stage. To this end a randomized controlled trial was performed to determine whether there were any differences in delivery rates between couples with a female partner younger than 36 randomly assigned to undergo transfer of a single blastocyst in a cycle without PGS and those assigned to undergo transfer of a single blastocyst in a cycle with PGS.

Materials and Methods

Study design
Between October 2004 and December 2006, ovulatory women undergoing infertility treatment were asked to participate in this RCT if fulfilling the following inclusion criteria: maternal age <36, need for ICSI and having motile sperm (sperm parameters: more than 1 x 10^6 sperm cells/ml) and both partners having a normal karyotype. Following written informed consent, eligible couples were randomly assigned to ICSI followed by blastocyst transfer either with or without PGS on the cleavage-stage embryo. Randomization was performed at the outpatient clinic, and each patient could be included only once. A computer programme was used to generate the allocation sequence and to assign participants to their treatment groups. The senior author (D.P.) enrolled the patients. The consent form mentioned that patients allocated to the PGS group would agree to donate embryos for research if these were chromosomally abnormal or unsuitable for transfer or cryopreservation. The study was approved by the Ethics Committee of the University Hospital and registered with the ClinicalTrials.gov (Protocol Registration System) and received registration number NCT00670059. The patients were aware of the treatment allocation and were informed at the moment of transfer concerning the chromosomal abnormalities observed and about the morphological quality of the embryos.

Ovarian stimulation, oocyte retrieval and embryo culture
Controlled ovarian stimulation was carried out in an antagonist protocol using recombinant FSH combined with a GnRH antagonist (ganimelix, Orgalutran, NV Organon) (Kolbianakis et al., 2004) or an agonist protocol, using GnRH analogues for pituitary desensitization (buserilin, Suprefact; Hoechst, Frankfurt, Germany), combined with an agonist protocol, using GnRH analogues for pituitary desensitization. Fertilization was assessed as the number of 2PN zygotes per cumulus oocyte complex (COC) retrieved. Embryo evaluation was performed on Days 2 and 3 by recording the number of blastomeres and the percentage of fragmentation. Grade A embryos were defined as embryos without anucleate fragments. Grade B embryos had blastomeres of equal or unequal sizes, with a maximum of 20% of the volume of the embryo filled with anucleate fragments. In Grade C embryos, anucleate fragments were present in 20–50% of the volume of the embryo. Grade D embryos had >50% of the volume of the embryo filled with anucleate fragments. The embryos were evaluated daily until Day 5. On Day 5, the embryos were classified into arrested embryos showing no signs of compaction on Day 5, compacting embryos (C1–C2), early blastocysts (B11–B12) and expanding blastocysts (B13–B17) according to the classification proposed by Gardner and Schoolcraft (1999). Embryos were considered for transfer at Day 5 only from the compacting stage on. In both groups, supernumerary embryos were cryopreserved on Day 5 or 6.

Embryo biopsy
Embryos of Grade A, B or C with at least five blastomeres were biopsied (Joris et al., 2003) on the morning of Day 3 after ICSI. These selection criteria to decide whether an embryo was suitable for biopsy were the same as those used to decide whether an embryo was transferable on Day 3 in the regular ICSI programme without preimplantation genetic diagnosis (PGD). Before biopsy of one blastomere, the blastomeres were checked for the presence of nuclei. If no nuclear material was found during fixation, a second blastomere was removed. Embryo biopsy was carried out in HEPES-buffered medium under oil using laser technology (fertilize) as has been described in detail earlier (Staessen et al., 2004).

Spreading of the interphase nuclei and FISH procedure
Using a mouth pipette, the individual blastomeres were first rinsed in medium and then transferred to a 1 µl droplet of 0.01 N HCl/ 0.1% Tween 20 solution (Coonen et al., 1994) on a Superfrost Plus glass slide (Kindler GmbH, Freiburg, Germany). A two-round FISH procedure was performed allowing us to detect the chromosomes X, Y, 13, 18, 21 (round 1) and 16, 22 (round 2), as has been previously described in detail (Staessen et al., 2004).

