Reimplantation genetic diagnosis (PGD) is a new test that can detect genetic abnormalities such as disease-causing mutations and chromosomal abnormalities to prevent the conception of an abnormal pregnancy or child. The use of PGD has made headlines recently in some controversial cases, such as in preventing the transmission of adult-onset diseases (eg, Alzheimer’s),\(^1\) and in helping a couple to conceive a human leukocyte antigen (HLA)-matched child to donate bone marrow for a sibling with Fanconi’s anemia.\(^2\) While PGD may seem to have little practical applications for the OB/GYN or primary care generalist, it may in fact be a reasonable option for many patients with recurrent first-trimester pregnancy loss or a history of a chromosomally abnormal pregnancy. For example, the test can be used to detect aneuploidy, which causes 50% to 70% of first-trimester miscarriages.\(^3,4\) By preventing the transfer of abnormal embryos, the risk of miscarriage and aneuploidy can be significantly reduced in these patients.\(^5\)

The Institute for Reproductive Medicine and Science at Saint Barnabas Medical Center in Livingston, NJ, is one of the few centers with significant experience in this technique, and this article reviews the authors’ experiences and results there. However, as the availability of PGD is increasing rapidly, it is important for the OB/GYN and other primary care physicians to understand which patients may benefit from this test. This article examines the indications, benefits, and limitations of PGD, and demonstrates why it should be considered for patients with recurrent miscarriage.

**TECHNIQUE**

As PGD requires the creation of embryos in the laboratory, patients must undergo in vitro fertilization (IVF). This involves ovarian stimulation of multiple oocyte development using injectable gonadotropins. While the patient is under sedation or anesthesia, oocytes are retrieved transvaginally via needle aspiration of the ovarian follicles under vaginal ultrasonographic guidance. The oocytes are then inseminated with the partner’s sperm in the laboratory. Once fertilization occurs, the embryos are cultured for 3 days, when the embryos should reach the 6- to 8-cell stage. At this point in development, prior to differentiation, analyzing the chromosomes of a single cell or blastomere should provide information about the chromosomes analyzed for all the cells in the embryo.

An embryo biopsy is performed by creating an opening in the zona pellucida using mechanical means, diluted acid solution, or a laser. A single blastomere is removed through this opening using gentle suction and a micropipette. The biopsy is undertaken using a special microscope with delicate micromanipulators (Figure 1). The blastomere is then fixed on a slide, and the embryo is returned to an incubator to await the results of the biopsy.

The diagnosis of aneuploidy uses fluorescence in-situ hybridization (FISH) (Figure 2). This technique
employs commercially available DNA probes attached to fluorescent labels that bind to specific chromosomes in the blastomere. Once the probes are bound, the signals are read under a fluorescent microscope so that the number and type of chromosomes present in that cell can be determined. The analysis takes approximately 1 day to complete. Embryos found to be normal are then transferred to the patient’s uterus on day 4 or 5 after oocyte retrieval.8,9 Due to the small amount of tissue available and the limited window of time in which to obtain a diagnosis, only eight of the 24 types of chromosomes can be analyzed. The chromosomes most commonly analyzed are 13, 15, 16, 18, 21, 22, X, and Y9,10 (ie, the chromosomes thought to be responsible for most clinical pregnancy losses).11

RESULTS

The use of PGD to detect aneuploidy can reduce spontaneous abortion rates. In a group of patients undergoing IVF matched for maternal age, number of previous IVF cycles, and response to fertility drugs, there was a significant decrease in the rate of spontaneous abortions in the group that underwent PGD for aneuploidy compared with the control group (23% miscarriage in the control group versus 9% in the PGD group, p < 0.05),7 along with a subsequent higher ongoing pregnancy and delivery rate in the PGD group.

The rate of trisomic pregnancies is also significantly reduced by PGD.12 In addition, PGD for aneuploidy in patients undergoing IVF may improve the chance of conception by increasing embryo implantation rates.7 It is thought that PGD improves the process of selecting embryos for transfer, allowing embryologists to choose embryos most likely to result in a normal pregnancy. By improving embryo

FIGURE 1. Embryo Blastomere Biopsy Sequence

In each photo, the large pipette to the left of the embryo holds it in place with gentle suction. First, a small glass pipette with dilute acid solution is used to create an opening in the zona pellucida. Then, a larger biopsy pipette is placed in the opening and gentle suction is used to remove a single blastomere for analysis.

