Idiopathic recurrent miscarriage is caused mostly by aneuploid embryos

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Objective: To determine any beneficial effects of preimplantation genetic screening (PGS) of all chromosomes by array comparative genomic hybridization (aCGH), with either day 3 or blastocyst biopsy, for idiopathic recurrent pregnancy loss (RPL) patients compared with their expected loss rate.
Design: Case series report.
Setting: Multiple fertility centers.
Patient(s): A total of 287 cycles of couples with idiopathic RPL (defined as two or more losses).
Intervention(s): PGS was done with day 3 biopsy (n = 193) or blastocyst biopsy (n = 94), followed by analysis with aCGH.
Main Outcome Measure(s): Spontaneous abortion rate, euploidy rate.
Result(s): A total of 2,282 embryos were analyzed, of which 35% were euploid and 60% were aneuploid. There were 181 embryo transfer cycles, of which 100 (55%) became pregnant with an implantation rate of 45% (136 sacs/299 replaced embryos) and 94 pregnancies (92%) were ongoing (past second trimester) or delivered. The miscarriage rate was found to be only 6.9% (7/102), compared with the expected rate of 33.5% in an RPL control population and 23.7% in an infertile control population.
Conclusion(s): Current PGS results with aCGH indicate a significant decrease in the miscarriage rate of idiopathic RPL patients and high pregnancy rates. Furthermore, this suggests that idiopathic recurrent miscarriage is mostly caused by chromosomal abnormalities in embryos. (Fertil Steril® 2012;98:675–80. ©2012 by American Society for Reproductive Medicine.)
Key Words: Recurrent pregnancy loss, preimplantation genetic screening, aneuploidy

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Recurrent pregnancy loss (RPL), defined as two or more failed pregnancies (1), has led to immeasurable grief for both the patient and the physician. RPL affects 2%–5% of all couples (2) and can be explained by many factors, such as genetic, anatomic, endocrinologic, and immunologic abnormalities (1, 3). However, >50% of those couples with RPL have a negative work-up and are labeled unexplained or idiopathic (4).

One possible cause for idiopathic RPL is that these couples are producing more aneuploid embryos, leading to more miscarriages. Marquard et al. found that chromosome analysis could explain 80% of unexplained RPL in women >35 years old (5). A higher rate of aneuploidy in RPL patients has been confirmed by many authors (4, 6–14). Therefore, the working hypothesis of preimplantation genetic screening (PGS) for the indication of idiopathic RPL is that euploid embryos could be selected for embryo transfer, leading to a decreased pregnancy loss rate in idiopathic RPL patients. Indeed, all studies using PGS for this indication that have evaluated the miscarriage rate after this procedure have shown a decrease in the miscarriage rate (15–18).

However, those earlier studies were typically performed with the use of fluorescence in situ hybridization (FISH) evaluation of cleavage-stage embryos and typically tested only 7–12 chromosomes. In one meta-analysis (19), four observational studies (18, 20–22) were evaluated in which fertile patients with RPL underwent day 3 cleavage-stage biopsy of 1–2 cells and were compared with natural-conception RPL patients. All four studies performed FISH (screening 3–9 chromosomes). The spontaneous abortion rate (SABR) ranged from 0 to 10% (mean 9%) in RPL patients with PGS compared with 14%–52% (mean 28%) with natural conception (P=.0013).

Finding the ideal control group is often difficult in RPL studies. Should

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the RPL couple be compared with other couples undergoing PGS, with or without infertility, or only those with a history of RPL (21)? To overcome this issue, Garrisi et al. (17) and Munné et al. (18) compared pregnancy loss with the expected rate based on Brigham et al. (23) and found that PGS using FISH significantly reduced miscarriage rates, from 36% expected rate to 13% (14). Patients that were offered PGS but rejected it had a 44% miscarriage rate, which is also another way to compare RPL patients using PGS with an appropriate control. This beneficial effect of PGS for RPL was observed in both fertile and infertile RPL patients undergoing IVF (17). However, these studies used FISH, evaluated a limited number of chromosomes and used day 3 embryo biopsy, which very recent evidence suggests it can negatively affect the implantation potential of the biopsied embryo, whereas blastocyst biopsy does not seem to be detrimental (24).

