Redefining advanced maternal age as an indication for preimplantation genetic screening

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Abstract In this retrospective study, the utility of preimplantation genetic screening (PGS) in patients with advanced maternal age is evaluated. The patient population consisted of women aged 38–44 years and included in a regular IVF programme with or without PGS analysis. Transfer rate, ongoing implantation rate and ongoing pregnancy rate were the main outcome parameters measured. A trend of better ongoing pregnancy rate per oocyte retrieval was observed in patients aged 38 and 39 years in the non-PGS group when compared with PGS groups, but better ongoing pregnancy rate per oocyte retrieval was observed in patients 41–44 years old in the PGS group. When patients with a low ovarian response accumulated oocytes in several stimulation cycles, clinical outcomes were comparable to those of normal-responder patients. These results show that, although PGS does not benefit patients less than 40 years of age, reproductive success increases more than two-fold in patients over 40 years, especially in patients with more than six metaphase II oocytes, as a result of a good ovarian response or gamete accumulation, suggesting a redefinition of advanced maternal age as indication for PGS.

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Introduction

Lifestyle changes now cause many women to delay motherhood until their thirties, when they are more psychologically and economically stable (Benzies et al., 2006; te Velde and Pearson, 2002). This new reproductive trend directly affects assisted reproduction centres, as most women erroneously believe that IVF can reverse the effects of ageing (Maheshwari et al., 2008). For example, a significant increase in advanced maternal age (AMA) patients attending the study clinic in recent years has been found compared with the years prior.
to 1995, shifting the mean age of patients from approximately 34 years to approximately 37 years.

Preimplantation genetic diagnosis (PGD) was initially developed as an option to conceive healthy children in couples with genetic disorders or with sex chromosome syndromes. The use of fluorescence in-situ hybridization (FISH) in PGD made the analysis of aneuploidies in embryos by preimplantation genetic screening (PGS) possible and, currently, the screening of aneuploidies by PGS covers a large number of patients visiting infertility centres all over the world. The inclusion of many of these patients under indications such as AMA, implantation failure and recurrent miscarriage is based on the high percentage of abortions cytogenetically studied from idiopathic miscarriages with chromosomal imbalances. Importantly, most of the embryos from patients with AMA, recurrent miscarriage and implantation failure are aneuploid (Baart et al., 2006; Bettio et al., 2008; Ferro et al., 2003; Findikli et al., 2006; Lathi et al., 2008; Munne et al., 2004, 2006; Pehlivan et al., 2003a,b; Rai and Regan 2006; Rubio et al., 2005a; Stephenson et al., 2002; Wilding et al., 2004). These results gave rise to the logical but not sufficiently supported hypothesis that the selection of euploid embryos for a selected set of chromosomes (representative of the most common aneuploidies found in miscarriages) would increase reproductive success while reducing the number of aneuploid conceptions and subsequent miscarriages or terminations of pregnancy. Several retrospective studies in support of this hypothesis describe the selection of euploid embryos as producing, in most of them, clinical benefits (Colls et al., 2007; Garrisi et al., 2009; Gianaroli et al., 1999, 2003, 2005; Grifo et al., 2007; Montag et al., 2004; Munne et al., 1999, 2003, 2006; Obasaju et al., 2001; Pehlivan et al., 2003c; Platteau et al., 2005; Rubio et al., 2005b).

In patients with AMA, the availability of transferrable embryos is affected by the low quantity and quality of oocytes retrieved after ovarian stimulation, which is closely related to the ovarian reserve (Broekmans et al., 2007). This problem is magnified in the case of patients included in PGS, where only those embryos that are properly developed and diagnosed as chromosomally normal are candidates for embryo transfer. Advances in technology, as well as new therapies for fertility preservation such as oocyte and embryo vitrification (Kuwayama 2007; Cobo et al., 2008a,b,c) create promising new possibilities for those patients with reduced ovarian reserve, allowing them to reserve gametes for a fertility treatment with a higher number of oocytes.

The present study analysed the utility of PGS in patients with AMA considering both female age (in a year-by-year fashion) and the ovarian response. This approach has allowed us to observe trends for different clinical parameters according to the age of patients and the number of metaphase II (MII) oocytes retrieved, revealing a new sub-group of patients in which PGS provides the best reproductive option with one’s own gametes.

