

# Improving pregnancy outcome for IVF patients with preimplantation genetic screening

Expert Rev. Obstet. Gynecol. 3(5), xxx-xxx (2008)

## Santiago Munné

Reprogenetics, LLC,  
3 Regent St., Suite 301,  
Livingston, NJ 07049, USA.  
Tel.: +1 973 436 5010  
Fax: +1 973 992 1423  
Munne@reprogenetics.com

More than 50% of cleavage-stage human embryos produced *in vitro* are chromosomally abnormal and this increases with advanced maternal age. Simultaneously, the increase in chromosome abnormalities with maternal age is linked with a decrease in implantation and an increase in spontaneous abortions. Thus, preimplantation genetic diagnosis has been offered to infertile couples of advanced maternal age, or with previous repeated miscarriages, based on the assumption that selecting chromosomally normal embryos for replacement should increase implantation rates, reduce spontaneous abortion rates and aneuploid conceptions and increase delivery rates. However, suboptimal methods may preclude the benefits of preimplantation genetic diagnosis. Some identified key factors are the number of cells biopsied, the number and type of chromosomes analyzed, an error rate based on reanalysis results of less than 10% reduced embryo damage during biopsies, and a minimum number of embryos produced for an effective selection. Thus, the difference between studies that produce positive results after preimplantation genetic diagnosis and those that do not is largely explained by the methodologies used.

**Keywords:** advanced maternal age • aneuploidy • embryo biopsy • miscarriage • preimplantation genetic diagnosis • recurrent pregnancy loss

The incidence of chromosomal abnormalities in cleavage-stage embryos produced *in vitro* is considerably higher than those occurring in spontaneous abortions; 50–70% depending on maternal age, which indicates that a sizable percentage of chromosomally abnormal embryos are eliminated before any prenatal diagnosis. Furthermore, data from oocyte donations show that in women of advanced maternal age (AMA), the decline in pregnancy is caused largely by failing oocyte quality [1]. Preimplantation genetic screening (PGS) selects chromosomally normal embryos and has come to be a useful tool for infertility treatment. When properly applied it can [2]:

- Reduce spontaneous abortion rates
- Reduce aneuploid conceptions
- Increase implantation rates
- Improve delivery rates

It is not yet fully acceptable to use PGS due to conflicting results published, reflecting the

differences between methods used and misunderstandings concerning the clinical application of PGS in various centers. This review discusses these differences.

### Regular embryo selection (non-PGS)

Programs using IVF depend heavily on embryo selection based on morphological and developmental characteristics [3–5] but the implantation potential of human embryos produced *in vitro* remains low, between 29% for patients aged under 35 years and 7.7% for patients aged 41–42 years [201]. The fact is that morphological and developmental characteristics do not reveal chromosomal abnormalities present in a large proportion of embryos and is therefore considerably limited [6–14]. A recent study showed that only 44% of the embryos with the best morphology in young patients (<35 years) were chromosomally normal. That proportion decreased subsequently with age and of course with decreasing embryo developmental characteristics [7]. It also

showed that selection by morphological and developmental characteristics could only improve implantation potential from an average of 37 to 44%.

Trisomies and other abnormalities occur in day-5 blastocysts up to 40% of the time even though pure monosomies and haploidies do not [15–17]. Thus extending culture to day 5 can improve implantation rates for patients with multiple day-3 embryos and good morphology, but day-3 embryos of lower quality can still implant and develop successfully while a delay to day 5 is often deleterious [3]. Noninvasive methods for embryo assessment are emerging but are unlikely to detect trisomies soon [18] because there are two main types of chromosome abnormalities with very different origins. One is aneuploidy, which is generated during gametogenesis and accounts for 20–50% of abnormalities depending on maternal age. The other is post-zygotic abnormality, accounting for 30% of chromosome abnormalities, this is not linked to maternal age but to dysmorphism [7] and is probably caused by spindle abnormality, which also causes dysmorphism. Spindle abnormalities are more correlated with abnormal development and can be screened against by noninvasive methods, morphological selection and extended culture, but not most trisomies, which can and do implant [19].

It appears that PGS can be used effectively in combination with other techniques to select the most euploid embryos and thereby improve assisted reproduction technology (ART) outcome, assuming patients have enough day-3 embryos. However, studies using different PGS methods have given different results, and the present review attempts to clarify which methodologies have been proven to work and which have not.

