In vitro fertilization with preimplantation genetic diagnosis for aneuploidies in advanced maternal age: a randomized, controlled study

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Objective: To determine the clinical value of preimplantation genetic diagnosis for aneuploidy screening (PGD-A) in women of advanced maternal age (AMA; between 38 and 41 years).

Design: This was a multicenter, randomized trial with two arms: a PGD-A group with blastocyst transfer, and a control group with blastocyst transfer without PGD-A.

Setting: Private reproductive centers.

Patient(s): A total of 326 recruited patients fit the inclusion criteria, and 205 completed the study (100 in the PGD-A group and 105 in the control group).

Intervention(s): Day-3 embryo biopsy, array comparative genomic hybridization, blastocyst transfer, and vitrification.

Main Outcome Measure(s): Primary outcomes were delivery and live birth rates in the first transfer and cumulative outcome rates.

Result(s): The PGD-A group exhibited significantly fewer ETs (68.0% vs. 90.5% for control) and lower miscarriage rates (2.7% vs. 39.0% for control). Delivery rate after the first transfer attempt was significantly higher in the PGD-A group per transfer (52.9% vs. 24.2%) and per patient (36.0% vs. 21.9%). No significant differences were observed in the cumulative delivery rates per patient 6 months after closing the study. However, the mean number of ETs needed per live birth was lower in the PGD-A group compared with the control group (1.8 vs. 3.7), as was the time to pregnancy (7.7 vs. 14.9 weeks).

Conclusion(s): Preimplantation genetic diagnosis for aneuploidy screening is superior compared with controls not only in clinical outcome at the first ET but also in dramatically decreasing miscarriage rates and shortening the time to pregnancy. (Fertil Steril® 2017;107:1122–9. ©2017 by American Society for Reproductive Medicine.)

Key Words: Aneuploidy, array-CGH, embryo biopsy, maternal age, PGD-A

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Advanced maternal age (AMA) is one of the most significant clinical bottlenecks in assisted reproduction. Fertility declines as women age, owing to both a diminished ovarian reserve and an impaired oocyte quality that leads to an increase in embryo aneuploidy [1]. Aneuploidy is the most common genetic abnormality in humans. Large data sets from comprehensive aneuploidy screenings of preimplantation embryos demonstrate

Received December 14, 2016; revised January 27, 2017; accepted March 3, 2017.
C.R. has nothing to disclose. J.B. has nothing to disclose. L.R. has nothing to disclose. G.C. has nothing to disclose. A.G. has nothing to disclose. C.V. has nothing to disclose. M.F. has nothing to disclose. J.G. has nothing to disclose. A.P. has nothing to disclose.

Partially supported by Igenomix, which covered the cost of the array comparative genomic hybridization (CGH) analysis. Illumina provided the arrays of CGH, and Instituto Valenciano de Infertilidad clinics covered the cost of embryo biopsies, so that patients in the preimplantation genetic diagnosis for aneuploidy screening (PGD-A) arm were not responsible for the cost of the PGD-A procedure.

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Fertility and Sterility® Vol. 107, No. 5, May 2017 0015-0282/$36.00
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http://dx.doi.org/10.1016/j.fertnstert.2017.03.011

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VOL. 107 NO. 5 / MAY 2017
that more than half of the embryos produced by IVF are aneuploid (2–4). This high human embryo aneuploidy rate does not exist in other mammalian species and has been implicated in poor embryo implantation, higher miscarriage rates, greater risk of congenital birth defects, and fetuses with chromosome abnormalities, including Down syndrome (5–8). Several techniques for embryo selection have been developed in IVF, including screening procedures for numeric or structural chromosome abnormalities from couples with normal karyotypes. These procedures are collectively known as preimplantation genetic diagnosis for aneuploidy screening (PGD-A).