Materials and methods

Study design

This retrospective study selected patients included in the study centre’s PGS programme with AMA as main medical indication ranging from 38–44 years and a normal karyotype. A written consent was compulsory to be included in the PGS programme. All patients within the same age range and attending the centre for regular IVF treatment without PGS or PGD within the same period of time were considered as the historical control group (non-PGS group). In the first part of this study, only cycles with fresh oocytes were included. Fertilization in all cycles was achieved by intracytoplasmic sperm injection (ICSI) with motile ejaculated spermatozoa displaying normal morphology. Data from patients with an azoospermic partner were excluded.

A second part of the study focused on a different group of PGS patients aged 41–44 years who accumulated oocytes by vitrification (patients not included in the former part) to increase their reproductive chances. Outcomes were analysed depending on the number of MII oocytes retrieved after a single ovarian stimulation or after accumulation of MII vitrified oocytes from several stimulation cycles. Since vitrification was first introduced routinely in this IVF laboratory in January 2007, this part of the study included only patients treated from January 2007 to May 2009 with the same inclusion/exclusion criteria exposed above.

IVF and embryo biopsy

Stimulation, oocyte retrieval and ICSI procedures were performed as described previously (Meseguer et al., 2003). Pronuclear zygote morphology was assessed at 16–18 h post-ICSI and embryo development was checked every 24 h. Embryo biopsy was performed on day-3 embryos with ≥5 nucleated blastomeres and ≤25% fragmentation. One or two blastomeres were analysed according to embryo development (one in embryos with 5 or 6 blastomeres; two in embryos with ≥7 blastomeres). For biopsy, embryos were placed on a droplet containing Ca2+ and Mg2+ free medium (G-PGD; Vitrolife, Göteborg, Sweden) and the zona pellucida were perforated using laser technology (OCTAX, Herbron, Germany). After the biopsy, embryos were carefully washed and co-cultured on a monolayer of endometrial epithelial cells (Mercader et al., 2006). In the PGS group, a mean ± SD of 1.4 ± 0.6 euploid embryos were transferred on day 5 at morula or blastocyst stage. In the non-PGS group 1.9 ± 0.6 good-quality embryos were transferred either on day 3 or day 5 of development. Arrested embryos or embryos with abnormal or inconclusive results were not transferred. For this study, pregnancies were defined as clinical pregnancies upon detection of a gestational sac on the vaginal ultrasound performed at week 5 of gestation.

Oocyte vitrification

The Cryotop method for oocyte vitrification was used as previously described by Kuwayama et al. (2005) and adapted to the study laboratory (Cobo et al., 2008a). In brief, oocytes were subjected to a solution containing 7.5% (v/v) ethylene glycol (EG) +7.5% (v/v) dimethylsulphoxide (DMSO) in TCM199 medium +20% (v/v) synthetic serum substitute (SSS) at room temperature for 15 min. Subsequently, oocytes were placed in a solution containing 15% EG +15% DMSO +0.5 mol/l sucrose. One minute later, they were placed on the Cryotop strip and immediately submerged in

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filtered liquid nitrogen (Brymill filter model 9409). For warming, the Cryotop was removed from the liquid nitrogen and instantly placed in 1.0 mol/l sucrose in TCM199 + 20% SSS at 37 °C. After 1 min, oocytes were placed in 0.5 mol/l sucrose in TCM199 + 20% SSS at room temperature for 3 min. Finally, two consecutive washes (5 min and 1 min) were performed with TCM199 + 20% SSS at room temperature before oocytes were incubated for 2 h preceding ICSI.

**FISH protocol**

Aneuploidy screening was performed by FISH as follows. The first round of hybridization was performed using probes for chromosomes 13, 16, 18, 21 and 22 (Multivision PB; Vysis, Downers Grove, IL, USA) and the second round included sex chromosomes and chromosome 15 (from January 2003 to December 2007) and chromosome 17 (from January 2008 onwards). The addition of chromosome 17 in the PGS panel did not produce a statistically significant change neither in aneuploidy rate nor in the reproductive parameters measured in this study. A third round with additional subtelomeric and locus-specific (LSI) DNA probes was performed for those embryos with unclear signals in the previous rounds and to decrease the risk of false monosomies for the tested chromosomes (Colls et al., 2007). The additional probes used were LSI 13q34 and TelVysion 15q, 16q, 17q, 18q, 21q and 22q (Vysis). The FISH protocol and signal scoring were carried out following manufacturer’s instructions.