### Differences in methods between optimal & poor PGS results

Preimplantation genetic screening starts with the biopsy of a single blastomere from each day-3 embryo, each having an optimum of eight cells (average 6–7); the method of biopsy is critical as it affects later development. Two-cell biopsy reduces the implantation potential of an embryo [20,21]. Biopsied cells must then be fixed for analysis using methods that minimize loss of DNA and chromosome overlaps. The FISH procedure needs to analyze at least eight pairs of chromosomes to provide a worthwhile result. Even so, PGS analysis errors between programs differ between 5 and 50% [22,23]. It is obvious that a high error rate obviates the entire process, as guesswork would be just as effective. Replacement skill also varies between doctors, especially when embryos have been biopsied.

Studies using standardized and successful methods with large numbers of patient procedures are still needed to show significant improvement in delivery rates. However, some reports have shown an increase in implantation rates and reduction in spontaneous abortion and trisomic conception [22,24–27]. Yet these studies reported significant differences in numbers of replaced embryos between the controls and test groups; therefore, it is still possible that they had improved deliveries.

### Which cells & how many to biopsy?

Polar bodies [28–30] and single blastomeres from day 3 are the cells most commonly used for assessment of euploidy [2]. More recently trials have included polar body biopsy combined with day 3 blastomere biopsy [31,32], day 3 two-cell biopsy [33] or blastocyst biopsy [34].

Each method has notable advantages and disadvantages concerning the damage caused and the quality and quantity of information obtained. Although first and second polar body biopsy and analysis are the least invasive techniques, especially if the biopsy is performed mechanically by a skilled operative, they do not provide information on post-zygotic abnormalities, which in any case affect 30% of embryos [28].

Trophectoderm blastocyst biopsy is probably more benign than cleavage-stage biopsy, but not all euploid embryos reach blastocyst stage, as mentioned previously, and therefore can only be applied in a limited number of cases. There are no direct reports on the effect of embryo biopsy performed without PGS selection on implantation. Only one study followed embryos up to blastocyst formation [35]. Theoretical assessment of the damage caused by embryo biopsy has been estimated by comparing the effect of cell loss after freezing and thawing, and indicated a modest reduction of implantation after one-cell biopsy but a severe decrease of potential after two-cell damage [20].

In some studies, where single-cell embryo biopsy reduced implantation potential, it was more than compensated for by the selection of euploid embryos [24–26], but on the other hand, it has also been shown to be very detrimental [36]. Indeed, this latter study is the only one to date that had a subgroup of patients who received biopsied embryos without PGS selection and the biopsy itself caused a more than 50% reduction in implantation potential [36]. In that study, many other factors were also suboptimal, therefore biopsy damage could not be compensated for by PGS [37–39].

There are very few publications comparing different biopsy methods in relation to ART outcome [40,41] but they suggest that specialized training is necessary for the correct performance of zona opening, regardless of the method used, and lack of such training is surely one of the primary reasons for the unsatisfactory implantation obtained in some centers after PGS [36].

Of the two largest randomized clinical tests that found no beneficial effect after PGS, one negative outcome was most likely caused by inappropriate biopsy [36] and the other has been shown to be caused by two-cell biopsy instead of one-cell biopsy [33] by the same team that performed the trial [21]. In this new study a highly significant decrease in embryo development potential after two-cell compared with one-cell biopsy ( $p < 0.007$ ) was determined [21]. The difference in implantation between one-cell biopsy (23.5%) and two-cell biopsy (17.3%) was not significant, and the difference in pregnancy rate was just below significance ( $p = 0.068$ ). Interestingly, the Goossens *et al.* study [21] and their previous one [33] both reported exactly the same implantation after biopsy of two-cells (17%). Thus if one compares the reported 11% implantation rate for the control group in Staessen *et al.* [33] with the 23.5% for one cell biopsy in Goossens *et al.* [21],

the differences in implantation rates are statistically significant. If Staessen *et al.* had biopsied only one cell, PGS would most probably have increased implantation significantly [33].

### Cell fixation

The purpose of fixation is to provide a nucleus with the highest chance of producing reliable FISH results.

For blastomere fixation, the modified method using Carnoy's fixative (method 1) [42,43] is the best, producing as little as 3% cell loss in expert hands. This method uses a mixture of fixative with a drop of hypotonic containing the blastomere and produces turbulences in which the cell may be lost. The actions require continuing practice to perform well, thus when performed sporadically, it is difficult to use successfully [44].