In the 1990s PGD-A version 1.0 used fluorescence in situ hybridization (FISH) on polar bodies or cleavage-stage embryos to visualize chromosomes. After the publication of several retrospective studies, the technique spread in popularity (9–12). Some studies focused on couples with AMA (13, 14). However, results of 11 randomized, controlled trials (RCTs) from 2004 to 2010 produced controversial results. Most of them demonstrated that FISH screening did not increase delivery rates and in some cases was associated with reduced delivery rates (15). Several factors were responsible for this outcome, including the inefficiency of the FISH procedure, the limited number of chromosomes analyzed, problems with the reported levels of chromosomal mosaicism at the cleavage stage, and the fact that most studies involved patients with poor prognosis who produced few, poor-quality embryos (16). Later an additional RCT with an optimized FISH protocol demonstrated a significant increase in live birth rates in women between 41 and 44 years of age (17).

In the last decade, new technology based on whole genome amplification from single cells, allowing for 24-chromosome screening, has invigorated the field, initiating the transition to PGD-A version 2.0 (18). Platforms using whole genome amplification include comparative genomic hybridization arrays (aCGH), single nucleotide polymorphism microarrays, and recently, next generation sequencing (NGS). Quantitative polymerase chain reaction (PCR) has also been applied to aneuploidy screening (19). These technologies have been routinely used since 2008 and aim to improve not only clinical results but also time to pregnancy and, most important, “healthy baby at home” rates. Using these new technologies in trophectoderm biopsies, three RCTs have been published involving patients with good prognosis (20–22). One of these RCTs showed an increase in ongoing implantation from 27% to 66% compared with controls, along with a reduction in miscarriage rates, although most were unable to claim statistical confidence. Only one RCT has been registered for AMA patients using 24-chromosome screening: an ongoing multicenter RCT of polar body biopsies designed and sponsored by the European Society of Human Reproduction and Embryology (23).

Because of the lack of RCTs for AMA patients using 24-chromosome screening—the most frequent indication of PGD-A in European countries—we designed a multicenter, randomized trial in women aged 38–41 years using day-3 embryo biopsies, the most extended biopsy type in the moment at which the study was designed. The control group underwent blastocyst transfers without chromosomal analyses, the primary endpoints were successful delivery and live birth rates. Secondary endpoints were implantation and clinical pregnancy, along with miscarriage and ectopic pregnancy rates at the first attempt and after cumulative attempts during the study and 6 months after closing recruitment. In addition, cost-effectiveness per live birth in Europe and the United States was determined.

MATERIALS AND METHODS

Design

Patients from four reproductive clinics were recruited to participate in this multicenter, prospective, randomized clinical trial between May 2012 and December 2014. Embryo transfers of the remaining cryopreserved embryos performed in the 6 months following the closure of recruitment were also included in the estimation of cumulative rates. Follow-up was conducted for all pregnancies carried to term. Full data analysis included records of deliveries and live births resulting from cryo-transfers performed during the 6 months following the study recruitment period.

Women included in the study were between 38 and 41 years of age, had normal karyotypes, were on their first or second intracytoplasmic sperm injection (ICSI) cycle, had a body mass index (BMI) <30 kg/m², had five or more metaphase II (MII) oocytes obtained from one or two cycles, and had sperm concentrations ≥2 × 10⁹/mL. Exclusion criteria were endocrine or systemic pathologies, a previous PGD-A/PGD cycle, and previous pregnancy or miscarriage due to chromosomal abnormalities. Embryo transfer was canceled in cycles without embryos reaching blastocyst stage or without euploid embryos in the PGD-A arm.

Sample size was estimated at 120 patients per arm for 15 points absolute difference in the primary endpoint of delivery rate (α 5%, β 20%). Before starting the treatment, patients were allocated through computer-generated randomization into two groups: one group would undergo a conventional ICSI cycle with morphologic embryo selection at the blastocyst stage, whereas the other would have embryo selection performed through PGD-A, with day-3 embryo biopsy and 24-chromosome screening by aCGH before blastocyst transfer.