FISH scoring criteria
The specific FISH signals detected in a given blastomere were interpreted as follows: (i) a blastomere was considered to be diploid when the two gonosomes and two chromosome 13-, 16-, 18-, 21- and 22-specific signals were present; (ii) a blastomere was considered to be haploid, triploid or tetraploid when one, three or four signals, respectively, for the investigated chromosomes were present; (iii) a blastomere was considered to be aneuploid when an extra (trisomic) and missing (monosomic) signal for one chromosome was observed, in the presence of two signals for the remaining chromosomes analysed; (iv) a blastomere was considered as combined abnormal when it was neither diploid nor haploid, triploid, tetraploid or aneuploid. After FISH analysis, only embryos found to be chromosomally normal in the corresponding biopsied blastomere were transferred on Day 5. FISH analysis was performed by experienced cytogeneticists trained in cytogenetics and FISH analysis. The results of the first and second round were analysed by two observers using a Zeiss Axiosplan 2 fluorescence microscope. When the two observers could not reach an agreement about the normality of the result, the embryo was considered as abnormal. The frequency of this fact was not recorded, but occurred rather exceptionally.
**Embryo transfer**

In the PGS group, the transfer was performed on Day 5 if at least one compacting embryo or early blastocyst was obtained from the embryos found to be genetically normal. In the control group, the transfer was also performed if at least one compacting embryo or early blastocyst was obtained on Day 5; four embryo transfers were erroneously performed at the cleavage-stage on Day 3. The mean number of embryos available for the patients receiving a transfer on Day 3 was 4.0 (SD = 1.8). The embryo transfers were performed without ultrasound guidance by clinicians and embryologists, who were blinded only with respect to the patient’s participation in the study.

**Patients characteristics and definition of events**

Baseline characteristics were documented, and the following events were assessed for each patient: biochemical pregnancy, clinical pregnancy, miscarriage and live birth (singleton or multiple) delivery. A biochemical pregnancy was defined as two consecutive positive rising (>15 IU/ml) serum HCG levels. A clinical pregnancy was defined as an intrauterine gestational sac with positive fetal heart activity seen at ultrasonography performed at least 6 weeks post embryo transfer. Multiple gestational sacs in one patient were counted as one clinical pregnancy. A miscarriage was defined as a pregnancy loss before 20 weeks of gestation. A live birth was defined as a birth in which a fetus was delivered with signs of life after complete expulsion or extraction from its mother, beyond 20 completed weeks of gestational age. Live births were counted as birth events, i.e. twins or multiple birth was counted as one birth event (Zegers-Hochschild et al., 2006).

**Primary and secondary outcome measures**

The primary outcome was live birth delivery rate per initiated cycle (treatment started), expressed as the number of live birth deliveries per 100 initiated cycles (treatments started).

Live birth delivery rates defined as the number of live birth deliveries per 100 patients randomized, aspiration cycles or embryo transfer cycles were considered supportive. Secondary outcome measures also included biochemical pregnancy rates, clinical pregnancy rates, miscarriage rates (expressed per 100 patients randomized, initiated cycles, aspiration cycles or embryo transfer cycles), frequency and type of genetic abnormalities detected and incidence of normal and abnormal embryos in relation to the cell stage on Day 3 and the developmental stage on Day 5.

**Statistical analysis**

Data for continuous variables are summarized with the use of means ± SD for each group of interest. The data for categorical variables are presented as the number of cases or percentages, including nominator and denominator values. Comparisons of percentages among groups are presented as absolute-between-group differences with corresponding P values and 95% confidence intervals (95% CIs) for each comparison made (Altman, 1991). In doing so, we provide information to the reader on the magnitude and precision of the difference between groups for each comparison, as well as the role that chance may play in the observed study results (Altman, 1991). We also performed statistical tests for categorical and continuous variables with the chi-square test and the t-test for independent samples, respectively. All tests were two-sided. We made no adjustments to P values for multiple comparisons. Therefore, readers become able to make their way through the many reported results.