Alternative methods have been developed [45–47] one using Tween-20 with HCl (method 2) and another using a combination of Tween-20/HCl and Carnoy's fixative (method 3), which each overcome the turbulence problem and are easily learned. However, when Velilla *et al.* compared all three techniques (methods 1–3), they found the average diameter of the fixed nuclei was 59, 31 and 46 microns, resulting in 14, 58 and 39% overlaps between chromosomes and 10, 30 and 17% errors, respectively [44]. The highest error rates (20–50%) reported in PGS have all used the Tween-20 method (method 2) [23,17,48], while studies reporting the lowest error rates (5–10%) have used Carnoy's fixative method [14,22,49]. Therefore, it appears that method 1 is more effective in reducing errors but has a higher learning curve and practice needs to be maintained to be effective.

### Number of chromosomes analyzed

FISH is the method of choice to analyze polar bodies (PBs) and blastomeres. However, because the human eye can reliably differentiate between only five colors in the visible spectrum, the number of chromosomes that can be detected simultaneously by fluorescent dyes is only five. Therefore, two or more rounds of hybridization are required to analyze additional chromosomes, but each round of hybridization on the same cells produces more errors, so that the practical limit is 15 chromosomes, although due to manufacturers' limitations the limit is currently 12 chromosomes. Tests outlined for the chromosomes most involved in aneuploidy at the cleavage stage are, in order, chromosomes 22, 16, 21 and 15 [50], and are usually combined with chromosomes whose abnormalities can reach term (X, Y, 13, 18, 21) to make eight in total, as 21 is in both groups. These are basically the minimum chromosomes used for PGS. Using the latter set of five chromosome probes that can reach term, only 28–31% of chromosome abnormalities found in fetuses can be detected. Nine probes that include the eight chromosomes plus chromosome 17 can detect 57–72%; and 67–80% can be detected with a 12-probe test (the eight plus 17, 14, 8 and 20) [51–55]; thus, any study that found improved implantation after PGS analyzed at least the eight critical chromosomes (X, Y, 13, 15, 16, 18, 21, 22) [24,26,27,56]. Studies that failed to analyze chromosomes 15 and 22 found no improvement after PGS [33,36]; these two chromosomes have since been proven to account for more than 20% of all abnormalities [55]. To

date, there is unfortunately no standard number of chromosomes analyzed in different IVF programs, which means yet another variation in PGS results among centers.

### No results, no result rescue, error rates & mosaicism

The rate of undiagnosed cells depends mainly on proper biopsy and fixation techniques. The rate of no diagnosis because of no result(s) ranges from 1 [57] to 20% [36], depending on center expertise. Size analysis and distance between signals have been used in the past as criteria for scoring dubious results [58] but a much better alternative is to use 'no result rescue' (NRR), which consists of reanalyzing a nucleus for which there is an uncertain signal for a specific chromosome, with a probe that binds to a different locus on that same chromosome [13,22,59]. NRR can reduce FISH errors from 13.6 to 4.7% [22]. Each PGS laboratory has a different error rate ranging from 4 to 7% (50, 23 and 15%) up to 50% [17,23,48], the latter being equivalent to no diagnosis at all. Thus it is paramount for a patient to be aware of the performing center's error rate.

When all or most cells in an embryo have different chromosomal make-up, it is termed mosaic and on average, it ranges between 25 and 30% [6–9,14,49,60,61]. This obviously poses problems for PGS, as some of the differences among studies are due to population, hormonal stimulation and the general quality of embryos produced by each center [62]. Despite its high frequency, some studies have shown that mosaicism can produce as little as 5–7% of all errors [22,49] because most mosaic embryos only have abnormal cells (TABLE 1). Therefore, the effect of mosaicism on PGS errors can easily be overestimated and should not affect an overall error rate of less than 10% [22]. Thereby, the problems of no results and mosaicism can largely be overcome.

### Results of PGS for aneuploidy: trisomic offspring, spontaneous abortions & implantation Reduction in trisomic offspring

The first proposed use of PGS for the detection of chromosome abnormalities was as an alternative to prenatal diagnosis and potential elective abortion. However, to prove that point, a large series of cases was needed. Munné *et al.* [27; UNPUBLISHED DATA] found that the expected risk of trisomic conceptions for chromosomes X, Y, 13, 18 and 21 in a group of 2300 PGS cycles was 2.6% [63] but after PGS, the observed rate was reduced to 0.5% ( $p < 0.001$ ).

