Preimplantation genetic screening in women of advanced maternal age caused a decrease in clinical pregnancy rate: a randomized controlled trial

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BACKGROUND: Advanced maternal age (AMA) is an important parameter that negatively influences the clinical pregnancy rate in IVF, in particular owing to the increased embryo aneuploidy rate. It has thus been suggested that only transferring euploid embryos in this patient group would improve the pregnancy rate. The purpose of this study was to test whether employing preimplantation genetic screening (PGS) in AMA patients would increase the clinical pregnancy rate. METHODS: We conducted a two-center, randomized controlled trial (RCT) to analyze the outcome of embryo transfers in AMA patients (>38 years of age) after PGS using FISH analysis for chromosomes X, Y, 13, 16, 18, 21 and 22. The PGS group was compared with a control group. The primary outcome measure was clinical pregnancy rate after 6–7 weeks of gestation per randomized patient. RESULTS: The study was terminated early as an interim analysis showed a very low conditional power of superiority for the primary outcome. Of the 320 patients calculated to be included in the study, 56 and 53 patients were randomized into the PGS and control groups, respectively. The clinical pregnancy rate in the PGS group was 8.9% (95% CI, 2.9–19.6%) compared with 24.5% (95% CI, 13.8–38.3%) in the control group, giving a difference of 15.6% (95% CI, 1.8–29.4%, P = 0.039). CONCLUSIONS: Although the study was terminated early, this RCT study provides evidence against the use of PGS for AMA patients when performing IVF. Trial registration number: ISRCTN38014610.

Keywords: AMA; PGS; embryo biopsy; RCT; IVF

Introduction

It is well established that maternal age is one of the most important variables predicting live birth after in vitro fertilization (IVF) (e.g. Piette et al., 1990; Schieve et al., 1999; Elizur et al., 2005). Consequently, and despite the development of and increasing success rates in assisted reproductive technology (ART), women aged >35 have a much reduced chance of reaching childbirth. Women in this age group constitute a substantial proportion of those who are treated with IVF. According to the latest report from the American Society of Reproductive Medicine/Society of Assisted Reproduction Technology Registry (ASRM/SART) reflecting the results for 2001, the overall success rate for women <35 years was 38.9% deliveries per oocyte retrieval, whereas for women >40 years, it was 11.1% deliveries per oocyte retrieval (The practice Committee of the Society for Assisted Reproductive Technology and the Practice committee of the American Society for Reproductive Medicine, 2007).

Although both hormonal, uterine and oocyte factors (e.g. Meldrum, 1993; Pellicer et al., 1995; Saldeen et al., 2007) have been suggested to explain the decline in ART results for women of advanced age, one major factor appears to be the decline in oocyte quality, with chromosomal abnormalities probably being one important aspect. Increased rates of chromosomal abnormalities have been reported in oocytes from older women (Angell et al., 1993; Battaglia et al., 1996; Anahory et al., 2003; Pellestor et al., 2003; Zhivkova et al., 2007). In addition, it has been shown that embryos from older women have more chromosomal abnormalities (Munne et al., 1995; Dailey et al., 1996), and that embryo morphology and development rates and maternal age all correlate with chromosome abnormalities (Munne et al., 1995; Hardarson et al., 2001, 2003; ziebe et al., 2003; Magli et al., 2007). A decline in oocyte quality with age is further supported by studies showing that older women failing to conceive with their own oocytes may conceive by using donor oocytes from younger women (Navot et al., 1991; Borini et al., 1995).
Selection of embryos for transfer is routinely based on the developmental rate and embryo morphology evaluated using light microscopy. However, the correlation between these variables and chromosomal status is not complete.

Originally, preimplantation genetic diagnosis (PGD) was developed in order to identify non-affected embryos in couples with a known severe hereditary disease (Handyside et al., 1989, 1992). However, more recently the use of the technique has been broadened to include IVF performed in couples without genetic diseases, with a purpose of identifying chromosomally normal embryos for transfer in order to improve pregnancy and delivery rates (Verlinsky et al., 1995, 1996a,b; Gianaroli, 1997; Munné, 1998, 1999; Kahraman et al., 2000). Theoretically, the selection and transfer of chromosomally normal embryos ought to improve pregnancy and delivery rates and reduce abortion rates, particularly in women of advanced age. Observational studies with matched controls have shown an increased implantation rate after preimplantation genetic screening (PGS) in this group of patients (Gianaroli et al., 1999; Munne et al., 2003, 2007a,b).

