Preimplantation genetic diagnosis reduces pregnancy loss in women aged 35 years and older with a history of recurrent miscarriages

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Objective: To determine whether preimplantation genetic diagnosis (PGD) and transfer of euploid embryos would decrease spontaneous abortion rates in recurrent miscarriage (RM) patients.
Design: Controlled clinical study.
Setting: In vitro fertilization centers and PGD reference laboratory.
Patient(s): Recurrent-miscarriage patients with three or more prior lost pregnancies with no known etiology.
Intervention(s): Biopsy of a single blastomere from each day 3 embryo, followed by fluorescence in situ hybridization analysis.
Main Outcome Measure(s): The rate of spontaneous abortions in RM subjects undergoing PGD were compared with [1] their own a priori expectations and [2] a comparison group of women undergoing PGD for advanced maternal age (≥35 years).
Result(s): Before PGD, RM patients had lost 87% (262/301) of their pregnancies, with an expected loss rate of 36.5%. After, they only lost 16.7% pregnancies. This difference was mostly due to reduction in pregnancy loss in the ≥35-years age subgroup, to 12% from an expected 44.5%.
Conclusion(s): Preimplantation genetic diagnosis aneuploidy screening has a beneficial effect on pregnancy outcome in RM couples, especially those in which the woman is aged ≥35 years. Our data indicate that PGD reduces the risk of miscarriage in RM patients to baseline levels. (Fertil Steril® 2005;84:331–5. ©2005 by American Society for Reproductive Medicine.)

Key Words: FISH, PGD, recurrent miscarriage, RPL

Recurrent miscarriage (RM) typically is defined as three or more spontaneous abortions (1, 2). The phenomenon is not well demarcated but appears genuine. If the likelihood of any given clinical pregnancy being lost is 10% to 15%, the probability of three consecutive miscarriages occurring by chance is say, 10^{-3}(0.1%) to 15^{-3}(0.34%). This clearly is lower than the observed 1% in the general population (3–5).

Recurrent miscarriage has been attributed to a host of anatomic, endocrine, and immunologic causes (6), but except for genetic (chromosomal) factors, neither sporadic nor recurrent pregnancy losses usually can be explained with certainty. The case for a preponderant genetic role is, however, large.

At least 50%–60% of early losses show number of chromosomal abnormalities. Conversely, 90% of chromosomally abnormal pregnancies abort (7), compared with 7% of the chromosomally normal (8). It is reasonable to postulate that recurrent losses are caused by the same phenomenon responsible for sporadic cases. Consistent with this, rates of chromosomal abnormalities in RM and sporadic abortuses are nearly identical in most studies (9–12). In young patients, factors other than chromosome abnormalities could be relatively more likely to cause RM. When data were stratified by age, Stephenson et al. (12) found slightly higher rates of euploid karyotypes in RM (64%) than in control (51%) women aged ≤35 years; in women aged >35 years, rates were more similar (40% and 37%, respectively). In addition, women with two to four consecutive pregnancies showed 60% chromosomally abnormal abortuses, whereas women with >4 RM showed only 29% (10).

Still, evidence is mounting that the prevalence of chromosomal abnormalities is higher than the traditionally stated 50%–60%, perhaps as high as 80%–90%. Chromosomal abnormalities of missed abortuses that are subjected to chorionic villi sampling (CVS) show 80%–90% abnormalities, albeit in an older cohort (13–16). When comparative genome hybridization (17, 18), a technique that does not require tissue culture, is used, ≤72% of abortuses are chromosomally abnormal (18). By using transcervical embryoscopic and

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cytogenetic analysis of missed abortions, Philipp et al. (19) found 75% of 233 missed abortuses to be chromosomally abnormal; only 7% were chromosomally and developmentally normal. Perhaps ostensible nongenetic or maternal causes are merely epiphenomena, not truly causative.