FISH error rate with 1 or 2 analysed blastomeres did not show statistical differences in the PGS programme (with 1 blastomere, error rate in re-analysed day-5 abnormal embryos was 5.6%; with 2 blastomeres, error rate was 5.3%).

**Statistical analysis**

Non-PGS and PGS groups were compared with chi-squared analysis with Yates correction and Fisher’s exact test. Maternal age and the mean number of transferred embryos were compared by Welch’s t-test. The statistical analysis was carried out using the Graphpad Instat v. 2.05a package (Graphpad Software, San Diego, CA, USA).

### Results

This retrospective study have reviewed clinical outcomes for a total of 2253 IVF cycles (1848 patients) performed between January 2003 and May 2009. The study group included 1117 cycles (corresponding to 919 patients) in which PGS was performed on day 3. The non-PGS group comprised 1136 IVF cycles (corresponding to 929 patients). The infertility profile of all patients is shown in Table 1. Results were separated according to patient age at the time of treatment: (i) 38 years: 109 cycles (96 patients) in PGS versus 448 cycles (359 patients) in non-PGS group; (ii) 39 years: 195 cycles (166 patients) in PGS versus 332 cycles (269 patients) in non-PGS group; (iii) 40 years: 305 cycles (240 patients) in PGS versus 167 cycles (139 patients) in non-PGS group; (iv) 41 years: 229 cycles (186 patients) in PGS versus 79 cycles (66 patients) in non-PGS group; (v) 42 years: 161 cycles (130 patients) in PGS versus 49 cycles (42 patients) in non-PGS group; and (vi) 43–44 years: 118 cycles (101 patients) in PGS versus 61 cycles (54 patients) in non-PGS group.

The percentage of chromosomal abnormalities in embryos from our study group was analysed according to both maternal age and the number of MII oocytes retrieved (Figure 1). Embryo chromosomal abnormalities increased as women got older (Figure 1A), but were constant in all groups of patients irrespective of the number of MII retrieved after ovarian stimulation (Figure 1B). Importantly, there is a large difference in aneuploidy rate between patients of 38 years and those ≥43 years of age.

Figure 2 depicts year-by-year data. The percentage of transfers was inversely related to age in both non-PGS and PGS groups (Figure 2A). The selection of euploid embryos in the PGS group produced a significant reduction of embryo transfers only in women up to 39 years of age when compared with the non-PGS group (90.2 versus 65.5% in 38-year-olds, \(P < 0.0001\); 88.6 versus 65.1% in 39-year-olds, \(P = 0.0033\); non-PGS versus PGS group, respectively). Transfer rates were comparable in women ≥40 years old. The selection of euploid embryos produced higher ongoing implantation rate in all PGS age groups, although the difference was only statistically significant for the 41 and 43–44 years age groups (2.6% versus 21.7% in 41 years, \(P < 0.0001\); 0% versus 12.0% in 43–44 years, \(P = 0.0453\); non-PGS versus PGS group, respectively; see Figure 2B). This observation was further reflected in better ongoing pregnancy rate per oocyte retrieval as women got older (Figure 2C), although the differences were only statistically significant at the age of 41 years (2.4% versus 11.8%, \(P = 0.0082\); non-PGS versus PGS group, respectively). Taking all the clinical parameters together, these results indicate a turning point in clinical outcome at 40 years of age.