The design of these studies has varied concerning day of transfer, number of chromosomes analyzed and number of embryos transferred, as well as the indications for PGS. The lack of randomization, however, gives these studies low evidence levels, making it difficult to draw conclusions. In fact, the authors of the latest SART/ASRM practice committee report find that the evidence does not support the use of PGS with AMA as an indication (The practice Committee of the Society for Assisted Reproductive Technology and the Practice committee of the American Society for Reproductive Medicine, 2007).

In contrast to the observational studies, the results from the three randomized controlled trials (RCTs) (Staessen et al., 2004; Stevens et al., 2004; Mastenbroek et al., 2007) do not show PGS to significantly improve implantation or clinical pregnancy rates per started cycle as compared with controls. In these studies, a maximum of three embryos could be replaced.

In Sweden, as a rule, single embryo transfer is performed with the exception in women of advanced maternal age (AMA), where two embryos may be replaced. We hypothesized that aneuploidy screening could be more important when only one or two embryos were replaced. The aim of this randomized study was therefore to investigate whether PGS of embryos on Day 3 would increase the clinical pregnancy rate per randomized patient after IVF in women of advanced reproductive maternal age (≥38 years).

Materials and Methods

Study design

The study was performed as a randomized non-blinded, controlled, two-center study comparing the PGS group with a control group. The study took place at Sahlgrenska University Hospital and Fertility Center Scandinavia, Carlanderska hospital, Göteborg, between November 2003 and February 2007. The study population consisted of all consecutive patients at the participating clinics who fulfilled the inclusion criteria for the study and were willing to take part. Ethical approval was obtained from the ethics committee of Göteborg University, and all patients signed informed consent.

The primary outcome of the study was clinical pregnancy rate, shown as fetal heart activity per randomized patient. Secondary outcomes were pregnancy rate per transfer, and rates of implantation, spontaneous abortion and delivery.

The randomization was performed centrally in a 1:1 fashion, after the embryo evaluation on Day 3 post-insemination/microinjection. Randomization into two groups was performed using a computerized randomization program and balanced for the following prognostic variables: age (mean), age (>40 years (yes/no)), number of earlier IVF cycles leading to transfer (fresh and frozen), tubal factor (yes/no) and number of good-quality embryos on Day 3.

In the PGS group, all good quality embryos were analyzed using FISH in order to select chromosomally normal embryos for transfer. In the control group, no FISH analyses were performed.

Sample size calculation

A retrospective analysis of Day 2 transfers, using the databases from the two IVF clinics, had shown a clinical pregnancy rate per aspirated cycle of 11.9% (56/470) in women ≥38 years as compared with 24.4% (465/1908) in women <38 years. Our hypothesis was that PGS would increase the clinical pregnancy rate in women ≥38 years to the same rate as in women below this age. With 160 patients randomized to each arm, there would be 80% power to detect a 12% difference (12 versus 24%) at the 5% level of significance. Recruitment of patients was planned to take 2 years, including patients from both clinics. The study was planned to take a total of 3 years.

Inclusion criteria and randomization

Couples with infertility of female or male origin, intending to undergo IVF or ICSI, who had signed a written consent form and in which the age of the woman was ≥38 years were eligible for randomization. The couple had to have at least three embryos of good morphological quality (GQE). After an amendment, owing to the introduction of SET in Sweden in 2003, only two GQE were required if the patient only wanted one embryo back. Patients were randomly assigned on Day 3 by computer, with the use of minimization allocation, in ratios (1:1) to PGS or control.

Exclusion criteria

Patients who had previously been randomized to either of the two study groups in this trial.

Ovarian stimulation and embryo culture

Following the down-regulation with buserelin (Suprecur®) or nafardin (Synarel®) nasal spray starting in the mid-luteal phase of the previous cycle, ovarian stimulation was performed with recombinant FSH (Gonal-F®). Follicular aspiration was performed 36–38 h after the hCG (Ovitrelle®) administration, using vaginal ultrasonography. Fertilization was performed by IVF or ICSI following standard techniques. Thereafter embryos were cultured for 3 days. Embryos were scored prior to randomization according to our grading system (Hardarson et al., 2001; Ziebe et al., 2003).

