

Effect of infertility, maternal age, and number of previous miscarriages on the outcome of preimplantation genetic diagnosis for idiopathic recurrent pregnancy loss

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Objective: To determine whether preimplantation genetic diagnosis (PGD) would decrease spontaneous abortion rates in patients with idiopathic recurrent pregnancy loss (RPL).

Design: Controlled clinical study.

Setting: IVF center and PGD reference laboratory.

Patient(s): Patients with RPL with no known etiology.

Intervention(s): Preimplantation genetic diagnosis by fluorescence in situ hybridization analyzing nine chromosomes.

Main Outcome Measure(s): The spontaneous abortion rate was compared to patients' own expectations. Patients were evaluated according to the number of previous losses, fertility, and maternal age.

Result(s): Preimplantation genetic diagnosis significantly reduced spontaneous abortions in patients with RPL, especially for patients with more than two previous losses (12.8% vs. 35.9% expected). The PGD significantly reduced the rate of spontaneous abortion in both fertile (15.2% vs. 33.8% expected) and infertile patients (13.0% vs. 29.5%), which also achieved similar delivery rates (37% and 34%, respectively). The beneficial effect of PGD was less pronounced in patients <35 years than in patients ≥35 years old (13.6% vs. 34.0% expected). Overall, the PGD reduced the miscarriage rate to a similar baseline of 14%–16% across all maternal ages.

Conclusion(s): Preimplantation genetic diagnosis improves pregnancy outcome for women with idiopathic RPL, especially those with more than two previous losses, and >35 years of age, and that benefit is not affected by fertility status. (Fertil Steril® 2008; ■: ■–■. ©2008 by American Society for Reproductive Medicine.)

Key Words: FISH, PGD, recurrent miscarriage, idiopathic RPL, infertility

Recurrent pregnancy loss (RPL) is usually defined as three or more spontaneous abortions at less than 20–28 weeks' gestation (1–3), although some studies consider RPL if two or more consecutive losses have occurred (4, 5).

Recurrent pregnancy loss has been attributed to anatomic uterine pathologies, genetic defects (mostly translocations) (2.7%–4.7%) (6–10), endocrine and endometrial defects, prothrombotic state, inherited thrombophilia, endocrinologic disorders, polycystic ovaries (PCO), high androgen levels, and immunologic factors, infections, and environmental factors, but 40%–50% of cases remain classified as having unknown etiology (11, 12).

In patients with normal karyotype there are two major predictive factors of unsuccessful pregnancy (13)—number of

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miscarriages and maternal age. Women with previous pregnancy losses have a higher abortion risk (25%) than women with previous successful pregnancies (5%) (14). In addition, with increasing numbers of previous miscarriages comes an increasing risk of losing the next pregnancy (12, 13, 15). Kupka et al. (16) found a miscarriage rate of 21% in couples with no previous miscarriage, compared with 27% with a single previous loss, and 31% with three previous losses (16). The other factor affecting pregnancy outcome, maternal age, contributes not only to an increase of chromosomally abnormal miscarriage but also increases the risk of chromosomally normal ones (12, 13).

Chromosomally or genetically abnormal embryos have been assumed to cause a large percentage of losses in RPL because chromosome abnormalities are the major cause of sporadic miscarriage, with 90% of chromosomally abnormal pregnancies miscarrying (17) compared with 7% chromosomally normal (18). However, conventional karyotyping does not support a higher rate of chromosome abnormalities in fetus of couples with RPL than in those from women with sporadic pregnancy loss. These studies have found similar chromosome abnormality rates in miscarriages of RPL as in those from couples with a single miscarriage (17, 19–22).

112 However, conventional karyotyping requires tissue culture,
113 which fails up to 25% of the time, especially for chromoso-
114 mally abnormal samples (23), and is prone to maternal
115 contamination, thus over-reporting euploid karyotypes
116 (21, 24).

117 Studies using comparative genome hybridization (25, 26),
118 which do not require tissue culture, report up to 72% of chro-
119 mosomally abnormal abortuses, indicating that chromosome
120 abnormalities play a major role in RPL. Also, using transcervical
121 embryoscopic and cytogenetic analysis of missed
122 abortions, Philipp et al. (27) detected that 93% were either
123 chromosomally or morphologically abnormal. Chromoso-
124 mally normal abortuses may carry other genetic abnormali-
125 ties, such as differential X-inactivation (28), cryptic
126 translocations or deletions (29), mutations or imprinting. Al-
127 lelic variations of the HLA-G (30) and p53 tumor suppressor
128 gene (31) have been associated with RPL.