It follows from the high frequency of aneuploidy in abortuses and from both sporadic and recurrent abortuses that aneuploid meiotic perturbation actually cause RM (20–22). This phenomenon may extend to preimplantation embryos and thus manifest as infertility or low assisted reproductive technology (ART) success. Indeed, Vidal et al. (20) found more chromosomally abnormal embryos in couples with RM than in controls characterized by no history of RM but who were infertile or were fertile carriers of X-linked diseases. Given this, RM patients should benefit from preimplantation genetic diagnosis (PGD) because selection can allow transfer only of chromosomally normal embryos. This should increase implantation rates, reduce spontaneous abortion, and eventually increase live-birth rates.

When PGD is applied to infertile patients undergoing IVF, implantation and pregnancy rates are indeed improved, and spontaneous abortion is reduced (23–27). The purpose of the present study was to clarify whether PGD and transfer of euploid embryos reduces the frequency of miscarriages in RM patients.

MATERIALS AND METHODS

Subjects

The study sample consisted of women who had had three or more previous miscarriages (RM patients). All were identified from women who presented for PGD during the 27-month interval between January 1, 2001 and March 31, 2003 at The Institute for Reproductive Medicine and Science, Saint Barnabas Medical Center, or The Center for Reproductive Medicine. Of the potential subjects who cycled for PGD, 11.5% canceled the PGD procedure because of low number of embryos or for other reasons. The group studied did not differ significantly from those RM patients who did not participate in the study. Of the 58 who entered into our study, 24 had a prior live birth, and 35 had undergone a prior ART cycle. The mean number of prior abortuses was 3.9 ± 1.1. No subject had an accepted explanation for RM, and none were carriers of chromosomal rearrangements.

Implantation was defined as presence of a gestational sac. A spontaneous abortion was defined as loss of a pregnancy after presence of a gestational sac.

Biopsy, Fixation, Fluorescence In Situ Hybridization Procedure, and Embryo Classification

After informed, signed consent was obtained from patients in accordance with institutional review board protocol, ovulation stimulation and IVF were instituted.

On day 3, each embryo had a single blastomere biopsied. If the nucleus could not be located after fixation, a second cell was biopsied; fixation was performed as described elsewhere (28). Usually, up to four normally developing embryos that were classified as presumptively chromosomally normal by PGD were transferred on day 4 or 5. In rare cases with poor morphology and advanced maternal age, up to five embryos were replaced. Not replaced were embryos arrested in development or that were in excess of the appropriate number of replaceable embryos.

Cells were analyzed by personnel from Reprogenetics and Saint Barnabas by using DNA probes for chromosomes X, Y, 13, 15, 16, 17, 18, 21, and 22 (Vysis, Downer's Grove, IL). Protocols published elsewhere (29, 30) applied.

Embryos were classified by criteria detailed elsewhere (31): chromosomally normal, aneuploid, or other chromosome abnormalities. To assess efficiency of FISH analysis, some nonreplaced embryos were reanalyzed after disaggregation. All or most of their cells were fixed individually, again as described elsewhere (32). If reanalysis of all or most cells of a given embryo could be performed, we applied the classification criteria for normal, aneuploid, extensive mosaic, polyploid, or haploid as described elsewhere (29). Not all nonreplaced embryos could be reanalyzed because of time constraints, lack of patient consent, or embryo degeneration.

Expected Vs. Observed Miscarriage Rate

The effects of PGD on the RM group were studied by comparing pregnancy loss of each subject with that expected on the basis of the individual's history, according to prediction parameters from the study by Brigham et al. (33). Those investigators had conducted logistic regression, taking into account patient age and number of previous miscarriages as regressor variables and deriving a formula for probability of a successful pregnancy.

This formula was as follows: $\mathrm{Ln}(p/[1 - p]) = 2.00 - 0.0828\ (\mathrm{Age} - \mathrm{Mean}) - 0.2467\ (\mathrm{Nprev})$, where mean age was 32 years and Nprev was number of previous miscarriages. For a nominated set of variables (Age, Nprev), the probability was computed as follows:

$$p = e^{\theta}/(1 + e^{\theta}),$$

where θ is the value calculated when the variables are entered into the above equation. This expression is simply the back-transformation of the logistic transform; thus, $\theta = \mathrm{Ln}(p/[1 - p])$.