The aim of this superiority trial was to test an absolute difference of 10% in live birth delivery rate with PGS, using a Group Sequential Trial Design methodology with four interim analyses (including the final one). Based on a live birth delivery rate of 30% obtained after single blastocyst transfer without PGS in couples with female partners younger than 36 years (control group), an expected increase to 40% after single blastocyst transfer with PGS (intervention group) could be considered as beneficial and clinically meaningful. Using a group sequential trial design methodology with four interim analyses, including the final one, we calculated that the enrolment of 447 patients in each group would give the trial a statistical power of 80% to detect an absolute difference of 10% in the live birth delivery rate between the groups at an alpha level of 0.05 with the use of a two-sided z-test. The alpha levels were 0.017, 0.031 and 0.041 for the first three analyses, respectively.

**Result**

**Study conduct**

Study recruitment was terminated prematurely on the basis of futility at the first pre-specified interim analysis, after the enrolment of 120 of the projected 447 patients in each group. The observed interim differences in outcomes between the intervention (PGS) and control groups were so unimpressive that any prospect of a positive result with the planned sample size was very unlikely. A conditional power analysis indicated that, given the interim data in the primary outcome measure, there was only a 2.7% chance of success of meeting the prospectively defined objective of a significant 10% increase or decrease in live birth delivery rate with PGS.

**Patient characteristics**

At the time of early termination, a total of 240 couples were recruited: 120 in the control and 120 in the PGS group (Fig. 1). An oocyte pick-up was effectively performed in 198 couples: 98 control cycles and 100 PGS cycles. The reasons for cancelling the intended treatment cycle were similar in both the control and the PGS group.

As shown in Table I, the mean female age in the control group was 29.7 ± 3.7 versus 30.0 ± 4.1 years in the PGS group. The patients included in the control and PGS groups were similar also in terms of duration of infertility, number of previous gestations (G-status), number of previous parturitions (P-status), number of failed previous cycles and the indication for infertility treatment. In both groups, the major indication for fertility treatment was an andrological factor. A mean of 11.7 ± 5.9 COCs was retrieved in the control group versus 12.1 ± 7.0 COCs in the PGS group. In the control cycles, a mean fertilization rate of 57.8 ± 20.3% per COC retrieved was obtained resulting in a mean of 6.8 ± 4.4 fertilized (2PN) oocytes per cycle. In the PGS group, the fertilization rate was 63.3 ± 20.4% resulting in a mean of 7.6 ± 5.0 fertilized oocytes per cycle. The cleavage rate on Day 3 defined as embryos reaching at least the 5-cell stage was also similar in the control and the PGS groups. Significantly more embryos could be cryopreserved in the control group than in the PGS group; 2.1 ± 2.5 versus 1.4 ± 2.2 (P < 0.01), respectively.

**Clinical results**

Transfer was cancelled in nine cycles of the control group: in one cycle, no fertilization was observed; in seven cycles, no
compacting or blastocyst stage embryos were available on Day 5, and in one cycle, no transfer could be performed because of ovarian stimulation syndrome (Table II). In the PGS group, transfer was cancelled in 15 cycles: in two cycles, no fertilization was obtained; in four cycles, no embryos suitable for biopsy were obtained; in six cycles, no normal embryos were available after FISH diagnosis, in two cycles, the genetically normal embryos did not reach the morulae or blastocyst stage and in one cycle, all the normal embryos were cryopreserved due to ovarian hyperstimulation syndrome.

In both groups, a relatively high number of patients had an initial positive human chorionic gonadotrophin test: 52 for controls and 47 for PGS. In the control group, 35 women delivered a singleton and two delivered twins. From the PGS group, 37 deliveries occurred: 36 singleton and one twin pregnancy deliveries. In the control and PGS groups, we observed twin pregnancies after single-embryo transfer. In the control group, both twins observed were monozygotic and in the PGS group, a dizygotic twin was delivered. The most plausible explanation is the occurrence of a natural conception of one of non-retrieved oocytes. In all the other cases of the PGS group, the sex of the child born is corresponding to the sex of the transferred embryo.

In the PGS group, all miscarriages were found to be preclinical, whereas in the control group, eight are found preclinical and seven clinical, including one child stillborn at 19 weeks with normal karyotype and one induced abortion due to trisomy 21 conception. With the small numbers available no statistical differences were observed.