This RCT was approved by the institutional review board at the Instituto Valenciano de Infertilidad and registered at ClinicalTrials.gov as NCT01571076. Written, informed consent was obtained from all patients before entering the study.

Ovarian Stimulation, Embryo Culture, and Biopsy

Controlled ovarian stimulation (COS) was carried out with a GnRH antagonist protocol. Ovarian stimulation was performed using 150–300 IU of recombinant FSH (Gonal-F, Merck-Serono) or recombinant FSH plus hMG (Menopur, Ferring Pharmaceutical) daily, according to female age, basal hormone levels, ovarian pattern at ultrasound, and BMI. After a normal basal ultrasound, gonadotropins were administered from day 2 to 3 after menstruation. Serial transvaginal ultrasound examinations and serum E₂ determination were initiated on day 5 of COS and repeated every 48 hours to monitor the ovarian response. Cetrotide
PCR tubes were immediately frozen at phosphate-buffered saline. After blastomere loading, the system (LifeGlobal) was used with tri-gas incubators (7% O2 5. In the remaining laboratories, a global sequential culture was subsequently cultured in CCM medium until day 5, and then embryos were subsequently cultured in CCM medium until day 5. In the remaining laboratories, a global sequential culture system (LifeGlobal) was used with tri-gas incubators (7% O2 and 5% CO2).

Embryo biopsy was performed on day 3 as follows: embryos were placed in a droplet containing Ca2+/Mg2+-free medium (G-PGD, Vitrolife or LifeGlobal), the zona pellucida was perforated using laser technology (OCTAX), and a single blastomere was withdrawn from each embryo. Only embryos with six or more nucleated blastomeres and <25% fragmentation were biopsied. For blastomere washing and handling, 10% polyvinylpyrrolidone was used. Individual blastomeres were placed in 0.2 mL PCR tubes containing 2 µL phosphate-buffered saline. After blastomere loading, the PCR tubes were immediately frozen at –20°C and kept in the freezer until transportation to the genetic analysis laboratory. Properly developed euploid embryos were transferred on day 5, and surplus euploid blastocysts were vitrified either on day 5 or 6.

Array CGH for 24-Chromosome Aneuploidy Screening
Chromosomal analysis was centralized in a reference genetic laboratory. To perform blastomere aCGH analysis, a single cell from each embryo was amplified using the Sureplex DNA amplification system (Illumina), and the amplification quality confirmed by gel electrophoresis (Lonza). Sample and male/female control DNA were labeled with Cy3 and Cy5 fluorophores following the manufacturer’s instructions. Labeling mixes were combined and hybridized on 24sure arrays (V2 and V3, Illumina) for 6–12 hours. Each probe was specific to a different chromosomal region and occupied a discrete spot on the slide; the technique involves the competitive hybridization of differentially labeled test and reference DNA samples, and chromosomal loss or gain is revealed by the color taken on by each spot after hybridization. Fluorescence intensity was detected using a laser scanner (Powerscanner, TECAN), and BlueFuse Multi software was used for data processing (Illumina). As specified by the manufacturer, the 24sure platform has an effective 10-Mb resolution when using this software; therefore, only full chromosome aneuploidies and segmental aneuploidies affecting a chromosome fragment larger than 10 Mb were able to be visualized. The entire protocol was completed in less than 24 hours, such that ET and vitrification of surplus euploid embryos could take place on day 5 (25).

Statistical Analysis
The ongoing implantation rate was defined as the percentage of embryos transferred that produced an evolutive implanted embryo up to week 12 of pregnancy. Clinical pregnancy rates were calculated according to the presence of a gestational sac regardless of the number of embryos transferred. Delivery rates per transfer and per patient were calculated as the percentage of clinical pregnancies that ended in a live birth. The miscarriage rate was defined as the percentage of intrauterine clinical pregnancies that were missed before the 12th week of pregnancy. The percentage of live births was calculated considering all live births per patient. The total number of missed sacs represented the loss of the implanted sacs in a singleton pregnancy plus the loss of one of the fuses in a twin vanishing pregnancy. In the tables, analysis is expressed as the percentage of probability and OR, with 95% confidence intervals (CIs).