That is, if a patient aged 30 years had experienced three previous miscarriages, the derived value of θ is 1.426, leading to a predicted probability of a successful pregnancy of 0.806. The predicted probability of a pregnancy loss would therefore be 0.194.

The probability of maintaining pregnancy, according to Brigham's formula (33), was calculated for each pregnant patient. Thus, the cumulative mean value of those independent probabilities would represent the predicted proportion

of continuing pregnancies in the relevant group. Difference between this proportion and unity would of course be a prediction of the incidence of pregnancy loss, to be compared with the actual rate as obtained from our data.

Chromosome Abnormalities

A second comparison was between embryos of RM patients aged 35 years and older and a comparison group consistent for women undergoing PGD for advanced maternal age (35 years or older; n = 112). Inclusion criteria restricted entry to encompass subjects with no more than one previous pregnancy loss. This comparison group underwent IVF at our two infertility clinics during the same period of time, as did the RM group (January 1, 2001 to March 31, 2003). None were carriers of chromosomal rearrangements. The PGD cancellation rate, because of small number of embryos to biopsy, was 43% in this group.

Recurrent-miscarriage patients younger than 35 years of age were not included because no suitable comparison group was found among our PGD patients.

RESULTS

Comparison of Miscarriages to Prior Expectations

Recurrent-miscarriage subjects had experienced an average of 3.9 previous pregnancies before the PGD cycle, of which 87% (262/301) were lost (Table 1). After PGD, 15.7% pregnancies were lost ($P<.001$). In the whole RM group, the a priori expected losses were 36.5%; after PGD, the observed loss rate was only 16.7% (5/30; $P=.028$). In the RM subgroup with age ≥35 years, the expected loss rate for the next pregnancy was 44.5%, whereas the observed loss after PGD was 12% (2/17; $P=.007$). For the RM subgroup with age <35 years, there were no differences between expected and observed loss rates (29% vs. 23% [3/13]).

Comparing the results in those RM patients with and without previous live births, with similar average maternal ages (37.5 and 36.7 years), the observed pregnancy loss was similar: 11.1% and 13.8%, respectively.

Comparison of Chromosome Abnormalities

The frequency and types of chromosome abnormalities detected in PGD cycles of the RM group and comparison PGD group are shown in Table 2. There were no differences in chromosome abnormality rates between groups. There were no differences in the chromosome-specific aneuploidy rates between the RM and the comparison group. Overall, aneuploidy for chromosome 22 was most common (20% of all aneuploidies), followed by chromosomes 16 (18%), 21 (16%), 15 (15%), 13 (11%), 18 (9%), 17 (8%), and sex chromosomes (3%).

DISCUSSION

The purpose of this study was to evaluate whether PGD could improve pregnancy outcome in RM. The biologic

TABLE 1

Pregnancy outcome of RM patients before and after PGD.

Variable	Maternal age group (y) <35	≥35	Total
No. subjects	21	37	58
Average maternal age	32.6	39.5	37.0
Before PGD			
Previous number of pregnancies	96	205	301
Previous number of lost pregnancies	86	176	262
Average previous losses	3.7	4.1	3.9
Previous lost pregnancies (%)	90[a]	86[b]	87[c]
Expected loss in next pregnancy[d] (%)	29	45[e]	37[f]
After PGD			
Cycles	25	44	69
Cycles replaced	23	37	60
Cycles pregnant	13	17	30
Pregnancy rate per retrieval (%)	52	39	43
Pregnancy rate per transfer (%)	57	46	50
Pregnancies lost	3	2	5
Pregnancy loss rate (%)	23[a]	12[e,b]	17[f,c]
Take-home baby rate/ retrieval (%)	40	34	36
Embryos replaced	55	84	139
Sacs	22	28	50
Lost sacs (without FHB)	2	3	5
Embryonic loss rate (%)	9	11	10
FHBs	21[g]	26[g]	47
Lost FHBs	3	3	6
Fetal loss rate (%)	14	12	13
Implantation rate[h] (%)	38	31	34
Selective reductions (multiple pregnancies)	0	3	3
Fetus ongoing >2nd trimester	4	0	4
Babies delivered	14	20	34

Note: FHB = fetus with fetal heart beat.