**Table 1. Error rate and percentage of abnormal cells in mosaics.**

Abnormal cells in mosaics	Number of reanalyzed abnormal embryos out of 592
Normal	13
Mosaic <38% abnormal	15
Mosaic 38–49% abnormal	12
Mosaic 50–99% abnormal	124
Mosaic 100% abnormal	297
Homogeneously abnormal	131
False-positive error with 38% threshold as abnormal = 4.7%	
False-positive error with 50% threshold as abnormal = 6.8%	
Obtained with permission from [22].	

Although the prevention of trisomic conceptions is usually grouped together with the concept of improving ART outcome, it is an indication in itself. For example, a recent survey by Twisk *et al.* indicated that if 80% of Down's were accurately screened and no change in pregnancy rate occurred after PGS, 75% of subfertile women would use PGS [64]. That is akin to the 81% screening potential reported by Munné *et al.* [27] and shows that patients believe PGS to be worthwhile, even if the error rate were 20% or approximately 1 in 5.

### Decrease in spontaneous abortions

Most studies on pregnancy loss agree that prior miscarriages and AMA are the major risk indicators for spontaneous abortion [65–68].

The Society for ART (SART) data indicate that 13.3% of ART pregnancies in patients younger than 35 years of age result in miscarriage, increasing to 53.3% in patients aged 43–44 years [201]. Chromosome studies in spontaneous abortions of ART patients indicate high rates of chromosome abnormalities (65–71%) [55,69], increasing with maternal age, from 65% in women aged 39 years and younger to 82% in women aged 40 years and older [69].

Owing to the high rate of chromosome abnormalities in spontaneous abortions, PGS should reduce the rate of miscarriage in infertile patients undergoing ART. Indeed, before it was understood that two rounds of hybridization were possible, analysis of five chromosomes X, Y, 13, 18 and 21 [25] revealed a significant reduction in spontaneous abortions from 23% in the controls to 9% in the PGS group ( $p < 0.05$ ) and a significant increase in ongoing pregnancies (10.5 vs 16.1%,  $p < 0.05$ ). In another study, 9% of pregnancies were lost after PGS [70,71] instead of an expected 16%.

Two studies compared PGS outcome with previous pregnancy history, both reporting significant reduction in pregnancy loss rates [57,72]. However, these two studies have been criticized on grounds of comparing a self-selected population with prior negative reproductive history with their next cycle, which could have overestimated the true baseline of spontaneous abortions.

A more recent study comparing SART data [201] on spontaneous abortions with PGS data from 100 IVF centers, revealed a significant decrease in spontaneous abortions after PGS, from 19 to 14.1% in women 35–40 years, and from 40.6 to 22.2%

( $p < 0.001$ ) in women over 40 years old [27]. However, this study had the limitation that not all centers in the SART database contributed results of their PGS. To solve the problem, Munné *et al.* compared SART data from five IVF centers with extensive experience in embryo biopsy, with the PGS data from those same centers, same time period and maternal ages, and again found a significant reduction in spontaneous abortions from 30% in non-PGS cycles to 21% in PGS cycles ( $p < 0.01$ ) (TABLE 2) [73]. Although this paper could also be criticized on the grounds that PGS cycles usually have large cohorts of embryos, the literature indicates higher rates of chromosome abnormalities in larger cohorts of embryos than in small ones [74,75], which should in fact affect PGS cycles more negatively.

Pregnancy loss is itself an indication to undergo PGS. Patients who have had three or more consecutive spontaneous abortions are considered to have recurrent pregnancy loss (RPL) [76,77], and 50% of cases remain classified as having unknown etiology (idiopathic RPL) [78,79,80]. It cannot be assumed that only chromosome abnormalities are involved. The frequency of abnormal embryonic karyotypes has been found to be higher in sporadic abortions (63–76%) than in RPL (40–60%) [81,82]. Furthermore, women aborting more than four miscarriages had only 29% abnormal fetuses [81]. However, these studies were performed on products of conception from clinically recognized pregnancies. Cleavage-stage embryos from RPL patients have consistently been found to have either more chromosome abnormalities than the control embryos [83–86] or similar rates in fertile RPL patients than infertile ones [27,56].