Recent evidence demonstrates that there is an increase in accuracy using array comparative genomic hybridization (aCGH), where all 24 chromosomes can be evaluated, ruling out aneuploidies that would not otherwise be identified (24–27). In addition, the use of blastocyst biopsy, in which more than one cell is biopsied, could further reduce misdiagnosis, both by analyzing more cells and because there seems to be less mosacism in blastocysts than in cleavage-stage embryos, and when mosacism is present, it seems to be similarly allocated to both the inner cell mass and the trophectoderm (25, 26). Blastocyst culture is becoming more common, and combined with full chromosome analysis it is producing high pregnancy rates after PGS (27–30).

Although the previous PGS technology of FISH already demonstrated a significant reduction in miscarriages in patients with RPL, current advances in technology, such as blastocyst biopsy and aCGH may allow for further reduction in miscarriage risk while simultaneously increasing pregnancy rates, eventually moving toward single-embryo transfer. The objective of the present study was to determine any beneficial effects of PGS by aCGH for RPL patients compared with the expected loss rate in RPL patients and a control infertile population.

MATERIALS AND METHODS

Patient Population

Patients with normal karyotypes, without uterine anomalies or endocrine disorders, and with a history of two or more previous unexplained (idiopathic RPL) miscarriages that occurred after $\leq$20 weeks of gestation were included in the study. All translocation carriers were excluded. Patients included 287 cycles of both fertile and infertile couples. Couples were undergoing assisted reproductive technologies (ART) at multiple fertility centers (mainly NYU Fertility Center, New York, NY; ART Reproductive Center, Beverly Hills, CA; Fertility Centers of Illinois, Highland Park, IL; Lifequest Centre for Reproductive Medicine, Toronto, ON; and Main Line Fertility and Reproductive Medicine, Bryn Mawr, PA). PGS was done using day 3 biopsy (n = 193) or day 5 biopsy (n = 94), followed by analysis with aCGH at Reprogenetics, Livingston, NJ. All day 3 biopsied embryos were transferred on day 5.

In addition, the observed spontaneous abortion rate after PGS in each subject was compared with the expected rate on the basis of the individual's history, according to: 1) the predictive parameters (age, number of prior losses) from the study by Brigham et al. (23), which has been used in similar previous RPL studies (17, 18); and 2) with the expected rate of miscarriage in a control infertile population as reported in the United States to the Society of Assisted Reproduction Technology (SART) according to maternal age and clinical center (excluding five patients from Lifequest Centre for Reproductive Medicine, Toronto, ON).

Variables in the study groups were compared by χ^2 analyses and Fisher exact t test, as appropriate. Specific outcome measures included rates of euploidy, implantation (IR), clinical pregnancy, and ongoing pregnancy plus live birth. The percentage of euploid embryos was calculated using the number of euploid embryos divided by the total number of embryos that were biopsied. The IR was calculated as the number of intrauterine gestational sacs visualized on ultrasound per total number of embryos transferred. A clinical pregnancy was defined as the presence of intrauterine gestational sac(s) with fetal cardiac activity as documented by ultrasound. An ongoing pregnancy was defined as a pregnancy past the second trimester, and a spontaneous abortion was considered to be a loss after <20 weeks.

PGS Procedure

Day 3 biopsy was performed using a variety of methods, depending on each fertility center. Overall, all centers used either acid or laser to breach the zona pellucida, using common techniques described elsewhere (31, 32). For blastocyst biopsy, all centers hatched the embryos on day 3 or day 5 of development, and isolated a piece of the extruded trophectoderm on day 5 and cut using laser (several models and manufacturers). The biopsied cells were placed in Eppendorf tubes, frozen in dry ice, and then transported the same day or overnight to Reprogenetics for PGS analysis. This analysis was performed with the method described in Gutierrez-Mateo et al. without modification (25). With trophectoderm biopsy, several cells are sampled, compared with the one cell (at most two cells) typically taken from a cleavage-stage embryo (33).