On Day 3, embryos that had been biopsied were transferred into a blastocyst medium for culture until Day 4 when their morphology was re-evaluated. Embryos from this group were selected for transfer first based on the PGS results and secondly the morphology on Day 4. An important variable was continued cleavage after biopsy. In the biopsy group, only embryos that showed normal chromosomal setup were transferred. Embryos that were found to be abnormal were later fixated for subsequent re-analysis. Embryos with an undetermined chromosomal constitution were not transferred but were
cultured until Day 5 and cryopreserved if they reached the blastocyst stage. Embryos in the control group were transferred on Day 3.

One or two embryos were transferred according to the patients’ wishes and the availability of normal embryos. Luteal support was given as vaginal progesterone (400 mg × 3). Pregnancy was defined as positive hCG in serum or urine measured at the earliest 14 days after embryo transfer. Clinical pregnancy was defined as a gestational sac with fetal heartbeats at least 5 weeks after embryo transfer.

**Embryo biopsy procedure**

Embryos of good quality were biopsied on Day 3 after oocyte retrieval using a previously described procedure (Hanson et al., 2001). In short, laser assisted biopsy was performed on all embryos that had >5 cells and <20% fragmentation. A single blastomere was biopsied in the majority of cases. Two blastomers were obtained if the first one did not contain a nucleus as observed during fixation.

**Fixation of interphase nuclei, FISH procedure and scoring**

The blastocysts were fixed on glass slides using 0.01 N HCl + 0.1% Tween 20 as first introduced by Coonen et al. (1994). Care was taken to remove as much of the cytoplasm as possible from the nuclei to improve the FISH probe penetration.

The slides were washed for 1 min in 1 × PBS followed by an ethanol series of 70, 85 and 99.5% for 1 min each. For simultaneous detection of five chromosomes, a multicolor kit was used (Multi-Vision™ PGT multi-color Probe Panel, Vysis Inc, USA) that includes chromosomes 13, 18, 21, X and Y. A small amount (0.3 μl) of the probe was pipetted onto the nuclei, a cover glass applied and glued, and the preparation was denatured at 80°C for 3 min on a heating plate. The slides were incubated at 38°C overnight in a moist chamber, the cover glasses were then removed and the slides washed at 73°C. 0.4 × SSC (2 min) followed by 2 × SSC + 0.1% NP40 for 10–15 s to remove unspecific staining. The slides were thereafter air-dried, antifade solution was applied and the preparation was sealed with a cover glass. The nuclei were observed with an epifluorescence microscope equipped with appropriate filters. Before the second FISH round, the cover glasses were carefully removed and the slides were then washed for 10 min in 1 × PBS. After dehydration in ethanol series, a probe solution for chromosomes 16 and 22 was applied (Vysis Inc, USA). The FISH procedure was performed as described above except that the incubation time was shortened to 5 h.

**FISH scoring criteria**

FISH signals had to be at least one signal’s width apart to be scored as two separate signals. The embryos were categorized according to the following criteria:

- A nucleus was considered:
  - Normal: when all seven analyzed chromosomes were present in the correct numbers.
  - Abnormal: when an uneven number of chromosomes was found.
  - Undetermined: when very weak or no FISH signals were found or when no nucleus was found.

**Statistical methods**

The statistical analysis was performed according to intention to treat, i.e. in the analysis the patients belonged to the group to which they were randomized. Comparisons between the groups were analyzed for continuous variables with Mann–Whitney U-test and for proportions with Fisher’s exact test. For the main outcome, 95% CIs were given for estimates and differences between groups. For descriptive statistics mean, SD, median and range were used. All tests were two-sided and the level of significance was 0.05.

**Results**

A total of 331 patients (from 386 cycles) satisfied the inclusion criteria concerning patient characteristics and signed informed consent. Of them, 109 (32.9%) fulfilled all the inclusion criteria, including numbers of good-quality embryos, and were randomized (Table I and Fig. 1). Fifty-six patients were randomized to blastomere biopsy and 53 patients to the control group. The main reason for a patient not being included in the study was that she had very few embryos of good quality. Significantly more GQE were found in the PGS group on Day 3 as compared with that found in the control group. This would normally have been corrected for in the statistical analysis, but since the PGS group did significantly worse than the control group despite this fact, it was decided that a correction was not needed.