129 Studies on products of conception are seldom performed in
130 cases of very early losses and thus may underestimate chro-
131 mosome abnormalities incompatible with further develop-
132 ment. Studies on cleavage stage embryos of patients with
133 RPL has consistently found more chromosomally abnormal
134 embryos in couples with RPL when compared with fertile
135 controls (32–34), and similar to those of infertile patients
136 (35), although most patients with RPL were fertile.

137 Although the main goal of most patients undergoing pre-
138 implantation genetic diagnosis (PGD) for RPL is to conceive
139 a baby without having to undergo a miscarriage, few studies
140 have evaluated spontaneous abortion rates after PGD in
141 patients with RPL (35). Other studies were uncontrolled
142 (36) or did not assess miscarriage rates (34, 37).

143 Munné et al. (35) offered PGD to idiopathic RPL patients
144 and compared pregnancy loss of each subject with that
145 expected based on their previous number of miscarriages
146 and maternal age, according to prediction from the study
147 by Brigham et al. (13). In the RPL group of women ≥ 35
148 years old, the expected loss in the next pregnancy was
149 44.5% compared with a significantly lower observed rate of
150 12% after PGD. That study concluded that RPL with idi-
151 opathic etiology in women of advanced maternal age is mostly
152 a problem of recurrent chromosomally abnormal embryos.
153 This is in agreement with previous observations in translocat-
154 ion carriers in which PGD of translocations significantly
155 reduced spontaneous abortions (38, 39).

156 Previous studies on RPL have not evaluated the effect of
157 the number of previous miscarriages on the pregnancy out-
158 come of PGD cycles. Ogasawara et al. (20) found a decrease
159 of chromosomally abnormal abortuses with increasing num-
160 ber of previous conceptions, indicating that nonchromosomal
161 factors may have a higher impact on RPL than chromosome
162 abnormalities when the number of previous miscarriages is
163 five or more.

164 The first goal of this study was to determine whether there
165 is a relationship between the number of previous miscar-

riages in patients with idiopathic RPL and their pregnancy
outcome after PGD.

The effect of fertility, understood as the ability of a woman
to become pregnant without assisted reproductive technology
(ART), irrespective of the outcome of the pregnancy, is
another factor that has not been evaluated in PGD for RPL.
Infertile patients may suffer RPL after undergoing several
treatments of ART. Thus, the second goal of this study is to
determine whether PGD will improve pregnancy outcome
in fertile, infertile, or both groups of patients.

MATERIALS AND METHODS

Test Subjects

Patients with normal karyotypes and a history of two or more
previous miscarriages, consecutive or not, that occurred ≤ 20
weeks of gestation were included in the study. None had an
acceptable explanation for their miscarriages (idiopathic
RPL). Patients were tested in the following areas to evaluate
other treatable causes of recurrent pregnancy loss: uterine
factors, infectious disease, genetic causes such as chromo-
somal translocation, and thrombogenic/immune factors. If
the testing performed in all of these areas was normal or neg-
ative, patients were classified as “idiopathic,” meaning no
known cause could be found for their recurrent loss and hence
aneuploidy was suspected.

Uterine factors were assessed by cavity imaging with
hysterosalpingogram or saline sonohysterogram. Cervical
cultures were performed for *Mycoplasma hominis* (as well
as *Chlamydia* and gonococcus for tubal infertility factors).
Karyotypes of both the husband and wife were obtained to
exclude a chromosomal translocation. Thrombogenic/im-
mune testing included the following: anticardiolipin anti-
bodies, lupus anticoagulant, antithrombin III mutation,
Factor V Leiden mutation, methyl tetrahydrofolate reductase
mutation and homocysteine levels, prothrombin or Factor II
gene mutation, protein C and protein S (total and free).

Baseline levels of FSH, E_2 , TSH, and PRL were measured.
Any underlying endocrinologic abnormality was treated
before initiation of stimulation. The FSH levels were less
than 14 mIU/mL, unless there was a history of adequate
oocyte recruitment during previous stimulation.

Patients were either fertile or infertile, the later having
conceived and miscarried after ART treatment.

These patients were offered PGD at the Institute for Repro-
ductive Medicine and Science of Saint Barnabas, Livingston,
NJ. For purposes of comparison, these patients were classi-
fied into a fertile test group if they had conceived the previous
lost pregnancies naturally or an infertile test group if they
have conceived them through IVF. Patients with PGD were
also stratified by previous number of miscarriages. Table 1
shows the characteristics of these two groups of patients.