[a,b,c] $P<.001$.

[d] According to Brigham et al. (1999) based on age and previous number of pregnancies lost.

[e] $P=.004$.

[f] $P=.028$.

[g] One monozygotic twin.

[h] FHB/embryos replaced.

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TABLE 2

Chromosome abnormalities in RM and comparison groups after PGD.

Group	RM group (y) <35	RM group (y) ≥35	Comparison group (≥35 y)
Analyzed	241	409	1,295
% Normal	43	33	32
% Aneuploid	28	34	38
% Other abnormal[a]	29	33	30

[a] Polyploidy, haploidy, complex abnormal, and extensive mosaics (if the embryo was reanalyzed).

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plausibility was based on most, perhaps ≤80%–90%, pregnancy losses caused by numerical chromosomal abnormalities and aneuploidy that are known to exist in both sporadic and recurrent abortuses. We did not expect to prevent all miscarriages because PGD screens for a limited number of chromosomes, specifically those responsible for about 70% of numerically abnormal aberrations. Duplications and deletions also cannot be assessed.

The key finding was that for women aged 35 years and older, PGD significantly reduced losses and increased number of viable pregnancies, benefiting 46% of patients aged ≥35 years, per cycle. Sample size precludes definitive statements on live-birth rates, but with experience, there is every reason to believe that results will be similarly salutary.

Recurrent-miscarriage patients had an average of 3.9 previous pregnancies before the PGD cycle, of which 87% (262/301) were lost. Their predicted loss rate was 36.5%, according to the calculation of Brigham et al. (33), which takes into account prior losses and maternal age. After PGD, the pregnancy loss was only 16.7% (P=.028). This difference was mostly due to reduction in pregnancy loss in the ≥35-years age subgroup of RM: the expected loss in these PGD cycles was 44.5%, but the observed loss after PGD was 12% (P=.007). There were no differences in the RM group of women aged <35 years.

Our data are consistent with those of previous studies (20–22). This group repeatedly has shown more chromosomally abnormal embryos in RM couples than in comparison couples. In their latest work, they compared RM patients with a fertile control group undergoing PGD for X-linked diseases; embryos from the RM group showed 70% chromosome abnormalities vs. 45% in the control PGD group. Differences were even greater in patients aged <37 years (70% vs. 33%) (22). In the present work, we found that for patients aged 35 years and older, RM patients had similar rates of chromosome abnormalities as infertile patients.

Women aged ≥35 years showed the most significant reduction in spontaneous abortion, compared with a priori predictions of Brigham et al. (33). This is expected, given that PGD reduces the frequency of pregnancy loss in the RM group and given that chromosome abnormalities are the primary cause of embryonic wastage in the ≥35 years RM patients. This also is in agreement with Stephenson et al. (12), who found lower rates of chromosome abnormalities compared with controls in RM patients aged ≤35 years but found similar rates in RM patients aged >35 years. Overall, it is reasonable to conclude that RM with idiopathic etiology in women of advanced maternal age is mostly a problem of recurrent chromosomally abnormal embryos.

Our findings indicate that PGD can be recommended to RM patients who are 35 years and older and show no clear etiology of RM. After PGD, implantation rates are higher and embryonic loss rates lower. Predictably, fetal loss rates (≥9 gestational weeks) were not changed. Randomized clinical trials are desirable to verify this premise, to show that liveborn rates are increased, and to identify the subset of patients most likely to benefit from PGD.

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