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

Santiago Munné, Ph.D.,[a,b] *Serena Chen, M.D.,*[b] *Jill Fischer, M.Sc.,*[a,b] *Pere Colls, Ph.D.,*[a] *Xuezong Zheng, M.D.,*[a] *John Stevens, M.Sc.,*[c] *Tomas Escudero, M.Sc.,*[a] *Maria Oter, M.Sc.,*[a] *Bill Schoolcraft, M.D.,*[c] *Joe Leigh Simpson, M.D.,*[d] *and Jacques Cohen, Ph.D.*[a,b,e]

[a] Reprogenetics, LLC, Hoboken, New Jersey; [b] the Institute for Reproductive Medicine and Science, Saint Barnabas Medical Center, Livingston, New Jersey; [c] the Colorado Center for Reproductive Medicine, Englewood, Colorado; [d] the Department of Obstetrics and Gynecology, Baylor College of Medicine, Houston, Texas; and [e] Tyho-Galileo Research Laboratories, LLC, Hudson, New York

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

Received September 30, 2004; revised and accepted February 7, 2005.
Reprint requests: Santiago Munné, Ph.D., Reprogenetics, 101 Old Short Hills Road, Suite 501, West Orange, New Jersey 07052 (FAX: 973-322-6235; E-mail: munne@embryos.net).

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