So far, a few studies have evaluated spontaneous abortion rates after PGS [56,87], while other studies were uncontrolled and/or did not assess miscarriages [85,88,89].

The study by Munné *et al.* predicted a loss rate of 36.5% in the next pregnancy (based on Brigham *et al.* [90]), but after PGS, the observed loss rate was only 16.7% ( $p = 0.028$ ) [56]. The effect was more significant in the 35 years of age and over subgroup, where the expected loss in the next pregnancy was 44.5% compared with an observed 12% ( $p = 0.007$ ) after PGS.

Another study by Garrisi *et al.* [87] examined the effect of PGS reduction on spontaneous abortions in relation to the number of previous miscarriages and fertility status. They found that PGS significantly reduced miscarriage rates in patients with three to

**Table 2. Comparison of non-PGS and PGS cycles from five IVF centers.**

IVF clinic	Age group (years)	Non-PGS cycles (n)	Loss rate (%)	Live birth rate (%)	PGS cycles (n)	Loss rate (%)	Live birth rate (%)
1	38–42	505	27	35	70	22	40
2	38–42	210	36	14	72	27	15
3	38–42	1204	34	12	120	15	23
4	38–42	509	29	15	236	26	22
5	38–42	191	25	17	208	16	25
Total	38–42	2619	30	18	706	21	24

Total PGS results were significantly better with regards to pregnancy rates ( $p < 0.01$ ), miscarriage rates ( $p < 0.01$ ) and pregnancy to term ( $p < 0.001$ ).

PGS: Preimplantation genetic screening.

Obtained with permission from [73].

five previous miscarriages and while the beneficial effect of PGS was more accentuated in fertile patients, it also helped infertile ones undergoing IVF.

Based on these two studies, it is reasonable to conclude that RPL with idiopathic etiology in women of AMA is mostly a problem of recurrent chromosomally abnormal embryos. This coincides with results after PGS for translocations, where RPL is a major result and where PGS substantially increases a couple's chances of sustaining a pregnancy to full term [56,58,72,91–96].

The practice committee of SART and the American Society for Reproductive Medicine (ASRM) 2007 guidelines only took into consideration randomized clinical trials and therefore do not believe that PGS can reduce spontaneous abortions [97]. Fortunately, a better informed group currently indicates that PGS should be offered for idiopathic RPL [98].

### **Increase in implantation, pregnancy & take-home-baby rates**

Reviewing the literature there are two sets of investigators, one group that supports the hypothesis that PGS for aneuploidy improves implantation and reduces miscarriage rates [22,24–27,56,57,72,73], and a second group which did not demonstrate any improvement [33,89] or showed a negative effect [36].

In addition, there have been three types of studies, depending on the type of control used:

- Type I – Those that were retrospective or used prior reproductive history as a control [27,56,57,72,73,99];
- Type II – Those that were prospective, comparing patients that accepted PGS with those that declined it [22,24–26];
- Type III – Prospective randomized studies [33,36,89].

The limitations and parameters of some of the retrospective studies have been discussed above [27,57,72,73]. Regarding prospective, nonrandomized and randomized studies, there are significant differences between them (TABLE 3). Studies in group II used inappropriate methodology which appears to have caused a lack of positive results. These methodology, problems principally involved biopsying two cells per embryo [33] rather than one, which as mentioned previously, seriously reduces implantation potential [21,22].

Two type III studies have less than six embryos on average per patient [33,36], resulting in a limited potential selection. Other studies indicated that in order to improve implantation rates, a larger number of two pro-nuclei embryos is necessary [26,100]. This is because when the total number of replaceable day-3 embryos is low (four or less), most control group embryos would be replaced anyway, thus the total number of normal embryos replaced is similar in the control and PGS groups. PGS is a selection tool to improve IVF outcome, if there are not enough embryos to select from, evidently, no improvement can be achieved. In fact, PGS can then have a negative effect because of the biopsy procedure.

Another methodology problem in the same two type III studies was that chromosomes 15 and 22 were not tested, although these chromosomes actually account for 24% of abnormalities detected

in spontaneous abortions after IVF [55] and 10% of day-3 abnormal embryos [50]. In addition, the fixation method used in these two studies was not optimal [44].