Cumulative pregnancy and delivery rates included the number of pregnancies and deliveries achieved following the first transfer plus the cryo-transfers of some remaining embryos performed in the 6 months after the close of study recruitment.

A geometric distribution was used to present a theoretical explanation of the number of transfers and the expected time required for the first delivery. Two possible outcomes for each transfer (trial) were considered: pregnancy ending in a delivery (success) or no delivery (failure).

Fisher’s exact test was used to compare the study groups with respect to percentages. A Student t test was used to compare noncategorical variables. A P value of <.05 was considered statistically significant.

RESULTS
A flow chart of the patients initially recruited and finally treated in the study is presented in Figure 1. In total, 326 couples were informed of the study, and 48 refused to participate. Of the 278 remaining, 73 (26.2%) initiated COS but were ultimately excluded because they did not reach all the inclusion criteria, mostly owing to low MII oocyte number or ovum donation treatment (16.5% of the patients who accepted participation in the study were ultimately excluded).

In the PGD-A group, there were 18 patients who did not reach the MII number inclusion criteria. In eight of them, despite that at least one MII was obtained in the first attempt, couples decided not to go ahead with an additional stimulation to reach the required MII number. In four of them, no oocytes were obtained; in the remaining ones the cycle was cancelled, and two of them directly decided to go for ovum donation. In the control group, 28 patients did not reach this criterion. Half of them rejected the second stimulation to achieve the required MII number, whereas in the other half the cycle was cancelled and patients were lost for follow-up.

In addition, 5 couples were excluded owing to spontaneous pregnancy during treatment (1.8%), 7 owing to low sperm count (2.5%), and 14 because of previous recurrent miscarriages or implantation failures (5.0%). A uterine septum was detected in one of the patients after recruitment.
In total, 73.7% of the recruited patients completed a cycle in their corresponding allocation arm.

The intention to treat analysis taking into consideration all patients who initially accepted participation showed live birth rates of 31.9% (44 of 138) in the PGD-A group vs. 18.6% (26 of 140) in the control group (P = .0031; OR 2.381, 95% CI 1.343–4.223). The intention to treat analysis for delivery rates was 26.1% (36 of 138) for PGD-A vs. 16.4% (23 of 140) per recruited patient (P = .0568; OR 1.795, 95% CI 0.9984–3.229).

The epidemiologic characteristics of the couples included in the study are presented in Supplemental Table 1 (available online). Patients included in both arms of the study had similar maternal age, BMI, sperm parameters, number of previous IVF failures, and previous miscarriages. Additionally, a comparison of the embryologic outcome in both groups demonstrated similar numbers of MII oocytes retrieved, 2-pronuclei zygotes, good-quality day-3 embryos, mean blastomere numbers, and fragmentation degrees, as well as similar percentages of arrested embryos, morula, and blastocyst stage embryos (Table 1). A two-sided Fisher exact test and Student t-test for noncategorical variables did not show any statistical differences between groups. No differences between PGD-A and non–PGD-A were observed in the percentage of patients who underwent several COS cycles to accumulate oocytes via vitrification to achieve the minimal inclusion number (five MII; 14 patients in the PGD-A group and 12 in the non–PGD-A group).