A total of 332 embryos satisfied the biopsy criteria (Fig. 2). From these, a successful FISH analysis was performed on 302 embryos (91.5%). Of these analyzed embryos, 98 (32.4%) had normal chromosome content for the chromosomes analyzed, whereas 204 embryos had an abnormal number of chromosomes. The single most common aberration was monosomy 16, which was found in 10% of all embryos analysed. Of the 30 embryos where no FISH results were obtained, the most frequent reason was that no nucleus and/or only a nuclear fragment was present on the slide (25/30). Of the 338 biopsied embryos, 31 embryos (9.2%) had two blastomeres biopsied, whereas a single blastomere was biopsied from the remaining embryos. Of these, four embryos were transferred in four

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**Table I. Demographics of randomized patients.**

<table>
<thead>
<tr>
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<th>PGS group (n = 56)</th>
<th>Control group (n = 53)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean female age (SD) (Median, Range)</td>
<td>40.5 (2.0) (40.0, 38–46)</td>
<td>40.6 (1.8) (40.0, 38–45)</td>
<td>0.57</td>
</tr>
<tr>
<td>Tubal factor (%)</td>
<td>11 (19.6%)</td>
<td>11 (20.8%)</td>
<td>1.00</td>
</tr>
<tr>
<td>No. of previous fresh transfers (Median, Range)</td>
<td>1.39 (1.5) (1.0, 0–6)</td>
<td>1.34 (1.3) (1.0, 0–5)</td>
<td>0.87</td>
</tr>
<tr>
<td>Routine IVF (%)</td>
<td>37 (66.1%)</td>
<td>36 (67.9%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Mean no. of oocytes (SD) (Median, Range)</td>
<td>12.2 (5.5) (10.5, 4–30)</td>
<td>12.5 (5.2) (12.0, 3–26)</td>
<td>0.63</td>
</tr>
<tr>
<td>Mean no. of 2PN (SD) (Median, Range)</td>
<td>8.1 (3.9) (7.0, 3–19)</td>
<td>7.9 (4.0) (7.0, 2–20)</td>
<td>0.51</td>
</tr>
<tr>
<td>Mean no. of GQE Day 2 (SD) (Median, Range)</td>
<td>5.8 (3.1) (6.0, 1–16)</td>
<td>5.1 (2.4) (4.0, 2–11)</td>
<td>0.23</td>
</tr>
<tr>
<td>Mean no. of GQE Day 3 (SD) (Median, Range)</td>
<td>5.9 (2.8) (5.0, 3–14)</td>
<td>4.9 (2.1) (4.0, 2–11)</td>
<td>0.037</td>
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</table>
patients. All the embryos that were analysed as abnormal were fixated for reanalysis, which is currently ongoing.

One chromosomally normal embryo (1.4%) was not transferred in the PGS group due to that it arrested in development between Days 3 and 4.

The clinical pregnancy rate/randomized patient was 8.9% (95% CI, 3.0–19.6%) and 24.5% (95% CI, 13.8–38.3%) in the PGS and the control groups, respectively, with the absolute difference being 15.6% (95% CI, 1.8–29.4%, \(P = 0.039\)). The pregnancy rates/embryo transfer in the two groups were 22.2% (95% CI, 11.2–37.1%) and 30.2% (95% CI, 18.3–44.3%), difference of 8.0% (95% CI, −9.4 to 25.3%); \(P = 0.34\) (Table II). The live birth rate in each group was 5.4% (95% CI, 1.12–14.9%) and 18.9% (95% CI, 9.4–32.0%), a difference of 13.5% (95% CI, 1.4–25.6%; \(P = 0.039\)). No difference was found in the live birth rate per randomized patients between two groups divided according to the method of fertilization.

**Interim analysis**

Owing to the lower than expected recruitment rate, an external Data Monitoring Committee (DMC) was appointed to perform an interim analysis. The O’Brien–Fleming boundary truncated at \(z = 3.5\) for benefit and Pocook boundary (\(z = −2.5\)) for harmful effect and conditional power for benefit <20% (lack of benefit) were used as group sequential boundaries. At the interim analysis when 34% of the projected subjects had been enrolled, the \(z\) value was −1.26 and the conditional power for benefit was 10%. In summary, the interim analysis showed no difference in baseline data between the groups, no heterogeneity between sites in a subgroup analysis, significantly fewer embryo transfers in the PGS group, and a very low conditional power to show superiority for the PGS group, for the primary outcome. The DMC recommended early termination of the study, and it was agreed to by the steering group.