Table II shows that the observed live birth delivery rates after ART were 37 of 120 (30.8%) versus 37 of 120 (30.8%) per randomized patient, 37 of 107 (34.6%) versus 37 of 107

![Figure 1](image-url)
was also anuclear (78.2%) of 78 embryos, a diagnosis was obtained based on
0.91) and 2.1% (34.6%) per cycle initiated, 37 of 98 (37.8%) versus 37 of
100 (37.0%) per aspirated cycle and 37 of 89 (41.6%) versus 37 of
85 (43.5%) per embryo transfer cycle for the control versus the PGS
group, respectively, with absolute between-group differences (95% CI; P value) of 0% (−11.7 to 11.7; P = 1.00), 0% (−12.7 to 12.7; P = 1.00), 0.8% (−14.2 to 12.7; P = 0.91) and 2.1% (−12.7 to 16.7; P = 0.79), respectively.

Logistic regression analysis with live birth delivery (and, in turn, biochemical pregnancy) as the dependent variable and maternal age, years of infertility, gestation status, parity status, rank of trial, cause of infertility, treatment cycle, number of COCs, sperm concentration, percentage motile sperm, number of 2PN and treatment group as the independent variables indicated that none of these independent variables was statistically significant.

Genetic analysis
Of 523 embryos suitable for biopsy, we obtained a FISH result in 499 (95.0%) embryos. One blastomere was removed in 445 embryos from which in 438 (98.5%), we obtained a FISH diagnosis. In 78 embryos, we removed two blastomeres because during spreading the first biopsied blastomere was found to be anuclear (n = 12), multinuclear (n = 50), nucleus in metaphase (n = 10) or the nucleus was lost (n = 6). Finally, in 61 (78.2%) of 78 embryos, a diagnosis was obtained based on the second blastomere. In 17 embryos, the second blastomere was also anuclear/multinuclear/nucleus in metaphase/lost and the embryo remained without diagnosis. In 304 embryos (61.0%), we observed a normal diploid status, in 60 embryos (12.0%) a trisomy was observed, in 65 embryos (13.0%) a monosomy was observed, 11 embryos (2.2%) had an abnormality of the ploidy status and in 59 (11.8%) embryos combined abnormalities were observed. From the normal embryos (n = 304), 85 (27.9%) were transferred and 124 (40.8%) were frozen. The remaining 95 embryos were developmentally or morphologically not suitable for transfer and/or cryopreservation and therefore discarded or given for research.

Fig. 2 represents the incidence of normal and abnormal embryos in relation to the cell stage on Day 3 (Fig. 2A) and the developmental stage on Day 5 (Fig. 2B). On Day 3, from
the total number of 499 embryos on which we obtained a diagnosis, 31 had less than six cells, 87 were at the 6-cell stage, 86 were at the 7-cell stage, 277 were at the 8-cell stage and 68 had more than eight cells. The overall distribution of the abnormal embryos was not significantly different in relation with the cell stage on Day 3, although if we compared the results for each cell stage separately embryos at the 5-cell stage have a significantly lower incidence of being normal (chi-square test, P = 0.037). From the 499 embryos, the development was documented until Day 5 and a significant relative increase of the percentage of normal embryos was observed in relation to the further developmental stage on Day 5 (chi-square test for trend; P < 0.001). The results indicate that among the 230 embryos (43% of the 523 embryos suitable for biopsy) that became an expanded blastocyst on Day 5, 80% of them are genetically normal for the chromosomes tested.

Discussion
Common indications for PGS included advanced maternal age, recurrent failure of implantation following IVF, recurrent miscarriage and severe male infertility (for review: Donoso et al., 2007). The application of the PGS technique has been advocated in all IVF cycles, especially given the increasing pressure to perform elective single-embryo transfer. According to Belgian legislation, single-embryo transfer is mandatory in patients under 36 years of age for their first treatment cycle.