In the PGD-A arm a total of 538 cleavage stage embryos were biopsied and analyzed; 97.2% were informative and 78.6% were aneuploid. Only 33.6% of the informative embryos had a single aneuploidy; 14.9% showed a chaotic pattern, and only 1.1% presented segmental/partial

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**TABLE 1**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PGD-A</th>
<th>Non–PGD-A</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cycles performed</td>
<td>100</td>
<td>105</td>
</tr>
<tr>
<td>Mean no. MII oocytes (SD)</td>
<td>10.2 (5.3)</td>
<td>10.0 (4.6)</td>
</tr>
<tr>
<td>Mean no. 2-pronuclei zygotes (SD)</td>
<td>7.6 (4.2)</td>
<td>7.1 (3.1)</td>
</tr>
<tr>
<td>Mean no. day-3 good-quality embryos*</td>
<td>5.4 (2.9)</td>
<td>5.8 (3.2)</td>
</tr>
<tr>
<td>Mean no. day-3 blastomeres (SD)</td>
<td>7.9 (1.5)</td>
<td>8.0 (1.5)</td>
</tr>
<tr>
<td>Mean day-3 fragmentation degree (SD)</td>
<td>6.4 (5.4)</td>
<td>6.7 (5.4)</td>
</tr>
<tr>
<td>No. of arrested embryos/day-3 embryos (%)</td>
<td>76/538 (14.1)</td>
<td>111/581 (19.1)</td>
</tr>
<tr>
<td>No. of morula/day-3 embryos (%)</td>
<td>127/538 (23.6)</td>
<td>115/581 (19.8)</td>
</tr>
<tr>
<td>No. of blastocyst/day-3 embryos (%)</td>
<td>335/538 (62.3)</td>
<td>355/581 (61.1)</td>
</tr>
</tbody>
</table>

* Day-3 embryos with six or more blastomere and with fragmentation degree <25%.


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**FIGURE 1**

Assignment, treatment, and analysis of couples. PGD-A = preimplantation genetic diagnosis for aneuploidy.

aneuploidies. Detailed information on the chromosomal status of the embryos analyzed is provided in Supplemental Table 2.

The distribution of the aneuploidies in the 330 remaining abnormal embryos after excluding chaotic embryos was 54.9% uniform chromosome monosomies, 37.5% uniform chromosome trisomies, and 7.5% segmental aneuploidies (Fig. 2). Chromosomes with higher aneuploidy rates were as follows: chromosomes 15 (9.2%), 16 (11.8%), 21 (7.1%), and 22 (13.6%). Chromosome 1 had the highest frequency of segmental aneuploidies; chromosomes 2, 3, 9, and 10 followed. Single aneuploidies compatible with life were present in 22 of 411 embryos: 13 embryos carried trisomy 21, 3 embryos carried trisomy 18, 4 embryos carried trisomy 13, and 2 embryos had monosomy X.

Clinical outcomes at the first ET performed in each patient (intention to treat analysis and per-protocol analysis) are presented in Table 2. In the PGD-A group, a significant decrease in the percentage of cycles reaching ET was observed compared with the control group (68.0% vs. 90.5%, respectively; \( P = .0001 \)). The mean number of embryos transferred per cycle was also significantly lower in the PGD-A group.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PGD-A</th>
<th>Non–PGD-A</th>
<th>( P ) value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cycles performed</td>
<td>100</td>
<td>105</td>
<td>–</td>
<td>0.22 (0.10–0.48)</td>
</tr>
<tr>
<td>No. of cycles with transfer (%)</td>
<td>68 (68.0)</td>
<td>95 (90.5)</td>
<td>.0001</td>
<td>0.22 (0.10–0.48)</td>
</tr>
<tr>
<td>Mean no. embryos/transfer (SD)</td>
<td>1.3 (0.5)</td>
<td>1.8 (0.4)</td>
<td>–</td>
<td>0.35–0.65</td>
</tr>
<tr>
<td>Implantation rate (IR), n (%)</td>
<td>47/89 (52.8)</td>
<td>48/174 (27.6)</td>
<td>&lt; .0001</td>
<td>2.94 (1.72–5.0)</td>
</tr>
<tr>
<td>Clinical pregnancy rate/transfer (%)</td>
<td>37/68 (54.4)</td>
<td>41/105 (43.1)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Clinical pregnancy rate/patient (%)</td>
<td>37/100 (37.0)</td>
<td>41/105 (39.0)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>No. of miscarriages (%)</td>
<td>1 (2.7)</td>
<td>16 (39.0)a</td>
<td>&lt; .0001</td>
<td>2.94 (1.72–5.0)</td>
</tr>
<tr>
<td>No. of ectopic pregnancies (%)</td>
<td>0</td>
<td>2 (4.9)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>No. of missed sacs (%)</td>
<td>0</td>
<td>22/48 (45.8)c</td>
<td>&lt; .0001</td>
<td>5.57 (3.09–10.03)</td>
</tr>
<tr>
<td>Ongoing IR</td>
<td>44/89 (49.4)</td>
<td>26/174 (14.9)</td>
<td>&lt; .0001</td>
<td>5.57 (3.09–10.03)</td>
</tr>
<tr>
<td>Delivery rate/transfer</td>
<td>52.9</td>
<td>24.2</td>
<td>.0002</td>
<td>3.52 (1.80–6.87)</td>
</tr>
<tr>
<td>Delivery rate/patient</td>
<td>36.0</td>
<td>21.9</td>
<td>.0009</td>
<td>2.00 (1.08–3.71)</td>
</tr>
<tr>
<td>No. of live births/transfer (%)</td>
<td>44/68 (64.7)</td>
<td>26 (27.4)</td>
<td>&lt; .0001</td>
<td>4.86 (2.49–9.52)</td>
</tr>
<tr>
<td>No. of live births/patient (%)</td>
<td>44/100 (44)</td>
<td>26 (24.8)</td>
<td>.0050</td>
<td>2.39 (1.32–4.32)</td>
</tr>
</tbody>
</table>