**Discussion**

The main finding in this study was that PGS as a tool for selecting embryos for transfer did not improve the clinical pregnancy rate per randomized patient in women of advanced age. On the contrary, the clinical pregnancy rate was lower for the PGS group. Regarding the secondary outcomes, no difference was seen in pregnancy rate per transfer, implantation rate or spontaneous abortion rate between the groups, while the live birth rate was significantly lower for the PGS group. The interim analysis indicated that the chance of showing superiority for the PGS group according to our hypothesis was very low (conditional power 10%), even if we had continued the study. The clinical pregnancy rate in the control group (24.5%) was higher than observed in the retrospective analysis from our clinics in women \(\geq 38\) years of age (11.9%). The reason for this might be that both clinics have improved their results compared to these retrospective data. Another reason could be that the extended embryo culture to Day 3 is of advantage for this group of patients. It could, of course, also be a chance finding due to the limited number of patients included.

In our study, the sample size calculation was based on clinical pregnancy rate as the primary outcome. In this respect, our study design is similar to that of Mastenbroek et al. (2007) although they included women \(\geq 35\) years, while our patients were \(\geq 38\) years of age. In the Mastenbroek study, 408 women were randomly assigned to PGS or control, with ongoing pregnancy rate as the primary outcome measure. They found a significantly reduced pregnancy and live birth rate in the PGS group. Our data support these findings. The two other RCT’s that have been published, do not show any benefit from using PGS for the indication of AMA (Staessen et al., 2004; Stevens et al., 2004). In the study by Staessen et al. (2004), 400 women aged \(\geq 37\) were randomized. No statistical difference was found in the primary outcome, implantation rate, pregnancy rate per transfer or pregnancy rate per
cycle between the PGS and the control groups. Similarly, in the prospective study by Stevens et al. (2004), 40 women over 35 years of age were randomized, with no significant differences in either clinical pregnancy or implantation rates found in the PGS group as compared with the controls.

The reasons for these results are not clear. Theoretically, a selection of chromosomally normal embryos would improve pregnancy rates and reduce abortion rates. However, as shown in the present and previous randomized trials, no improvement in efficacy has been observed. Several possible mechanisms have been suggested for this finding: the biopsy may be detrimental to embryo development: either the removal of 1–2 cells as such or the handling during the biopsy procedure. Choosing a chromosomally normal embryo might not always guarantee that the embryo will be developmentally viable. In addition, the developmental potential of mosaic embryos is not known. This chromosomal mosaicism is also a potential source of error when performing PGD/PGS, which might lead to transfer of abnormal and/or discarding of (mainly) normal embryos. In fact, by removing an abnormal cell from a mosaic embryo, the percentage of normal cells will increase, but the embryo will be considered abnormal. At the same time, if a normal cell is removed from a mosaic embryo, the percentage of normal cells will decrease, but the embryo will be considered normal and might well be transferred. This could be one reason for the lower than expected implantation rate. Furthermore, not all chromosomes are currently being analyzed, owing to the limitations in the techniques used. Finally, it has been shown that the transfer of embryos showing both mono- and trisomic chromosomal status after PGD/PGS can generate unaffected children, (M. Hughes, personal communication), which suggests some kind of repair mechanism and/or exclusion of abnormal blastomeres.

In our opinion, it is important to bear in mind that an embryo is comprised not only of chromosomes and that poor results obtained after PGS cannot solely be explained in terms of suboptimal performance by the PGD/PGS center(s) as has been proposed (Munne et al., 2007a,b). An ideal set of chromosomes does not necessarily go hand in hand with an ideal metabolic state. There is an increasing number of publications concerning cytoplasmic maturation and metabolic turnover rates (e.g. Brison et al., 2004; Seli et al., 2007), which may provide useful information about how to select embryos based on their different metabolic competence in the near future.