Informed signed consents were obtained from patients in accordance with Institutional Review Board (IRB) protocol. It was determined that this study, being a retrospective analysis of deidentified data, was exempt from IRB approval.

RESULTS

In total, 2,282 embryos were analyzed (1,710 biopsied at day 3, 572 biopsied at the blastocyst stage) from 44 centers and 287 cycles. The average maternal age at biopsy was 36.7 $\pm$ 4.2 (range 21–45) years, and these patients had an average of 3.3 $\pm$ 1.2 (range 2–7) prior losses. When comparing those that had a day 3 biopsy to those that had a day 5 biopsy there was no statistical difference in the maternal age at biopsy or number of prior losses. In addition, when comparing those <35 years old and those $\geq$35 years old, there was no difference in the number of prior losses or the day of biopsy.

TABLE 1

Chromosome abnormalities in women with recurrent pregnancy loss.

Day of biopsy	Maternal age, y	No. of cycles	Average no. of embryos biopsied[a]	Euploid embryos[a]
Day 3	36.5 ± 4.2	193	8.8 ± 4.9	31.2% (534/1,710)
Day 5	36.9 ± 4.0	94	6.1 ± 3.6	47.0% (269/572)
Total	36.7 ± 4.2	287	8.0 ± 4.7	35.2% (803/2,282)

[a] $P<.001$ comparing day 3 and day 5.

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Aneuploidy Results

Of those 2,282 embryos, 35.2% (n = 803) were euploid, 60.8% (n = 1,388) were aneuploid, and 4.0% (n = 91) were not analyzable (Table 1). On average, 8.0 ± 4.7 (range 1–35) embryos were biopsied and 2.8 ± 2.9 (range 0–21) were found to be normal. A significantly larger portion of euploid embryos were found on day 5 biopsy compared with day 3 biopsy (47.0% vs. 31.2%; $P<.0001$; risk ratio 1.51, 95% confidence interval 1.35–1.68; Table 1). Of note, there were 52 cycles (18.1%) where there were no available euploid embryos for transfer. Thirty-four of those cycles were after day 3 biopsy. The chance of not having aneuploid embryo increased with age from 5% (4/80) in women <35 years old to 23% (48/207)in those ≥35 years old ($P<.001$).

Transfer and Pregnancy Outcomes

Of those 287 biopsy cycles, there were 181 transfer cycles (one patient had two transfers from one biopsy cohort), 52 cycles where there were no normal embryos to transfer, 4 cycles where an embryo transfer had not taken place at the time of submission, and 51 cycles (17%) where the transfer and pregnancy outcome data were not available. Reprogenetics does not have access to pregnancy records of all the centers referring to them, only of those volunteering that information. The patients in the cycles lost to follow-up were significantly younger (35.4 ± 4.5; $P=.002$), had more embryos biopsied (9.7 vs. 7.6; $P=.004$), and had more normal embryos (3.9 vs. 2.6; $P=.003$) than the larger sample, but there was no difference in the number of prior losses. Nevertheless, the cycles lost to follow-up were included in this study to calculate the above aneuploidy rates.

The cycles with follow-up information were similar to the larger sample, with an average age at biopsy of 36.9 ± 4.1 years and a history of 3.3 ± 1.2 prior losses. In the 181 transfer cycles, an average of 1.65 ± 0.65 (range 1–4) embryos were transferred. The overall pregnancy rate where an implantation had occurred was 56.4% (n = 102). The overall implantation rate was 45.5% (136/299) per embryo transferred. There was a fetal heartbeat in 133 of the 136 sacs noted and an overall clinical pregnancy rate of 55.2% (100/181 transfers). At the time of writing, the combined ongoing pregnancy plus live birth rate was 92.1% (94/102).