* One fetal loss (Down syndrome).
* One miscarriage + two vanishing twins.
* Sixteen miscarriages + six vanishing twins.

compared with the control group (1.3 vs. 1.8; \( P < .0001 \)). Interestingly, a total of 37 pregnancies were achieved in the PGD-A group, with only one clinical miscarriage, resulting in a delivery rate per transfer of 52.9%. In contrast, a total of 41 pregnancies were obtained in the control group, with 16 miscarriages (1 of them was a lethal loss with Down syndrome at week 14) and 2 ectopic pregnancies, with a delivery rate per transfer of 24.2% \( (P = .0002) \). Delivery rates per cycle after the first attempt were also significantly increased in the PGD-A group compared with the control group (36.0% vs. 21.9%; \( P = .0309 \)). Finally, in the PGD-A group, eight of the ongoing pregnancies were twin pregnancies (22.2%), and the remaining were singletons; there were 44 healthy live births. In the control group there were 26 live births from 20 singleton pregnancies and three twin pregnancies (13.0%). The number of implanted sacs was similar between groups; however, there were only 3 missed sacs in the PGD-A arm compared with 22 missed sacs in the control group.

Cumulative clinical outcomes after transfer of cryopreserved blastocysts (Supplemental Table 3) revealed that in the PGD-A group only one additional cryo-transfer was performed, resulting in a live birth. The cumulative delivery rate per patient was 37%, with 45 total live births (in total 15 surplus blastocysts were vitrified after first transfer). In the control group, 35 additional cryo-transfers were performed (in total, 154 blastocysts were vitrified), with 13 additional live births. In the control group, there were a total of 39 live births and a cumulative delivery rate per patient of 33.3%. No significant differences in the cumulative delivery rate per patient were observed between groups.

Supplemental Table 4 shows the differences in time to pregnancy in the present study, with a mean of 4.5 weeks in the PGD-A group vs. 5.8 weeks in the control group. There were significantly more transfers in the control compared with the PGD-A group (1.3 vs. 1.0; \( P < .0001 \)). A theoretical model, using a geometric distribution and assuming the observed “cumulative delivery rate/transfer” for PGD-A patients of 53.6%, showed that the expected mean number of transfers that would have been required to achieve the final goal of a “healthy baby at home” would have been 1.8. Using the same model, with the observed “cumulative delivery/transfer” of 26.9% in the control group, the expected mean number of transfers needed for a “healthy baby at home” without PGD-A would have increased to 3.7. Taking into consideration these differences, the theoretical time to achieve a live birth (expressed in weeks and considering 4 weeks when the first pregnancy was achieved in the first transfer) could be estimated as 7.7 weeks in the PGD-A group and 14.9 weeks in the control group, with a difference of 7.2 weeks between both arms of the study.