The present randomized controlled trial shows that PGS at Day 3 does not increase the delivery rate in young women aged <36 receiving a single-blastocyst transfer. Our results nevertheless confirm the high delivery rates among women aged <36 undergoing ICSI followed by the transfer of a single blastocyst as described earlier (Pananikolaou et al., 2006). This indicates that although we have a significant contribution of male factor infertility, the results of the study may reasonably be generalized to IVF outcome. Using the design methodology of a randomized clinical trial, we were unable to confirm the results of a retrospective case–control study describing a reduction in miscarriage rate associated with PGS (Munné et al., 1999). Our finding of a not significant between-group difference in this regard should be interpreted

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<th>Table II. Pregnancy outcome after ART among women randomized to receive either standard care (active control group) or standard care with PGS (intervention group)</th>
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<td>Biochemical pregnancies (positive serum HCG) after ART</td>
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<td>per initiated cycle (treatment started)</td>
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Study recruitment was terminated prematurely on the basis of futility at the first pre-specified interim analysis, after the enrolment of 120 of the projected 447 patients in each group. The data are presented as number of cases or percentages.

Barynning typically results in confidence intervals not as precise as originally intended.
with some caution as our prospective study was not powered to specifically detect a difference in this outcome measure.

Even in this younger age group, only 61% of the embryos had a normal diploid status. At least two other studies also reported the results of PGS analysis in women aged <35.

Munne et al. (2004) published PGS data from young women (maternal age <35) carriers of X-linked diseases (20 cycles; mean maternal age 31.6), patients with two or more previous IVF failures (48 cycles; mean maternal age 32.3) or patients with a previous aneuploid conception (18 cycles; mean maternal age 33.0). After analysis of a single cell for 6–9 chromosomes, the percentages of normal embryos were

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**Figure 2:** Relationship between the FISH diagnosis and the developmental stage.

**(A)** On Day 3. Chi-square test, $P = 0.037$. **(B)** On Day 5. Chi-square test, $P < 0.001$. 

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<th>C1-C2</th>
<th>B1-B12</th>
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<td>Total number of embryos</td>
<td>84</td>
<td>70</td>
<td>115</td>
<td>230</td>
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<td>Haploid/Triploid/Tetraploid</td>
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<td>1 (1.4)</td>
<td>2 (1.7)</td>
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<td>Trisomy</td>
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<td>11 (15.7)</td>
<td>20 (17.4)</td>
<td>20 (8.7)</td>
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<td>Monosomy</td>
<td>10 (11.9)</td>
<td>18 (25.7)</td>
<td>20 (17.4)</td>
<td>17 (7.4)</td>
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<td>Combined Abnormalities</td>
<td>23 (27.4)</td>
<td>13 (18.6)</td>
<td>14 (12.2)</td>
<td>9 (3.9)</td>
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<tr>
<td>Normal</td>
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<td>27 (38.6)</td>
<td>59 (51.3)</td>
<td>184 (80.0)</td>
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found to be 52, 47 and 29%, respectively. The subgroup of the carriers of X-linked disease is very close to the population we analysed; the 52% euploidy rate is probably not significantly distinguishable from 61%. Baart et al. (2006), investigating embryos from relatively young IVF patients (mean maternal age 33.1 years) with FISH targeting for 10 chromosomes, found that only 36% of the embryos were normal after analysis of both, one or two blastomeres. This low incidence of normal embryos in this study is probably due to the fact that they investigate for more chromosomes.

Our results confirm the potential benefit of the genetic constitution on further embryonic development. In line with other studies (Magli et al., 2000; Sandalinas et al., 2001; Staessen et al., 2004), we observed that in patients in whom expanded blastocysts are obtained, the chance of selecting a chromosomally normal embryo increased from 61% on Day 3 to 80% on Day 5. Therefore, extending embryo culture to the blastocyst stage may allow an improved selection of the best morphological quality embryo as well as the selection of embryos with a euploid status, which possibly explains why adjunctive PGS does not enhance delivery rates beyond any improvement provided by blastocyst transfer. Although a significant degree of embryonic wastage occurs between Days 3 and 5, due to intrinsic genetic aberrations, transfer of Day 5 embryos does not ensure the absence of chromosomal or other abnormalities. No obvious chromosomal abnormalities were detected in the children born in our study, but we acknowledge that pre- or post-natal karyotyping results were available in only a small number of cases.