Few studies have been performed, regarding the potential impairments from performing embryo biopsy. In a study by Hardy et al. (1990), it was shown that blastomere biopsy did not impair subsequent in vitro development. However, considering the lower than expected pregnancy results in the randomized studies, the biopsy process might still play a negative role. In our regular PGD program, we have a clinical pregnancy rate/started cycle of 18% (28% per embryo transfer) for PGD with X-linked disease as an indication. In comparison, in the ESHRE PGD consortium (Harper et al., 2006), the overall success rate for PGD with X-linked disease as the indication is 19% clinical pregnancy rate per oocyte retrieval (24% per embryo transfer). These results indicate that the low success rate observed after PGS in the current study is probably not attributable to technical problems in the embryo biopsy technique used in our laboratory. However, embryo biopsy is an invasive procedure that may potentially disturb viability and subsequent development of the embryo. The frequency of normal embryos found in our study (32%) was in accordance with both the figures from ESHRE for PGS with maternal age as the indication in the ESHRE PGD consortium (Magli et al., 2007) who found a rate of 34% normal embryos out of 4665 embryos analysed.

The day of the biopsy may be another possible issue. It might be that blastocysts are better suited for biopsy as more cells can then be harvested and analyzed with good clinical results (McArthur et al., 2005). However, in general, the most common techniques for performing PGS today are either cleavage stage biopsy of one or two blastomeres (Handyside, 1989) or polar body biopsy from the oocyte (Verlinsky et al., 1990, 1996a,b).

Transfer was made on different days in the two study groups. In the control group, transfer was performed on Day 3. The reason why we chose to transfer on Day 3 was that we wished to keep each step for the control group as similar as possible to our ordinary routines. This means Day 2 or Day 3 for transfer but not Day 4. Such a strategy will increase generalizability or external validity of the results. In the intervention group, transfer was performed on Day 4 of practical reasons.

Although never more than two embryos were transferred, the numbers transferred were significantly higher in the control group. This has been observed in all comparative PGS studies. Since in this study a rather ‘old’ group of patients were treated, the total number of embryos available will be

<table>
<thead>
<tr>
<th>Table II. Results.</th>
<th>PGS group (n = 56)</th>
<th>Control group (n = 53)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal embryos; Mean (SD) (Median, Range)</td>
<td>1.75 (1.3) (1.5, 0–5)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Non-diagnosed; Mean (SD) (Median, Range)</td>
<td>0.68 ± 0.99 (0, 0–4)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>No. of transfers (% per randomized)</td>
<td>45 (80.3%) 53 (100%)</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Embryos transferred/ET; Mean (SD) (Median, Range)</td>
<td>1.5 (0.5) (1.0, 0–2)</td>
<td>1.8 (0.4) (1.0, 1–2)</td>
<td>0.003</td>
</tr>
<tr>
<td>Pregnancy rate (% per ET)</td>
<td>10/45 (22.2%)</td>
<td>16/53 (30.2%)</td>
<td>0.34</td>
</tr>
<tr>
<td>Pregnancy rate (% per randomized)</td>
<td>10 (17.9%)</td>
<td>16 (30.2%)</td>
<td>0.18</td>
</tr>
<tr>
<td>Clinical pregnancy rate (fetal heart)</td>
<td>5 (8.9%)</td>
<td>13 (24.5%)</td>
<td>0.039</td>
</tr>
<tr>
<td>No. of live births (% per randomized)</td>
<td>3 (5.4%)</td>
<td>10 (18.9%)</td>
<td>0.039</td>
</tr>
<tr>
<td>Implantation rate (%)</td>
<td>8/70 (11.4%)</td>
<td>18/95 (18.9%)</td>
<td>0.19</td>
</tr>
<tr>
<td>Spontaneous abortions (%)</td>
<td>7/10 (70.0%)</td>
<td>6/16 (37.5%)</td>
<td>0.11</td>
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</tbody>
</table>
reduced. In addition, we have, in the biopsy group, excluded abnormal embryos, thus lowering the number of available embryos even further in this group.

In this study, two-thirds of the eligible patients did not fulfill the inclusion criteria owing to the lack of enough number of GQE on Day 3. It has been suggested that in order to have a successful PGS cycle with AMA as an indication the couple needs at least six embryos that can be biopsied (Munne et al., 2007a,b). In our study, this only applied to 17% of the eligible patients. This clearly shows the difficulties in performing PGS in this patient group and, in light of the results found here, the patients might have done better receiving all their embryos, one at a time.

In conclusion, this study gives no support to the hypothesis that PGS improves the clinical pregnancy rate in women of advanced age. Thus, based on the data from this trial and previous RCTs, routine use of PGS for the indication AMA should not be recommended.

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