Cost-effectiveness per live birth in Europe and the United States was also calculated in each arm of the study. This assessment considered the cost of the different procedures involved in a PGD-A cycle with blastocyst transfer vs. standard blastocyst transfer, including the cost of clinical treatment, drugs, genetic analysis, cryopreservation, and additional cryo-transfers and clinical management of miscarriages and ectopic pregnancies (Supplemental Table 5). The cost per baby considering the methodology used in the present study was slightly higher in the PGD-A group (8%) compared with regular blastocyst transfer. However, with new NGS technologies routinely used in 2016, the price for PGD-A could decrease the cost per baby by 12% in Europe and by 10% in the United States, compared with a non-PGD-A cycle. It is worth noting the emotional cost advantages to PGD-A. The non-PGD-A group included 21 miscarriages and two ectopic pregnancies, whereas the PGD-A group had only one miscarriage.

**DISCUSSION**

This multicenter, prospective, randomized clinical trial in patients between 38 and 41 years of age demonstrates the superiority of PGD-A in achieving live birth at the first attempt, both per transfer and per cycle. These results were possible despite a significant decrease in the percentage of cycles reaching ET in PGD-A vs. control patients (69.0% vs. 90.5%) due to significantly fewer miscarriages in the PGD-A group vs. control (2.7% vs. 39.0%). At the first attempt there were 26 live births in the control group and 44 in the PGD-A group. Although the number of implanted sacs was similar in the two groups, there were only three missed sacs in the PGD-A arm, compared with 22 in the control group. This difference is most likely the result of the number of cycles with ET, emphasizing the utility of aneuploidy assessment in preimplantation embryos. The similarity in blastocyst development rates between groups (62.3% in PGD-A vs. 61.1% in controls) reinforces the concept that early embryonic development is not harmed by embryo biopsy even at the cleavage stage. On the basis of our results, clinicians must choose between a higher number of ETs with a greater risk of miscarriage; or decreased number of ETs with chromosomally normal embryos, reducing the chance of miscarriage and potential live births with chromosomal abnormalities. From the patients’ perspective this question has a straightforward response.

An independent prospective study showed that PGD-A miscarriage rates remain at approximately 7% regardless of patient age. Pregnancy loss in basic IVF (i.e., no PGD-A) was 11% in patients aged <35 years and increased to >35% in patients aged 41 to 42 years. Similarly, the percentage of embryos with successful implantation dropped from 36% to 9% in the same time period, according to women’s age, remaining constant at 50% when euploid embryos were selected for transfer using PGD-A (26). In our study the incidence of aneuploidies was 78.6%, with 33.6% single aneuploidies; most were uniform chromosome aneuploidy, and only 1.1% were segmental aneuploidies. All chromosomes were equally affected; uniform chromosome aneuploidies were most likely to result in higher incidences of miscarriages and live births carrying aneuploidies (27). Chromosomes 1 and 2 presented the highest rate of aneuploidies. Additionally, 18.3% of the embryos showed abnormalities that would not have been detected by FISH technology (9 chromosomes) published in previous RCTs in AMA patients (17).

We also considered cumulative pregnancy and live birth rates. Patients who produced multiple embryos had one or
two transferred and the rest cryopreserved for future use. If no pregnancy was achieved after the first transfer, we included the transfers performed from cryopreserved blastocysts during a 6-month period after closing recruitment. Following this strategy, because some patients underwent multiple embryo transfers, cumulative ongoing pregnancy rates per patient were assessed in PGD-A and control groups (37.0% vs. 33.0%, respectively), obtaining 45 and 39 live births in PGD-A vs. controls, respectively. Cumulative pregnancy and live birth rates were not significantly different; however, medical procedures related to miscarriages imply a significant emotional burden, carry substantial risks, and delay the time to live birth. In those patients in whom all embryos were chromosomally abnormal and failed to have ET in the PGD-A group, the psychological, physical, and economic burden of further stimulation cycles and oocyte retrieval procedures that have been avoided should also be considered.