In Table 2, patients are subdivided into two age-ranged subgroups as it was postulated that this turning point may reflect a general overall benefit of PGS to certain patients: 38–40 years of age in one subgroup and 41–44 years of age in the other. In both subgroups, the same trend was observed in terms of better ongoing implantation rate in PGS groups when compared with non-PGS groups. The

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Non-PGS</th>
<th>PGS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age in years (SD)</td>
<td>39.3 (1.5)</td>
<td>40.5 (1.5)</td>
</tr>
<tr>
<td>BMI (SD)</td>
<td>23.7 (4.1)</td>
<td>24.1 (4.1)</td>
</tr>
<tr>
<td>Cause of infertility (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tubal</td>
<td>3.9</td>
<td>1.1</td>
</tr>
<tr>
<td>Andrological</td>
<td>49.0</td>
<td>54.8</td>
</tr>
<tr>
<td>Anovulation</td>
<td>1.9</td>
<td>1.2</td>
</tr>
<tr>
<td>Endometriosis</td>
<td>3.3</td>
<td>0.9</td>
</tr>
<tr>
<td>Idiopathic</td>
<td>21.7</td>
<td>33.5</td>
</tr>
<tr>
<td>PCO</td>
<td>1.1</td>
<td>1.0</td>
</tr>
<tr>
<td>Low responder</td>
<td>4.8</td>
<td>1.0</td>
</tr>
<tr>
<td>Combined pathologies</td>
<td>14.3</td>
<td>6.5</td>
</tr>
</tbody>
</table>

BMI = body mass index; PCO = polycystic ovary; PGS = preimplantation genetic screening.
selection of normal embryos for the analysed chromosomes produced a significant reduction in the percentage of transfers in patients with 38–40 years in the PGS group ($P < 0.0001$), but there was no difference in patients 41–44 years of age when compared with the non-PGS population. In terms of ongoing pregnancy rate per oocyte retrieval, patients not included in the PGS group aged 38–40 years showed significantly better rates ($P = 0.0003$); importantly, in patients 41–44 years of age, the selection of euploid embryos increased the ongoing pregnancy rate per oocyte retrieval in the PGS group ($P = 0.0023$). This age-related and treatment-related association was masked when all the data were considered together (Table 2), likely because of the high number of cycles included from patients ranging from 38–40 years of age in the non-PGS group.

Low ovarian response is a frequent event in patients over 40 years of age. The same clinical parameters were further analysed according to ovarian response after ovarian stimulation in those patients 41–44 years of age included in the PGS programme (Table 3). Three groups of patients were studied according to the number of MII oocytes obtained: (i) women with a low ovarian response in a single cycle, considering as low responders those patients with $\leq 5$ MII oocytes retrieved after ovarian stimulation (87 PGS cycles, same number of patients); (ii) normal or high-responder patients ($\geq 6$ MII oocytes in a single stimulation cycle, 155

**Figure 1** Chromosomal abnormalities found in embryos from advanced maternal age patients. (A) The percentage of abnormal embryos increased progressively with maternal age. (B) The percentage remained constant irrespective of the number of metaphase II (MII) oocytes retrieved after ovarian stimulation.

**Figure 2** Clinical outcome according to maternal age. (A) The number of embryo transfers was significantly higher in patients 38 and 39 years old in the non-PGS group when compared with the PGS group ($P < 0.05$), but was comparable in patients $\geq 40$ years old. (B) Improved ongoing implantation rates (IR) were observed in the PGS group, with higher differences in patients $\geq 41$ years of age. (C) A trend towards higher ongoing pregnancy rate per oocyte retrieval (PR/OR) was observed for non-PGS groups in patients 38 and 39 years of age, whereas a change of trend was observed from 40 years onwards, with an improvement of ongoing pregnancy rate per oocyte retrieval with PGS. *$P < 0.0001$, # $P < 0.05$, /$P < 0.01$. 

PGS cycles, same number of patients); and (iii) patients with low ovarian response accumulating $\geq 6$ MII oocytes in several stimulation cycles using vitrification technology (105 PGS patients). Normal-responder patients had a significantly higher, by more than two-fold, number of embryo transfers ($P < 0.0001$) and, although not significantly different, a
two-fold higher ongoing pregnancy rate per oocyte retrieval when compared with low-responder patients. The accumulation of oocytes through vitrification in low-responder women significantly increased the percentage of embryo transfers in this group (i.e., a two-fold increase when compared with patients with ≤5 MII oocytes ($P < 0.0001$) and a similar transfer rate when compared with normal responders). Additionally, this process increased the mean number of embryos transferred and significantly increased the ongoing pregnancy rate per PGS cycle ($P = 0.0391$; more than two-fold increase when compared with patients with ≤5 MII oocytes in a single stimulation cycle).