Our findings have important implications related to the question of the potential benefit of PGS. Although our study shows that PGS does not improve pregnancy rate or delivery rate after IVF, our findings also indicate that PGS does not significantly impair ART outcome, lending support to three different hypotheses. First, the effect of aneuploidy screening on the implantation potential might be overestimated, especially in combination with an extended embryo culture extended until the blastocyst stage, since this strategy enables chromosomally normal embryos to be selected out. Secondly, PGS does select the best embryos, but because of the extra manipulation and removal of one cell at the 8-cell stage, the benefit of this methodology is potentially levelled out. Thirdly, the analysed cell might not be representative of the whole embryo, because of the high rate of mosaicism in cleavage stage embryos (Munne et al., 1994; Baart et al., 2006), so that a chromosome abnormality restricted to the biopsied blastomere condemns an otherwise healthy embryo to being discarded. On the other hand, a normal chromosomal status restricted to the biopsied blastomere would lead to the selection of an abnormal embryo with no chance to implant or on the birth of a child carrier of a chromosomal abnormality (Munne et al., 1998).

The argument of screening for more chromosomes, with other techniques such as comparative genomic hybridization (CGH) or array-CGH (Voullaire et al., 2000; Wilton et al., 2002) will indeed provide information for all the chromosomes but due to the mosaicism more embryos might incorrectly be rejected. Baart et al. (2006) demonstrated that re-analysis by means of FISH on Day 5 of the embryos that were first diagnosed on Day 3 provides an improved understanding of the fate of abnormal blastomeres during embryo development and a valuable insight into the mechanisms of aneuploidy formation. They showed that PGS after analysis of one blastomere is effective in detecting abnormal embryos resulting from a meiotic nondisjunction event. Nicolaidis and Petersen (1998) summarized the evidence about the origin of non-disjunction in human autosomal trisomies especially for chromosomes 13, 16, 18 and 21 accumulated during the last decade, by using DNA polymorphism analysis. Maternal meiosis I non-disjunction constitutes the single most important category, but chromosome-specific patterns exist. For the acrocentric chromosome 21, meiosis I errors predominate, in contrast to trisomy 18 where meiosis II errors predominate. For trisomy 16, virtually all cases are due to maternal meiosis I non-disjunction. Postzygotic (mitotic) non-disjunction constitutes 5–15% of cases of trisomies 18 and 21.

Despite the issue of mosaicism in preimplantation diagnosis, PGS may have a role as early prenatal diagnosis in avoiding viable trisomic pregnancies. Twisk et al. (2007) showed that most women needing IVF opt for PGS as an alternative to prenatal testing on condition that this would have no negative effect on their pregnancy chances, especially for Down Syndrome, even considering that PGS does not detect all Down Syndrome embryos.

Methodological strengths of our trial include the use of clearly defined and universally accepted events to describe outcome after ART (Zegers-Hochschild et al., 2006), a high recruitment (accrual) rate within a limited time frame, the virtually complete follow-up with no patients lost to follow-up after initiation of ART and the use of a pre-specified group sequential trial design aiming to detect an absolute increase of 10% in the live birth delivery rate with the use of PGS.

Our trial was stopped prematurely on the basis of futility, which refers to the situation in which the interim results make it clear that the hypothesized effect size does not seem to exist and that the final results at the scheduled end of the trial are unlikely to be statistically significant. Our calculated conditional probability value of 0.027 was much smaller than the threshold values of 0.33 (Ware et al., 1985), 0.30 (Pepe and Anderson, 1992; Betensky, 1997) and 0.10 (conservative threshold; Betensky, 1997) reported in the literature, below which the decision to stop the study early for futility and accept null hypothesis of no difference between treatment groups can be made. Our decision to discontinue the trial was also based on operational aspects of futility, because there was also a strong ethical imperative and an economic incentive to stop the trial early. Stopping a clinical trial prematurely typically results in wider confidence intervals of the estimated effect size for both primary and secondary end-points. Nevertheless, when a trial is stopped early, the effect estimates and their 95% confidence intervals observed at interim analysis should be published, because these data may contribute to future systematic reviews or meta-analyses. Because of ethical concern related to the PGS procedure, our trial was conducted in an open-labelled fashion. To reduce assessment bias, we used objective end-points, i.e. those on which independent reviewers would agree, and we determined follow-up status actively, i.e. with scheduled clinic visits and follow-up phone contacts.
Despite these concerns, we conclude that this randomized clinical trial provides no evidence in favour of PGS to improve delivery rates following single-embryo transfer in women under the age of 36.

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