Photos courtesy of the Saint Barnabas IRMS embryology laboratory and Dr Santiago Munne.
selection and reducing the number of embryos transferred, PGD can also help to decrease the frequency of high-order multiple births after IVF.7,12

RISKS AND LIMITATIONS
The risks of PGD include the possibility that the embryo will be damaged during the biopsy procedure. The current risk of embryo damage at the authors’ center is 0.9%, and depends on the experience and skill of the technician performing the biopsy.7

Because PGD for aneuploidy is currently limited to eight of the 24 types of chromosomes, an embryo that is deemed normal by PGD could have an abnormality in one of the 16 remaining types of chromosomes that were not analyzed by PGD. In addition, the error rate for the chromosomes analyzed (including mosaics and false-positive/false-negative results) is approximately 7% at the authors’ center, while the error rate for CVS and amniocentesis is typically less than 1%. Thus, PGD cannot be considered a substitute for prenatal diagnosis, and the decision to forgo CVS or amniocentesis should not be based on normal PGD findings. At this time, the authors recommend that patients at high risk for aneuploidy undergo CVS or amniocentesis even if PGD has been performed.

PATIENT SELECTION
Recurrent First-trimester Pregnancy Loss
Recurrent pregnancy loss (defined as three or more consecutive miscarriages) affects approximately 1% of the US population. The evaluation of these patients should first rule out genetic, anatomic, endocrine, and immunologic causes for recurrent miscarriage. Many clinicians will also test for inheritable thrombophilias, although this remains controversial.11 The work-up of these patients should be individualized, but typically includes history; physical examination; pelvic ultrasonography, hysterosalpingography, or saline hysterosonography; complete blood cell count; testing for thyrotropin, antithyroid antibodies, prolactin, lupus anticoagulant, antithrombin, and antiphosphati-dylserine antibodies; karyotyping of both partners; and possibly an endometrial biopsy and screening for inheritable thrombophilias.13

Approximately 5% to 8% of couples with a history of recurrent pregnancy loss have an abnormal karyotype, usually a balanced translocation. PGD can be performed for couples with a balanced translocation, allowing them to implant only chromosomally balanced embryos, thus reducing their risk of miscarriage.14,15 The use of PGD for translocations is technically more complicated than for aneuploidy. Patients with a translocation should be referred to a genetics counselor to review their options. A referral for PGD at a center with experience in this type of analysis can then be made if the couple desires it.

Even after undergoing a complete work-up, many couples have no identifiable cause for their miscarriages, and therefore no standard treatment options. Without treatment, couples with this history have a 55% to 70% chance of a successful live birth, depending on how many miscarriages they have had and whether they have any previous live births.13 Thus, expectant management with close follow-up is a reasonable course for these patients. For couples desiring a more aggressive approach, PGD may be offered for significant reduction (by more than 50%) of the risk of first-trimester loss due to aneuploidy.

History of Chromosomally Abnormal Child or Pregnancy
For patients with a previous child or pregnancy with a chromosomal abnormality, PGD can reduce the risk of certain abnormalities in the
patient’s next pregnancy. This may be an attractive alternative to post-conception testing for patients, as they may be able to avoid termination of an abnormal pregnancy.

**Advanced Maternal Age**
As a woman ages, her risk for both miscarriage and aneuploid pregnancy increases markedly. For women aged 37 years and older undergoing IVF, the authors’ center has demonstrated that PGD for aneuploidy significantly improves pregnancy rates, reduces miscarriage rates, and decreases trisomies if six or more embryos of good quality are available for analysis.16

**CONCLUSION**
The availability of PGD for aneuploidy is increasing quickly at IVF centers around the country. To undergo PGD, patients must conceive via IVF, and PGD for aneuploidy offers limited chromosomal analysis of early-stage embryos prior to implantation. Physicians should understand the technique, and discuss PGD for aneuploidy with patients who have a history of recurrent first-trimester loss that is due to a chromosomal abnormality or unexplained. The use of PGD can significantly reduce the risk of miscarriage in these patients. If a patient wants to consider PGD for aneuploidy, she should have a consultation with a genetics counselor for a more extensive discussion of the procedure and its limitations. The primary care physician, OB/GYN, or genetics counselor can then refer the patient to a center with experience in this technique. While PGD can have significant benefits, it is a limited genetic test, and is not a substitute for CVS or amniocentesis.

**REFERENCES**