Finally, although a low proportion of all miscarriages were cytogenetically studied, another aim of this study was to characterize the main reasons for fetal loss in the AMA population. In the non-PGS group, 67 miscarriages were registered and 12 of them (18%) were cytogenetically analysed. Nine out of 12 miscarriages were due to chromosomal abnormalities detectable by our PGS panel (75% of the analysed miscarriages), one out of 12 was due to trisomy 9, not detectable by our PGS panel (8.3%) and the remaining two miscarriages had a normal karyotype (16.7%). In the PGS group, a total of 46 miscarriages were registered and 19 of them (41%) were cytogenetically analysed. Two miscarriages were due to a chromosomal abnormality detectable by the current PGS panel but not tested in these cycles, 10 out of 19 miscarriages were due to chromosomal abnormalities not detectable by the PGS panel (53% of the analysed miscarriages: trisomy 3, trisomy 5, trisomy 7, trisomy 3 + 7, trisomy 10, three trisomy 14 and two trisomy 20), one miscarriage was due to a tetraploid embryo not detected during the diagnosis, possibly due to embryo mosaicism, and the remaining six miscarriages in the PGS group had a normal karyotype (30% of the analysed miscarriages).

### Table 2 Clinical outcomes of the non-PGS and PGS subgroups.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>38–40 years</th>
<th>41–44 years</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-PGS</td>
<td>PGS</td>
<td>P-value</td>
</tr>
<tr>
<td>No. of patients</td>
<td>767</td>
<td>502</td>
<td>–</td>
</tr>
<tr>
<td>No. of cycles</td>
<td>947</td>
<td>609</td>
<td>–</td>
</tr>
<tr>
<td>Mean age (SD)</td>
<td>38.7</td>
<td>39.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>(0.8)</td>
<td>(0.8)</td>
<td>(0.8)</td>
<td></td>
</tr>
<tr>
<td>Transfers</td>
<td>86.9</td>
<td>65.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Mean no. of embryos</td>
<td>1.9</td>
<td>1.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>transferred (SD)</td>
<td>(0.6)</td>
<td>(0.6)</td>
<td></td>
</tr>
<tr>
<td>PR/transfer</td>
<td>37.6</td>
<td>32.8</td>
<td>0.0002</td>
</tr>
<tr>
<td>IR</td>
<td>23.9</td>
<td>27.7</td>
<td>NS</td>
</tr>
<tr>
<td>Ongoing PR/transfer</td>
<td>28.8</td>
<td>25.4</td>
<td>NS</td>
</tr>
<tr>
<td>Ongoing PR/oocyte</td>
<td>24.1</td>
<td>16.4</td>
<td>0.0003</td>
</tr>
<tr>
<td>retrieval</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ongoing IR</td>
<td>19.8</td>
<td>21.8</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are percentages unless otherwise indicated. PGS = preimplantation genetic screening; IR = implantation rate; NS = implantation rate; PR = pregnancy rate.

### Table 3 Effect of oocyte accumulation by vitrification in the clinical outcome of low-responder PGS patients aged 41–44 years.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>≤5 MII oocytes</th>
<th>≥6 MII oocytes</th>
<th>Accumulated vitrified oocytes$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (SD)</td>
<td>42.1 (1.1)</td>
<td>41.7 (0.9)</td>
<td>42.1 (1.0)</td>
</tr>
<tr>
<td>No. of patients</td>
<td>87</td>
<td>155</td>
<td>105</td>
</tr>
<tr>
<td>Mean no. of MII oocytes retrieved (SD)</td>
<td>3.6 (1.2)</td>
<td>10.5 (4.5)</td>
<td>10.1 (3.3)</td>
</tr>
<tr>
<td>Transfers (%)</td>
<td>21.8</td>
<td>51.6$^b$</td>
<td>59.2$^c$</td>
</tr>
<tr>
<td>Mean no. of embryos transferred (SD)</td>
<td>1.0 (0)</td>
<td>1.4 (0.6)</td>
<td>1.4 (0.5)</td>
</tr>
<tr>
<td>PR</td>
<td>31.6</td>
<td>26.3</td>
<td>29.5</td>
</tr>
<tr>
<td>IR</td>
<td>31.6</td>
<td>24.3</td>
<td>25.6</td>
</tr>
<tr>
<td>Ongoing PR/transfer</td>
<td>26.3</td>
<td>18.8</td>
<td>29.5</td>
</tr>
<tr>
<td>Ongoing PR/oocyte retrieval</td>
<td>5.8</td>
<td>11.6</td>
<td>15.2$^d$</td>
</tr>
<tr>
<td>Ongoing IR</td>
<td>26.3</td>
<td>19.8</td>
<td>24.4</td>
</tr>
</tbody>
</table>