Implantation was defined as the presence of a gestational
sac and a spontaneous abortion as the loss of a gestational

TABLE 1

Population characteristics.		
	Accepted PGD	Rejected PGD but did IVF
Patients	238	40
Cycles (cycles/patient)	279 (1.17)	47 (1.75)
Previous pregnancies	1,000	122
Previously lost pregnancies	914 (91.4%)	114 (93.4%)
Average age	37.5	36.0
Average no. of biopsiable embryos	8.8	6.5
In pregnant patients	9.4	8.4
In nonpregnant patients	8.4	5.6
Average no. of embryos replaced	2.0	3.2
Pregnant cycles after IVF	116 (41.6%)	18 (38.3%)
Pregnancies miscarried	17 (14.7%)	8 (44.4%)

Note: PGD = preimplantation genetic diagnosis.
Garrisi. PGD for recurrent pregnancy loss. Fertil Steril 2008.

sac. Ectopic implantations were not counted as pregnancies. When comparing the patients with previous losses, the same criteria were used for patients who miscarried after IVF. Patients with previous miscarriages after natural conceptions obviously did not undergo the early pregnancy monitoring that occurs with IVF, as it was not available to them. Among these patients, pregnancies (and losses) were typically detected at or after sac formation.

For patients with RPL, PGD was made available regardless of embryo number.

Control Comparison

Patients with a history of RPL who declined PGD, but who underwent IVF either as a treatment for RPL or because they were infertile (Table 1) were not used as controls, because they were a small group and because the average number of embryos produced and the average age were different than the test group.

For purpose of determining whether PGD helped improve pregnancy outcome, the observed spontaneous abortion rate after PGD in each subject was compared with the expected rate on the basis of the individual's history, according to prediction parameters from the study by Brigham et al. (13). This type of comparison was previously used for similar studies on RPL (35). Briefly, Brigham et al. (13) had conducted logistic regression, taking into account patient age and number of previous pregnancy losses as variables and obtained a formula for probability of a successful pregnancy. This formula was: $\text{Ln}(p/[1 - p]) = 2.00 - 0.0828 (\text{Age} - \text{Mean age}) - 0.2467 (N_{\text{prev}})$, where mean age was 32 years and N_{prev} was number of previous miscarriages, and where the probability was calculated as follows: $p = e^{\theta} / (1 + e^{\theta})$, where θ is $\text{Ln}(p/[1 - p])$.

For each patient the probability of a pregnancy reaching term was calculated using this formula (13), and the cumula-

tive mean value of those independent probabilities was taken as the predicted proportion of a successful pregnancy, and that value subtracted from 1, as the prediction of the incidence of pregnancy loss. Expected and observed pregnancy losses were compared.

PGD Procedure

Embryo biopsy and cell fixation was performed at the Institute for Reproductive Medicine and Science. A single cell per embryo was biopsied from embryos with four or more cells using acid Tyrode's solution to produce a 25- to 30- μm aperture in the zona pellucida (ZP). A Ca/Mg-free biopsy media containing amino acids was used. Embryos were exposed to Ca/Mg-free medium for 2–5 minutes, including the time of loading, micromanipulation, and postbiopsy rinsing. Biopsy was accomplished by gentle suction. Once obtained the blastomeres were fixed using the modified Carnoy's method (40) with special attention to retaining DNA material by not adding fixation drops after cytoplasm breakage.

Fluorescence in situ hybridization was performed on fixed cells at Reprogenetics (Livingston, NJ). Due to improving techniques during the course of the recruiting period, cells were either studied using a nine-chromosome test (X, Y, 13, 15, 16, 17, 18, 21, 22) or the same test plus "No Result Rescue" (NRR) as recently described by Colls et al. (41). Briefly, NRR consist of reanalyzing those cells for which the nine-chromosome test was inconclusive for one or two chromosomes, but using probes for the uninformative chromosome that bound to different loci. Using NRR, error rate and no results are reduced. Probes were commercially available from Abbot, IL.

The PGD analysis was performed by two analysts, independently analyzing the sample and later agreeing on the results. If no agreement was reached for a specific chromosome

TABLE 2

Chromosome abnormalities in preimplantation genetic diagnosis cycles.