All this leads to the most serious shortcoming of the Mastenbroek *et al.* study, a 20% rate of undiagnosed embryos compared with 3–5% of other studies (TABLE 3) [36] and the 1.8–2.5% rate of Goossens *et al.* [21]. A third arm in the study was inadvertently created when these undiagnosed embryos were replaced, thus these embryos were biopsied but undiagnosed; this showed that their biopsy produced a 59% reduction in implantation (from 14.7% in controls to 6% in this group), something that has not occurred in other studies. Even taking into account the other shortcomings mentioned previously, PGS selection was able to compensate for the biopsy damage producing 16.8% implantation rates but not enough to be better than nonbiopsied controls. This study demonstrated the importance of embryo biopsy, an aspect of PGS that must not be overlooked for successful practice.

While none of the studies mentioned above is perfect, comparative studies using appropriate methodology do clearly indicate that PGS is can be beneficial for some patients. Randomized prospective studies with appropriate methods are about to start.

### **Recommendations & limitations of PGS at present**

Owing to the ongoing debate surrounding PGS and probably the lack of good understanding of the underlying technicalities, the practice committee of the SART and the ASRM produced guidelines stating that PGS for AMA and RPL are still experimental procedures [97]. These guidelines only took into account the CRT studies, without taking into account the quality of these studies. The statement that there is no evidence that the indication for RPL is the most puzzling, since there is only one CRT that did not study miscarriage rates [89], while two other studies clearly showed a reduction in miscarriages [56,87]. By contrast, the Preimplantation Genetic Diagnosis International Society (PGDIS) guidelines do recommend PGD for AMA and RPL [98].

Even with optimized PGS methods, current technology can only increase the implantation potential from 20 to 30% [22]. If 70% of embryos are abnormal, one would expect a much higher improvement rate. Error rates in optimized PGS studies are low, so embryo biopsy damage is the most probable cause of the missing expected improvement. In addition, as PGS is a selection tool, the technique is indicated only for patients with a sizable cohort of embryos, if the purpose is to improve take-home baby rates. Although, to prevent miscarriages or trisomic conceptions, the number of embryos is irrelevant.

Some of the above limitations may be solved by increasing selection further with comparative genome hybridization (CGH) or microarrays, and by improving biopsy methods or moving to polar body (PB) or blastocyst biopsy.

### **Future developments**

Current FISH tests can effectively analyze up to 12 probes but this is only 67% of known chromosomal abnormalities in spontaneous abortions [55]. Tests that can score all 24 chromosome types could produce a further and clearer improvement in ART outcome.

**Table 3. Summary of prospective studies comparing preimplantation genetic screening and control assisted reproduction technology outcome.**

Characteristics	Study						
	Munne <i>et al.</i> (1999)	Gianaroli <i>et al.</i> (1999)	Munne <i>et al.</i> (2003)	Werlin <i>et al.</i> (2003)	Staessen <i>et al.</i> (2004)	Colls <i>et al.</i> (2007)	Matenbroek <i>et al.</i> (2007)
Ref.	[25]	[24]	[26]	[88]	[33]	[22]	[36]
Cells biopsied (n)	1	1	1	1	2	1	1
Chromosomes analyzed (n)	4–8*	8	8	8	6	8	8
Analysis of 15, 22 chromosomes	No*	Yes	Yes	Yes	No	Yes	No
Fixation type appropriate	Yes	Yes	Yes	Yes/no <sup>†</sup>	No	Yes	No
No result rate (%)	N/A	3.1	4.4	N/A	4.7	4.7	20.1
Average embryos in PGS group (n)	N/A	6.7	8.9	N/A	5.9	7.2	5.4
Type of study	Comparative	Comparative	Comparative	Randomized	Randomized	Comparative	Randomized
<b>Results</b>							
Cycles retrieved in control (n)	117	127	138	28	141	100	402
Cycles retrieved in PGS (n)	117	135	138	29	148	100	Unclear <sup>§</sup>
Average embryos replaced in control (n)	3.6	3.0 (p = 0.001)	3.7 (p = 0.001)	N/A	2.8 (p = 0.001)	2.4 (p = 0.001)	1.9
Average embryos replaced in PGS (n)	3.1	1.8 (p = 0.001)	2.0 (p = 0.001)	N/A	2.0 (p = 0.001)	1.5 (p = 0.001)	1.8
Implantation rate in control (%)	13.7	12.4 (p = 0.001)	10.6 (p = 0.05)	N/A	11.5	20 (p = 0.025)	14.7
Implantation rate in PGS (%)	17.6	24.2 (p = 0.001)	17.6 (p = 0.05)	N/A	17.1	31 (p = 0.025)	16.8 to 6 <sup>¶</sup>
Pregnancy rate in control (%)	29.9	25.1	N/A	20.7	27.7	32	84
Pregnancy rate in PGS (%)	35.9	29.1	N/A	43.0	19.6	35	Unclear <sup>§</sup>
Pregnancy loss rate in control (%)	33.8 (p = 0.05)	20.6	N/A	N/A	25.6	28 (p = 0.025)	21.4
Pregnancy loss rate in PGS (%)	15.0 (p = 0.05)	5.4	N/A	N/A	25.0	6 (p = 0.025)	Unclear <sup>§</sup>
Ongoing implantation rate <sup>#</sup> control (%)	10.6 (p = 0.05)	10.2 (p = 0.001)	N/A	N/A	10.4 (p = 0.06)	14.1 (p = 0.025)	N/A
Ongoing implantation rate <sup>#</sup> in PGS (%)	15.9 (p = 0.05)	22.5 (p = 0.001)	N/A	N/A	16.5 (p = 0.06)	28.9 (p = 0.025)	N/A
Ongoing pregnancy rate in control (%)	22.2	20.0	N/A	N/A	20.6	26	16.4
Ongoing pregnancy rate in PGS (%)	32.5	27.6	N/A	N/A	14.9	31	Unclear <sup>§</sup>