Values are percentages unless otherwise indicated. IR = implantation rate; MII = metaphase II; PR = pregnancy rate.

$^a$Patients with low ovarian response accumulating ≥6 MII oocytes in several stimulation cycles using vitrification technology.

$^bP < 0.0001$ versus ≤5 MII oocytes.

$^cP < 0.0001$ versus ≤5 MII oocytes.

$^dP = 0.0391$ versus ≤5 MII oocytes.
Discussion

During the last 2 years, several randomized controlled trials (RCT) have been conducted to analyse the effect of PGS on clinical outcome for poor prognosis IVF patients, including AMA. Studies vary widely in age of target population, as well as in technical approaches. This methodological variability precludes drawing clear conclusions about the utility of PGS and, most importantly, does not identify patient subpopulations that benefit most by being included in a well-established PGS programme. It is believed, however, that a retrospective study provides a useful way to include a large number of patients for each single age group with enough statistical potency to successfully measure the ongoing pregnancy rate per oocyte retrieval as the primary outcome. This study separated clinical outcomes by age intervals in women aged 38–44 years because in this population the aneuploidy rate ranges from approximately 70% to approximately 90%. This difference is important in considering whether PGS is beneficial in a heterogenic and large population. Further, the range in aneuploidy rates is magnified if, as in several reports, patients aged 35 and older are included (Debrock et al., 2010; Mastenbroek et al., 2007; Schoolcraft et al., 2009).

Delineating clinical outcomes by age in single years clearly demonstrates that the selection of euploid embryos (for the analysed chromosomes) produced an increase in the ongoing implantation rate at every single age. However, the percentage of cycles reaching embryo transfer and the ongoing pregnancy rate per oocyte retrieval were only improved in PGS patients 41 years and older. Non-PGS patients 38 and 39 years of age had a significantly improved chance of having an embryo transfer than PGS patients; surprisingly, this trend disappeared in patients over 40 years old, with comparable embryo transfer rates in both non-PGS and PGS groups. The selection of euploid embryos for the analysed chromosomes produce, in this and in other studies, a reduction in embryo transfer rate when considering patients in toto or when young patients are studied (Blockeel et al., 2008; Hardarson et al., 2008; Jansen et al., 2008; Rubio et al., 2005a,b; Staessen et al., 2004; Taranissi et al., 2005). The trend observed in the current study in the group of patients ranging from 41–44 years, with no increase in transfer rate in the non-PGS group when compared with the PGS group, is likely due to the centre’s policy of embryo transfer in regular IVF cycles, where only developing embryos with acceptable morphology are candidates to embryo transfer. At this age range, the embryo aneuploidy rate is as high as 80% and therefore, it would not be possible to select euploid embryos based only on morphological parameters, with a high percentage of good-quality embryos being aneuploid.

The power of this retrospective study is to show subpopulations of patients by dividing into single years and grouping patients in age ranges with the same reproductive trends. When conclusions are obtained from the overall population (38–44 years), PGS not only is not beneficial in clinical terms but is detrimental and the benefits found in the subpopulation of patients from 41–44 years are hidden and lost. The global analysis would mask the benefits of euploid embryo selection for certain age ranges included in the general group defined as AMA.