Previous losses	Embryos	Previous birth	Fertile or not	Age informative	Embryos normal
2	Any	Either	Any	810	237 (29.3%) ^a
3–5	Any	Either	Any	1,524	455 (29.8%) ^a
>5	Any	Either	Any	110	43 (39.1%) ^a
≥2	Any	Fertile	Any	1,207	341 (28.3%)
≥2	Any	Infertile	Any	1,242	394 (31.7%)
≥2	Any	Either	<38	1,308	447 (34.2%) ^b
≥2	Any	Either	≥38	1,141	288 (25.2%) ^b
≥2	Any	Either	<35	575	212 (40.4%) ^c
≥2	Any	Either	35–39	1,113	352 (31.6%) ^c
≥2	Any	Either	≥40	715	154 (21.5%) ^c
3–5	0	Either	Any	1,087	340 (31.3%)
3–5	>0	Either	Any	442	115 (26.0%)

^a $P < .05$.
^b $P < .001$.
^c $P < .001$.

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result the diagnosis was “no result” for that chromosome. The NRR was used to resolve discrepancies or dubious results after it was introduced in 2004 (41).

Embryos were classified after PGD as normal or abnormal based on previous published criteria (42). Some abnormal embryos, chosen randomly, were fully disaggregated and the cells were fixed individually to assess PGD error rate. In that case a different scoring criteria was used that more finely classifies the chromosome abnormality (43).

Results were provided late on Day 3 and embryos replaced on Day 4. Up to four embryos were replaced depending on maternal age and embryo morphology. Embryos were considered suitable for replacement if any cleavage occurred between Day 3 and Day 4.

RESULTS

Table 2 depicts the rate of chromosome abnormalities in each subgroup of patients with PGD. As expected, overall and for patients with three to five previous miscarriages, more chromosome abnormalities were present in patients 38 years and older than in younger patients ($P < .001$). There were no differences in the frequency of chromosome abnormalities between fertile and infertile patients with PGD. Patients with five or more previous losses had slightly more chromosomally normal embryos (39.1%) than the rest of patients (29.6%) ($P < .05$).

Of the embryos not replaced, 187 were fully reanalyzed using NRR and of those 8% (15/187) were misdiagnosed by PGD. Of those, seven were embryos classified as monosomic, which after reanalysis were found to be normal or with low level mosaicism; one trisomic was found normal; two

embryos with complex abnormalities were found to have low level mosaic or normal, and five normal embryos, which arrested were found to be mosaics with >40% abnormal cells.

Overall, 279 cycles of PGD were performed on 238 couples (average 1.17 cycles per couple). These patients had previously 1,000 pregnancies, of which 914 (91%) resulted in a miscarriage. The timing of the losses, all before 24 weeks, is known precisely for 613 miscarriages. Of those, 44% occurred before 6 weeks, 52% between 6 and 12 weeks, and 4% between 12 and 20 weeks. The average maternal age at the time of PGD was 37.5 years.

A total of 561 embryos (average 2.0) were replaced, resulting in 179 sacs (31.9% implantation rate), of which 39 were lost, 15 as sacs without cardiac activity, and 24 after cardiac activity was detected. Of those lost, eight were karyotyped, of which six were 46,XX, one was 46,XY, and one was 47,XY +15.

The pregnancy and miscarriage rates for PGD cycles, stratified according to previous number of miscarriages, fertility groups, consecutiveness of miscarriages, and age, are shown in Table 3, and its statistical analysis concerning the effect of PGD on miscarriage compared with expected miscarriage rates is shown in Table 4.

Overall PGD significantly reduced the risk of pregnancy loss in patients with a history of RPL, from an expected 31.9% rate to an observed 14.2% rate ($P < .001$).

The beneficial effect of PGD was most significant for patients with more than two previous miscarriages, where losses were reduced from an expected 38.3% to an observed 12.8%, a threefold reduction ($P < .001$). For patients with two previous miscarriages PGD reduced the loss rate from an

TABLE 3

Pregnancy and miscarriage rates after preimplantation genetic diagnosis.

Previous losses	Pregnancies	Fertile or not	Average age	No. of cycles	Pregnancies ^a ongoing	Pregnancies lost
2	Either	Any	91	40	33 (36%)	7 (17.5%)
>2	Either	Any	184	76	66 (36%)	10 (13.2%)
≥2	No	Any	129	51	44 (34%)	7 (13.7%)
≥2	Yes	Any	150	65	55 (37%)	10 (15.4%)
≥2	Either	<35	64	31	26 (41%)	5 (16.1%)
≥2	Either	≥35	215	85	73 (41%)	12 (14.1%)
≥2	Either	Any	279	116	99 (35%)	17 (14.7%)

^a Nonectopic pregnancies. In addition of those in the Table, there were four more pregnancies that were ectopic.