\*Only 31/117 cycles with 8 probes.

<sup>†</sup>Different centers in this multicenter study used different fixation types.

<sup>§</sup>Pregnant cycles. Since PGS cycles were reported together with a subgroup of cycles with no PGS analysis (see <sup>¶</sup>), PGS pregnancy, miscarriage and ongoing pregnancy rates cannot be assessed properly.

<sup>¶</sup>There were three subgroups of cycles in the PGS group, the first with two normal embryos replaced (16.8% implantation rate), the second with two undetermined embryos replaced (6% implantation rate) and the remainder.

<sup>#</sup>Fetus ongoing  $\geq 12$  weeks/embryos replaced.

<sup>\*\*</sup>59% implantation reduction caused by the biopsy alone, when no PGS analysis was performed.

Comparative: Prospective non-randomized comparative study; PGS: Preimplantation genetic screening; Randomized: Prospective randomized study.

Comparative genomic hybridization is promising as a DNA-based method capable of accurately determining total or partial aneuploidy by detecting losses or gains in all 46 chromosomes [101] and has been applied to single cells and babies born after PGS using CGH [52–54,102–110]. However, CGH cannot be performed in time for day- replacement, and embryo freezing and thawing is needed, with some exceptions [105]. With the advent of improved freezing methods, such as vitrification [111,112], CGH has been re-evaluated. Recently, Sher *et al.* used improved oocyte freezing techniques combined with CGH for first PBs [110].

Currently, CGH is labor intensive as well as both costly and time consuming, requiring egg or embryo freezing, then thawing after the process. As this sets the embryos back substantially, it defeats the object. A more promising technique is microarray technology. The use of DNA microarrays in PGS to determine chromosomal copy number has been proposed [113,114] and recently tested on single cells [115–117], although not yet in the clinic. There are at least two DNA microarray platforms being tested currently: array CGH and single-nucleotide polymorphism arrays. It is still too early to determine if either will be useful.

A large fraction of embryos produced during IVF are chromosomally abnormal. Selection based on morphology and developmental assessment does not screen for the majority of these abnormalities. Thus, PGS during IVF attempts to select euploid embryos for replacement to improve implantation, reduce spontaneous abortions and trisomic offspring, and improve the number of take-home babies.

For these purposes, some circumstances seem to be better than others:

- An appropriate maternal age
- A minimum number of zygotes and embryos to be biopsied
- A small diagnosis failure rate
- A single cell biopsied by personnel with extensive experience in biopsy
- A fixation method that minimizes signal overlap and DNA loss
- An overall PGS laboratory error rate of under 10%
- Extensive and positive experience with PGS
- The analysis of eight or more chromosomes including X, Y, 13, 15, 16, 18, 21 and 22 with NRR.