It is important to point out that, despite embryo aneuploidy likely being the primary cause of reproductive failure in AMA, other contributing factors could be mitochondrial content (28, 29) and the endometrial factor (30). These additional factors are worth investigating after the first failed transfer of euploid embryos. The personalization of the window of implantation using the endometrial receptivity test to guide personalized ET of the remaining euploid embryos is a promising avenue in such cases (30).

Considering the clinical results of this RCT, we have performed a cost-effectiveness study per live birth based on figures relevant to both Europe and the United States. Our results demonstrate that, for a blastocyst biopsy and NGS for screening of aneuploidy, the estimated cost per live birth could be even lower in the PGD-A group than the cost of standard IVF with multiple untested blastocyst transfers. Regarding time to pregnancy, our empirical results and the theoretical calculations show that PGD-A decreased the number of transfers needed to achieve a live birth and therefore the number of weeks required for this final goal. Therefore, the argument moves from the efficacy in producing a healthy baby, decreasing miscarriages, and time to pregnancy to the scenario of no available embryos for transfer and cryopreservation after PGD-A, with the emotional burden of miscarriages and Down syndrome risk together with lower implantation rates in controls.

The main limitation of this study is the historical setting in which this RCT was designed. At the time of recruitment, day-3 embryo biopsies and aCGH were the standard for 24-chromosome screening. We designed the study on day-3 embryo biopsy on the basis of our previous published RCT (17). Our results demonstrate that, in our hands, blastocyst rate was not different between biopsied vs. nonbiopsied cleavage stage embryos (16, 17). More compelling data from another group (31) appeared after the initiation of this RCT, thus it is possible that trophectoderm biopsy would have shown even bigger differences.

At the time of publication the standard of care for PGD-A is a trophectoderm biopsy with NGS technology, which has been validated for screening whole chromosome and segmental aneuploidies (32). Despite these differences, we consider the conclusions derived from this study to be relevant to AMA patients with similar characteristics in which trophectoderm biopsy could be offered. On the other hand, the fact that the first transfer was performed during the controlled stimulated cycle (fresh cycle) in both arms of the study allowed us to estimate the success rates of the treatment in this particular condition without the potential bias of cryopreservation and deferred transfers commonly applied in blastocyst biopsies.

In conclusion, we found that PGD-A improved the likelihood of successful pregnancy and live birth compared with conventional morphologic embryo selection. Without the ability to screen for aneuploidy, AMA patients with a high percentage of aneuploid embryos may be subjected to multiple unsuccessful ETs for months, some of which may end in distressing miscarriages and associated medical risks. With the introduction of NGS, the cost of PGD-A is becoming increasingly affordable and enables embryo chromosome analysis in IVF (33, 34). Different factors contribute to patient-perceived determinants when choosing to accept or decline PGD-A, including cost, religion, ethical values, social and family support, provider influences, and past reproductive experience of the patient (35). In the light of the present results, with the current trend toward single-embryo transfer, it could be argued that failure to investigate the chromosomal constitution of the preimplantation embryo to be transferred may raise ethical questions of its own (36).

Acknowledgments: The authors thank the clinicians and nurses at the Instituto Valenciano de Infertilidad clinics in charge of informing and recruiting patients; the IVF laboratory and PGD teams performing the procedures; the molecular biologists of the preimplantation genetic screening group at Igenomix for their involvement with the study; Asunción Martinez for data collection; Dr. Ernesto Bosch and Mónica Clemente for statistical support; and Graham Snudden for his support in implementing and developing the study.

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