There were significantly more day 3 biopsied embryos (mean 1.76 ± 0.64) transferred on day 5 versus those that were biopsied at the blastocyst stage (1.47 ± 0.61; $P=.004$). There was no significant difference in the mean number of gestational sacs or fetal heartbeats between the two groups. There was a higher rate of pregnancies with an implantation (65% [43/66] vs. 51% [59/115] for day 3 biopsy; $P=.04$) and clinical pregnancy rate in the blastocyst biopsy group (Table 2). There was no significant difference between the rate of pregnancy with implantation per transfer, implantation rates or clinical pregnancies between the 5 SART age groups (<35, 35–37, 38–40, 41–42, and >42 years). This was also true when comparing those <35 years old at biopsy to those ≥35 years old.

Spontaneous Abortion Rates

There were seven losses in women that had a pregnancy with implantation. Therefore, the overall SABR was 6.9% (7/102 pregnancies). Of these seven pregnancies, three of them were twin pregnancies with two fetal heartbeats that were both lost. There were two twin pregnancies that were lost that only had a single fetal pole without a heartbeat. There were two singleton pregnancies lost: one without fetal heartbeat and one lost after a fetal heartbeat was documented. These seven patients were on average 37.0 years old at time of retrieval and all of them had a history of two prior losses, except for one who had 3 prior losses. Only one (twin) loss had the products of conception analyzed, which revealed a trisomy 6 and a mosaic 45XO/46XX miscarriage. Leftover amplified DNA from the embryo biopsy was reanalyzed by aCGH

TABLE 2

Pregnancy outcomes by day of biopsy.

Day of biopsy	Maternal age, y	No. of cycles with transfer and pregnancy data	Euploid embryos in transfer cycles[a]	Implantation rate	Clinical pregnancy rate[a]
Day 3	36.2 ± 3.9	115	33.2% (356/1072)	43.1% (87/202)	50.4% (58/115)
Day 5	36.4 ± 4.2	66	49.4% (229/464)	50.5% (49/97)	63.6% (42/66)
Total	36.2 ± 4.0	181	38.1% (585/1536)	45.5% (136/299)	55.2% (100/181)

[a] $P<.001$ comparing day 3 with day 5.

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TABLE 3

Pregnancy loss rate compared with the expected loss rate by day of biopsy.

Day of biopsy	No. of pregnancy cycles with implantation	Expected loss rate in RPL patients	Expected loss rate by SART data	Loss rate after PGS
Day 3	59	32.9%	22.9%	8.5% (5/59)
Day 5	43	34.6%	24.0%	4.7% (2/43)
Total	102	33.5%	23.7%	6.9% (7/102)

Note: PGS = preimplantation genetic screening; RPL = recurrent pregnancy loss; SART = Society for Assisted Reproductive Technology.

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and revealed again the same "euploid" diagnosis as the PGS one, suggesting mosacism as the cause of the misdiagnosis.

An expected loss rate was calculated based on Brigham et al.'s expected rates in those with recurrent pregnancy loss (17, 18, 23) (according to maternal age and number of prior losses) and then again according to the SART database based on age and clinic location. There was also an increased although not significant difference in pregnancy loss rate between day 3 biopsy (8.5%, 5/59 clinical pregnancies) and day 5 biopsy (4.7%, 2/27 clinical pregnancies). However, the pregnancy loss rate was considerably less than the expected rate according to Brigham et al. (33.5%) and SART (23.7%) rates for both day 3 and day 5 biopsies (Table 3). There was also an increased, though not significant difference, in pregnancy loss rate between those ≥35 years old (8.5%, 6/70 clinical pregnancies) and those <35 years old (3.3% 1/30 clinical pregnancies), again both less than the expected Brigham et al. and SART rates (Table 4).