Comparative genomic hybridization and microarrays have the promise of furthering the selection potential of the technique while minimizing error rates. Even so, for a fraction of patients choosing PGS, high take-home baby rates is not the only goal, as one survey showed that 83% of patients wish to prevent trisomic conceptions, as long as pregnancy did not decrease [64]. Furthermore, patients with idiopathic RPL are clearly reluctant to experience the trauma again and choose PGS to avoid pregnancy loss where possible.

PGS is hardly more than 15 years into clinical practice, so we are sure to see wider applications in the years to come.

### Expert commentary

Preimplantation genetic screening has been pushed mostly by geneticists trying to scale down technologies available in prenatal diagnosis to the single-cell level, and most publications on the subject have been on technology development. It only recently that differences among laboratories at the clinical outcome level have been compared and not until 2007 that the PGS community has realized that standardization and minimum quality of biopsy, as well as other factors are key to producing appropriate clinical results.

A doctor referring a patient for PGS should take into account the quality of the PGS laboratory to which the sample is sent. This laboratory should have a (preferably reported) low error rate and (preferably published) extensive experience. The biopsy and cell preparation at the IVF center and the IVF procedure itself are also key and are again a matter of experience and past results.

### Five-year view

Randomized studies with optimal methods are about to commence sponsored by centers with extensive experience in PGD [202]. Some of the characteristics of this study are:

- Maternal ages between 37–42 years, where chromosomes abnormalities are more frequent;
- A minimum of five embryos with six or more cells each on day 3;
- PGS laboratories with thousands of previous PGS cycles performed and with a low rate of undiagnosed embryos (<5%), including two persons scoring results;
- Biopsy performed by experienced personnel (>100 biopsies in the previous 3 years);
- Carnoy's fixation method used and performed by experienced biologists;
- PGS laboratories with error rates of less than 10%;
- IVF centers with extensive previous experience with PGS, showing positive results with comparison studies;
- Chromosomes analyzed are X, Y, 13, 15, 16, 18, 19, 20, 21 and 22, using a maximum of two hybridization steps; and also using 'no result rescue' to reduce error rates in a third hybridization if needed;
- Two embryos maximum replaced in each arm.

This will set-up the basis for moving forward clinically and define minimum methodological standards required.

Embryo biopsy techniques should be further studied, improved and standardized to be applied less detrimentally.

Simultaneously, new techniques such CGH are already being used clinically and in the coming 1 or 2 years, we will see if the advantages of full-chromosome analysis are superior to the

current 9–12-chromosome tests. If so, prices for those techniques will go down with volume increase and these techniques will substitute FISH altogether. It is not yet clear which micro-array DNA platform will work better, one based on bacterial artificial chromosomes or one based on single-nucleotide polymorphisms, the latter producing information on many other genetic characteristics of the embryo besides aneuploidy.

### Financial & competing interests disclosure

*The author has no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.*

*No writing assistance was utilized in the production of this manuscript.*

### Key issues

- There is little doubt that preimplantation genetic screening (PGS) can reduce the risk of miscarriage in couples with recurrent pregnancy loss of idiopathic origin in women aged over 35 years or translocation origin.
- For infertile couples of advanced maternal age, PGS has been shown to increase implantation rates, reduce miscarriage and trisomic conception rates, and/or increase take-home baby rates in some studies but not in others.
- The differences among studies are mostly due to methodology.
- Some appropriate parameters that have been identified are:
  - Single cell biopsy;
  - Expert biopsy procedure;
  - Fixation method that minimizes overlaps and loss of DNA;
  - PGS analysis of at least chromosomes 15, 16, 21, 22 plus four more;
  - PGS error rate below 10%;
  - Experienced PGS laboratory.
- New techniques, such as comparative genome hybridization and DNA microarrays, are being developed or already applied clinically; these will eventually substitute FISH as the technique of choice for chromosome analysis of embryos.

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### Website

- 201 SART  
[www.sartcorsonline.com/rptCSR\\_PublicMultYear.aspx?ClinicPKID=0](http://www.sartcorsonline.com/rptCSR_PublicMultYear.aspx?ClinicPKID=0)
- 202 ClinicalTrials.gov study NCT00646893  
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### Affiliation

- Santiago Munné, PhD  
Director, Reprogenetics, LLC,  
3 Regent St., Suite 301, Livingston,  
NJ 07049, USA.  
Tel.: +1 973 436 5010  
Fax: +1 973 992 1423  
[munne@reprogenetics.com](mailto:munne@reprogenetics.com)