In the case of ongoing pregnancy rate per oocyte retrieval, better rates were registered in the non-PGS group at the age of 38 years, similar rates were observed at 39 and 40 years and better rates were registered from 41 years onwards in the PGS groups (these differences did not reach statistical significance). These results indicate an important difference in the utility of PGS at specific ages. When the patients were divided into two groups based on this difference (patients 38–40 years and patients 41–44 years), a significantly higher ongoing pregnancy rate was observed in the non-PGS group at the age range of 38–40 years, but significantly higher in the PGS group at the age range of 41–44 years. Thus, it seems that there is no beneficial effect of PGS in terms of ongoing pregnancy rate per oocyte retrieval in patients from 38–40 years old but, importantly, a high proportion of the analysed miscarriages in the non-PGS group were due to abnormalities in chromosomes included in the PGS programme.

Therefore, it is concluded that, in patients between 38 and 40 years old, PGS does not improve ongoing pregnancy rate but can decrease miscarriages likely caused by aneuploidy for chromosomes analysed in the PGS panel. This finding suggests that patients and clinicians must weigh the costs and benefits of having more embryo transfers but more miscarriages and terminations of pregnancy due to chromosomopathies that could have been detected by PGS. Also, the current data indicate that, in patients 41–44 years of age, PGS significantly improves clinical outcomes, particularly by increasing ongoing pregnancy rate per oocyte retrieval by more than two-fold. The different sample size in different groups has no relevance for the overall results, given that exact tests were employed, in order to avoid any statistical bias caused by this fact. These results define a new subset of patients not included in most of the RCT published to date, but representing a large number of patients visiting infertility centres. Although the most effective reproductive option for women over 40, in clinical terms, is oocyte donation, most refuse this in favour of their own gametes. The current data suggest that PGS offers the safest and most successful therapeutic option with own oocytes for these patients.

The fact that PGS clearly benefits the 41–44 years population but not patients of 38–40 years could be explained by several key points. In one hand, one can expect that the classical strategy to analyse the chromosomal content in an embryo (biopsy on day 3 and FISH analysis for 9–12 chromosomes) could underestimate the total amount of normal embryos and thus reducing the real reproductive possibilities in a PGS cycle when compared with a regular IVF cycle without PGS. Munne et al. (2010) described that, using a standard panel of nine chromosomes, 86% of all studied embryos were correctly diagnosed, the remaining 14% of embryos being diagnosed as euploid and actually being aneuploid for a chromosome not included in the analysis. Also Magli et al. (2010) recently described that the aneuploidy rate for chromosomes 1 and 4 (mostly not included in the standard panels for chromosome screening) was comparable with that observed in chromosomes 15, 16, 21 and 22, producing in most cases implantation failures. Perhaps
the aneuploidy rate for these non-tested chromosomes could be more frequent (or not associated with aneuploidies for chromosomes included in this study’s PGS panel) in patients of 38–40 years. Further studies using new diagnostic procedures such DNA-based comparative genomic hybridization arrays will provide valuable information about this hypothesis.

On the other hand, this retrospective study is biased by the population characteristics. PGS patients included in the group of 38–40 years had the worst reproductive prognosis, since the indication for being included in the PGS programme was not only AMA but also previous miscarriages or implantation failures. This fact, rather than devaluing this study, is encouraging since a clear benefit in applying PGS has been found in older patients with the worst reproductive prognosis.

Patients with AMA not only produce a high percentage of aneuploid embryos, but fewer numbers of them as a direct consequence of poor ovarian response and oocyte quality. Several authors have suggested that patients with a normal or high ovarian response could obtain greater benefits from PGS (Gleicher et al., 2008; Platteau et al., 2005), improving their possibilities of obtaining euploid embryo transfers and, consequently, having more chances to achieve a term pregnancy. A retrospective analysis in this centre’s laboratory showed that a minimum of 6–8 MII oocytes retrieved are required to ensure at least one euploid embryo for transfer in patients with AMA (data not shown). The development and improvement of new cryobiology techniques such as vitrification, with oocyte and embryo survival rates that are close to 100% (Antinori et al., 2007; Cao et al., 2009; Cobo et al., 2008a,c; Katayama et al., 2003) allows the accumulation of gametes or embryos from several stimulation cycles in low-responder patients, thereby offering them a better reproductive outcome with their own gametes. In terms of embryo chromosomal abnormalities, the current results show that ovarian response is not associated with chromosomal imbalances when standard stimulation protocols are applied, since comparable aneuploidy rate in embryos from low, normal or high responders is observed. These results are consistent with previous reports (Gianaroli et al., 2000). The effect of the paternal contribution or mitotic instability on the observed aneuploidy rates cannot be excluded in this group of patients.