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expected 24.4% to an observed 16.7%, but that difference was not statistically different.

When the pregnancy loss rate of the infertile and fertile RPL subgroups were compared with their expected loss rate, the difference was statistically significant for both, with a reduced miscarriage rate in the infertile group from an expected 29.5% to an observed 13.0% ($P<.01$), and

a 33.8% expected to 15.2% observed ($P<.001$) in the fertile group. In both groups, fertile and infertile, the reduction in spontaneous abortions was the same (2.2- to 2.3-fold).

The beneficial effect of PGD was more accentuated in patients 35 years and older, with an expected 34.1% miscarriage rate and an observed 13.6% ($P<.001$), but although chromosome abnormalities increased with advancing maternal age

TABLE 4

Expected and observed miscarriage rates after preimplantation genetic diagnosis.

Previous losses	Age group		Previous fertility		
			No	Yes	Totals
2	<35 y	obs.	11.0% (1/9)	0 (0/2)	9.1% (1/11)
		exp.	19.0%	19.6%	19.1%
	≥35 y	obs.	18.2% (4/22)	22.2% (2/9)	19.4% (6/31)
		exp.	26.4%	26.0%	26.3%
Subtotal		obs.	16.1% (5/31)	18.2% (2/11)	16.7% (7/42)
		exp.	24.3%	24.8%	24.4%
>2	<35 y	obs.	0 (0/2)	21.1% (4/19)	19.1% (4/21)
		exp.	30.0%	29.5%	29.6%
	≥35 y	obs.	9.5% (2/21) ^b	11.1% (4/36) ^c	10.5% (6/57) ^c
		exp.	37.3%	38.9%	38.3%
Subtotal		obs.	8.7% (2/23) ^b	14.6% (8/55) ^c	12.8% (10/78) ^c
		exp.	36.7%	35.6%	35.9%
≥2	<35 y	obs.	9.1% (1/11)	19.1% (4/21)	15.6% (5/32)
		exp.	21.0%	28.6%	26.0%
	≥35 y	obs.	14.0% (6/43) ^b	13.3% (6/45) ^c	13.6% (12/88) ^c
		exp.	31.7%	36.3%	34.1%
Total		obs.	13.0% (7/54) ^b	15.2% (10/66) ^c	14.2% (17/120) ^c
		exp.	29.5%	33.8%	31.9%

Note: obs. = observed; exp. = expected according to Brigham et al. (1999).

^a $P<.05$.

^b $P<.01$.

^c $P<.001$.

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and therefore the PGD effect was more obvious in the >35 years of age group, the effect of PGD was to reduce the miscarriage rate to a similar baseline of 14%–16% across all maternal ages.

DISCUSSION

[Q5] In this study we characterized subgroups of idiopathic RPL for which PGD would be indicated. As reviewed in the introductory section, it seems plausible that chromosome abnormalities may have a role in idiopathic RPL and indeed, PGD seems to help reduce spontaneous abortions (35), but it also seems logical that it cannot be the sole cause of idiopathic RPL.

Overall, PGD reduced the risk of pregnancy loss by 2.2-fold in RPL patients ($P < .001$). The beneficial effect of PGD was more accentuated in patients with three or more previous miscarriages (2.8-fold reduction). The reduction in miscarriages after PGD in patients with two previous losses was not statistically significant, and it could be due to the small size of the sample ($n = 42$ pregnancies), or may reflect the more strict classification of RPL being three or more previous losses. However, the results are encouraging, and larger studies may still determine that couples with two previous miscarriages can also be helped by PGD.

The present study had not enough power to detect the effect of PGD in couples with more than five previous losses, and they were grouped with patients with three to five losses. Ogasawara et al. (13) showed that the higher the number of previous miscarriages, the higher the probability that the miscarriages were euploid, and thus, PGD in women with more than five miscarriages should be less effective than in those with five or less. The frequency of euploid embryos was identical in the 2–5 previous losses (29%–30%), but significantly higher in the >5 (39%) group (Table 2). The study by Ogasawara et al. (13) included not only idiopathic but all types of RPL patients, therefore more patients with idiopathic RPL and with more than five previous losses are needed to confirm the observation by Ogasawara et al., and a null effect of PGD in that group.