DISCUSSION

Earlier PGS results using FISH (15–18) showed that the miscarriage rate in idiopathic RPL patients was significantly reduced from 26% to 10% in patients <35 years old, and from 39% to 13% in older patients. However, no randomized control trial has ever been performed for this population, with the exception of one that, surprisingly, did not report on miscarriage outcome (21). The present PGS results with aCGH technology indicate a further significant decrease in the miscarriage rate of idiopathic RPL patients (to 5%–7%). This is most probably attributable to a lower error rate than FISH as well as to the ascertainment of more chromosome abnormalities. In a previous study, we reported an error rate for FISH of 5% (34) compared with 2% for aCGH (25), and aCGH ascertained 15% more chromosome abnormalities than FISH with 12 probes (35). In addition, day 5 trophectoderm biopsy allows for more cells to be examined and further decreases the error rate due to mosacism (33).

Day 5 trophectoderm biopsy exhibited a clear advantage over day 3 biopsy in the pregnancy rate, even though less embryos were transferred. Embryos that made it to day 5 and were able to be biopsied were significantly more likely to be euploid compared with all day 3 embryos. Trophectoderm biopsy with aCGH analysis offers the added advantage of a more reliable and accurate diagnosis due to the availability of more DNA while examining more chromosomes (33). In addition, preliminary studies suggest that compared with day 3 biopsy, blastocyst biopsy has no detrimental effect on embryo implantation (24, 36), and combined with full chromosome analysis, such as aCGH, it can significantly increase pregnancy outcome compared with control (15–18). The miscarriage and implantation rates from the day 5 biopsy group were improved compared with the day 3 group, though not significantly; perhaps with larger numbers of embryos and losses to analyze, the difference would be significant. Of note, had the 18% of patients with no euploid embryos after PGS not undergone the procedure, they would have had a transfer of an aneuploid embryo, leading to what would have been considered either a failed IVF cycle with a significantly lower pregnancy rate (43% [100/233]; $P<.006$) or, worse, another pregnancy loss. If these abnormal embryos implanted, the loss rate could have been as high as 38%. This descriptive study helps to enlighten us about what has been often labeled "unexplained" RPL and clearly shows that is mostly due to aneuploidy.

The present study has several limitations. It is a descriptive study with inherent disadvantages including lack of a control group, preventing statistical association, but hopefully our hypothesis will lead to a more sophisticated research study. The ideal control group for patients with RPL continues to be a challenge to determine. Even more difficult is the fact that most RPL patients undergoing IVF are offered PGS for

TABLE 4

Pregnancy loss rate compared with the expected loss rate by age.

Maternal age, y	No. of pregnancy cycles	Expected loss rate in RPL patients	Expected loss rate by SART data	Loss rate after PGS
<35	30	26.3%	14.4%	3.3% (1/30)
≥35	70	36.7%	27.1%	8.6% (6/70)

Note: Abbreviations as in Table 3.

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treatment. A randomized control trial in recurrent miscarriage patients has not been performed, and some might consider it to be unethical given the existing data, though not randomized, suggesting lower miscarriage rates with PGS in this population. Without an appropriate control group, there is no way to directly compare the rate of aneuploidy.

In addition, we are greatly limited by the loss to follow-up leading to selection bias. However, those with transfer data were similar in baseline characteristics to the larger sample and those lost to follow-up were younger, so their inclusion might have improved our results. These results can be extrapolated to a large population of RPL patients, because they came from centers from all over the country. However, this also leads to a great variability in treatment protocols and laboratory methods, which may affect outcomes and reproduction. Our overall SABR was small, which makes comparisons about methods and age difficult to perform.

This study does confirm that idiopathic RPL is mostly caused by chromosomal abnormalities, with only a residual 6.9% miscarriage rate. These losses demonstrate that a pregnancy loss can be a result of a factor beyond euploidy, mosaicism, or a genetic abnormality below the resolution of this technology. These new PGS technologies, aCGH and blastocyst biopsy, may allow us to finally provide RPL patients with not only an explanation but a cure.

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