Since the better prognosis among patients 41–44 years old was found in those patients with normal ovarian response, this study analysed if oocyte accumulation through vitrification techniques would be the best therapeutic approach to this important infertile and high-risk population. The observed increase of over two-fold in ongoing pregnancy rate per oocyte retrieval indicates that vitrification offers a viable approach to increase clinical outcomes. Although the number of patients included in this part of the study is small, the data show that low responders aged 41–44 would increase their reproductive success if over 6 MII oocytes are accumulated, reaching similar clinical outcomes when compared with normal-responder patients with the same number of oocytes obtained in a single stimulation cycle. Although not analysed in this study, similar final outcome is expected in both, accumulating gametes from several stimulation cycles and being all the resulting embryos included in one PGS analysis or when several PGS cycles are performed. Nonetheless, this centre’s experience is that patients are more prone to accumulate gametes in several stimulation cycles than repeating several PGS cycles, thus being a good alternative to improve clinical outcome in low-responder patients.

Since Mastenbroek et al. (2007) published on the negative impact of PGS on reducing pregnancy chances in AMA patients, controversial opinions have surfaced about the convenience of PGS programmes (Munne et al., 2009; Fauser 2008; Fritz 2008; Hernandez 2009; Mastenbroek et al., 2008; Rubio et al., 2009; Simpson 2008; Van Steirteghem 2008). Most of the RCT published to date show no beneficial effects in PGS groups. This body of evidence has encouraged some authors to dissuade the scientific community from including patients in PGS programmes, arguing ethical considerations (Mastenbroek et al., 2008; Van Steirteghem 2008).

 Conversely, some groups argue that most of these studies suffer from important methodological flaws: improper chromosome selection for analysis, absence of “no result rescue” in cases of doubtful diagnosis, high percentage of non-informative embryos after biopsy, large and surprising increase in miscarriage rate after PGS, minimum number of blastomeres forming the embryo at the time of biopsy, harmful biopsy procedure, and culture media selection issues (Munne et al., 2009; Rubio et al., 2009; Simpson 2008). Therefore, new, well-designed and properly conducted RCT are necessary to determine if PGS is beneficial in terms of ongoing pregnancy rate. The results reported here demonstrate the necessity of stratified studies to redefine each single clinical indication for PGS, and, if necessary, identifying those patients in which the reason for their reproductive problems is due to chromosomal imbalances in embryos.

Better ongoing pregnancy rates in AMA patients are desirable, but there are other benefits of PGS frequently not included in articles, such as a reduction in miscarriage rate, terminations of pregnancy and babies born with health problems. Patient attitudes toward PGS and the risk of a chromosomally abnormal pregnancy are not considered. Twisk et al. (2007) showed patient preferences towards using PGS to prevent Down syndrome (Twisk et al., 2007). Further, Shahine et al. (2008) examined the willingness of patients to participate in RCT to determine the benefits of PGS, with 84% of the patients preferring their embryos to undergo chromosomal analysis (Shahine et al., 2008). Psychological and physical complications due to miscarriages that could be avoided are not generally considered and a more global vision of the benefits as a whole should be applied when weighing the utility of PGS in clinical terms.

In conclusion, this study has described a new subset of AMA patients, those patients over 40 years of age, who benefit from being included in a PGS programme. Most importantly, those with a high number of MII oocytes retrieved as a result of a good ovarian response or due to oocyte accumulation strategies see greater increases in clinical outcomes. As widely described, not all patients visiting a reproductive centre will benefit from being included in a PGS programme, but new well-conducted studies must determine which subgroup(s) of patients from the current indications for PGS would truly benefit from chromosomal selection. This centre is currently performing a RCT to confirm the improved clinical outcomes seen in PGS patients, in particular the subgroup of patients aged 41–44 years.
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