Patients with RPL caused by translocations had a significant lower miscarriage rate PGD (38, 39, 44–49) than observed after no PGD treatment (6, 9, 10), with the added advantages that time to conception is much faster with IVF–PGD.

As in PGD for translocations, where almost 80% of embryos are chromosomally abnormal due to the translocation, the present study reinforces the hypothesis that idiopathic RPL is produced in part by chromosome abnormalities present in the embryos at a 70% rate, and that selecting for these abnormalities reduces the risk of miscarriage. Better PGD tests, such the use of comparative genome hybridization or DNA microarrays, which could detect all chromosome aneuploidies, should improve results further.

This study has also determined that the means of conceiving the previous losses (naturally or through ART proce-

dures) is irrelevant to the beneficial effect of PGD. Both fertile and infertile patients with idiopathic RPL showed a similar significant decrease in spontaneous abortions after PGD. This observation is of significant importance as the majority of RPL idiopathic patients are fertile, and very few are being currently treated using ART.

Regarding infertile patients, a previous study suggested a higher incidence of chromosome abnormalities for subfertile patients with RPL (8). However, the present study shows no difference in euploidy rates between fertile and infertile RPL embryos.

Similar to the findings in our smaller previous study (35), age was found to be a factor in determining the pregnancy outcome effect of PGD on RPL, with the <35-year age group not showing a significant improvement (1.7-fold reduction), although a larger cohort than in the present study ($n = 32$ pregnancies) may show the contrary. Interestingly, the rate of spontaneous abortions after PGD was similar for all ages (between 14% and 16%), whereas the expected rate of miscarriage increased with advancing maternal age. This means that in all maternal age groups the abnormal chromosome-dependent miscarriages that could be detected by this test were eliminated to the same baseline level, leaving other nonmaternal age-related factors as the only remaining cause of miscarriage.

This study supports the use of PGD for idiopathic RPL in patients with more than two previous losses, to reduce further miscarriages while achieving acceptable delivery rates, irrespective of their fertility status.

The use of PGD for idiopathic RPL to reduce spontaneous abortions has now been supported by two controlled studies (present study, 35), whereas other investigators have not reported on its effect on spontaneous abortions (34, 37) or those studies were not controlled (36). Based on available evidence at the time of writing, the Preimplantation Genetics Diagnosis International Society (PGDIS) recently published guidelines stating that it is acceptable to provide PGD for idiopathic or translocation-caused RPL (50). However, The practice committee of SART and ASRM (51), before the presentation of the results of the present study and based on the lack of randomized clinical trials on this subject, gave the opinion that PGD should not yet be considered an accepted practice for this indication. We agree that controlled randomized trials (CRT) should be performed when possible, but based on the lack of any study measuring the effect of PGD on miscarriage, the PGDIS guidelines are more helpful to prospective patients.

In addition, attention on the methodology used on CRTs is paramount. Previous reports on the use of PGD for advanced maternal age also indicated a positive outcome of PGD (52–55), but the CRT that succeeded them did not try to replicate the same methodology, and were not suitable as validation studies. For example, the study by Staessen et al. (56) biopsied two cells instead of one, which according to Cohen et al. (57) most probably eliminates any potential beneficial effect of the PGD selection. The same Staessen group later

showed in another CRT that a two-cell biopsy was more detrimental than a one-cell biopsy (58), specifically in embryos with poorer morphology. If one compares the study by Goessens et al. (58) to the one by Staessen et al. (56), both reported the same implantation rate after two-cell biopsies (17%), 11% for the control group (56), and (23.5%) for the one-cell biopsy group (58), thus indirectly showing that PGD with one-cell biopsy is indeed superior to no PGD for the indication of advanced maternal age. Other CRT (59) have been criticized extensively regarding the methodology and patients used (60–63).

In summary, this study and past evidence strongly indicates a beneficial effect of PGD in preventing miscarriages in couples with RPL. Controlled randomized trials replicating the methodology of studies showing positive results after PGD are desirable.

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903 **1** **Effect of infertility, maternal age, and number of**
904 **previous miscarriages on the outcome of**
905 **preimplantation genetic diagnosis for idiopathic**
906 **recurrent pregnancy loss**

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912 Preimplantation genetic diagnosis improves preg-
913 nancy outcome for couples with idiopathic recurrent
914 pregnancy loss, especially those with >2 previous
915 losses and >35 years of age, and that benefit is not
916 affected by fertility status.
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