

# Ten-year experience with preimplantation genetic diagnosis (PGD) at the New York University School of Medicine Fertility Center

We describe our experience of over 300 cycles of preimplantation genetic diagnosis (PGD) and report clinical pregnancy rates (35%–67%) that support using this technology to screen for genetic disorders and chromosomal abnormalities. In clinical practice for over ten years, PGD offers couples the earliest form of genetic screening and may help improve ongoing pregnancy rates in poor-prognosis patients. (*Fertil Steril*® 2007;88:978–81. ©2007 by American Society for Reproductive Medicine.)

Preimplantation genetic diagnosis (PGD) was first used on human embryos in the early 1990s to diagnose sex-linked genetic disorders (1–3). The clinical horizons for PGD have since broadened to include diagnoses for more than 100 different genetic disorders as well as for chromosomal aneuploidies (AS) and translocations (TS). As a result, over 1,000 healthy children have been born worldwide from PGD-IVF cycles (4, 5).

Originally, PGD was provided for couples at risk of having a child with a sex-linked disorder. Initially, single blastomeres were analyzed using multiplex polymerase chain reaction (PCR) to amplify X- and Y-chromosome-specific sequences. Now, fluorescence in situ hybridization (FISH) technology is routinely used for sex chromosome assessment (2, 6–8). For single gene defects (SGD), identification of the specific mutations have led to the development of specific PCR probes to identify affected, carrier, and normal embryos (9).

Other patients now routinely seek PGD to screen for chromosomal abnormalities. These include carriers of chromosomal translocations as well as women of advanced maternal age and women with repeated failed IVF cycles and recurrent miscarriage. For these patients, FISH technology has been applied to screen for AS or TS. Initially AS evaluated chromosomes 13, 16, 18, 21, X, and Y; the list subsequently broadened to include chromosomes 14, 15, and 22, allowing the evaluation of the most frequently observed chromosomal aneuploidies in human abortuses (10–12).

A final group seeking PGD includes patients with a child with a genetic disease who wish to create an unaffected HLA match for stem cell transplantation therapy. Preimplantation genetic diagnosis has been used to obtain HLA matches for children with Fanconi anemia, acute lymphoid leukemia, and acute myeloid leukemia (13, 14).

We have performed PGD at New York University (NYU) Fertility Center (formerly the Program for IVF, Reproductive Surgery and Infertility) at the NYU School of Medicine since 1995 and have completed over 300 PGD-IVF cycles through 2005. Performing PGD in a successful IVF program offers a unique opportunity to assess the outcomes of these cycles in an optimal setting. We completed a retrospective chart review for PGD patients from 1995 to 2005 (Institutional Board of Research Associates study no. 05–156). Cycles were reviewed for indications, patient histories, and outcomes.

All IVF, embryo biopsy, and transfer procedures were performed at our center as well as PCR and FISH analyses from 1995 to 2000. As genetic protocols became more specialized, biopsied cells were sent to Genesis Genetics (Detroit, MI) for PCR analyses and Reprogenetics (West Orange, NJ) for FISH analyses.

A patient's ovarian stimulation protocol was individualized to achieve adequate numbers of mature oocytes at retrieval. Most patients were down-regulated with a GnRH agonist and then treated with combinations of recombinant human FSH (rFSH) and/or hMG. Recent protocols used GnRH antagonists. When lead follicles reached a mean diameter of 17–18 mm, hCG was given, and about 34 hours later oocytes were collected by ultrasound-guided transvaginal aspiration and placed in human tubal fluid media (HTF; Irvine Scientific, Irvine, CA) supplemented with 6% Plasmanate (5% USP plasma protein fraction (human); Bayer, Elkhart, IN) overlaid with Sage mineral oil (Cooper Surgical Co., Trumbull, CT). Partner's sperm was collected on day of retrieval and washed. Oocytes were fertilized with routine insemination (4–6 hours after retrieval) or intracytoplasmic sperm injection (ICSI) if PCR analysis was indicated for SGD. The ICSI was routinely performed for SGD cases to eliminate DNA contamination by stray sperm cells at embryo biopsy. Fertilization was assessed 18 hours after insemination/ICSI by detection of two pronuclei (2PN).

Embryo biopsy was performed approximately 72 hours after retrieval in  $\text{Ca}^{++}/\text{Mg}^{++}$ -free media, supplemented

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with HEPES, BSA, and sucrose (Sage Sigma, In Vitro Fertilization Inc, Trumbull, CT). This medium disrupts cell adhesion and causes osmotic shrinkage of the cells from the zona pellucida, facilitating the removal of a single cell (2). The embryo was stabilized by a holding pipette and the zona pellucida breached using acidified Tyrode's solution. The single cell was gently removed by suction. For both PCR and FISH, biopsy was performed only on 2PN embryos that were  $\geq 5$  cells on day 3. If PCR was scheduled, the biopsied cell was rinsed and placed in a DNA-free prelabeled microfuge tube containing 5  $\mu$ L lysis buffer. If FISH was scheduled, the cell was rinsed and fixed to a glass slide as previously described (15, 16). Biopsy specimens were sent to the appropriate referral laboratory. In the majority of cases only a single blastomere was biopsied; if a nucleus was not present, a second cell was biopsied.

After biopsy, the embryos were rinsed and placed in Quinn's Blastocyst Media (Sage) supplemented with 10% Plasmanate and cultured under oil for an additional 24–72 hours. Following review of either FISH or PCR results (1 day after biopsy), the embryos were evaluated. The most advanced embryos that had either a normal chromosomal complement or were unaffected by the genetic mutation, were selected for transfer to the uterus on day 4, 5, or 6 after retrieval. In most cases,  $\leq 2$  embryos were suitable for transfer on day 4 and extended culture for selection purposes was not needed. Additional unaffected good-quality (grade 4BB or better per Gardner's criteria) embryos were cryopreserved on day 5 or 6 (17).

The methods used at our center for FISH were previously described for simultaneous enumeration of chromosomes 13, 18, 21, X, and Y in interphase cells (11, 12). At the time of this review, cells were routinely screened for nine chromosomes (13, 15, 16, 17, 18, 21, 22, X, and Y) at Reprogenetics. For many inheritable disorders for which the genetic mutations were determined, PCR was used to amplify the genetic material contained in a single cell; protocols for PCR on single cells have been described elsewhere (18).

From 1995 to 2005, 304 PGD-IVF cycles were performed for 190 patients; 181 (60%) were performed for SGD, and 123 (40%) were performed for AS and TS. Embryos were transferred in 158 of 181 cycles (87%) for SGD, 87 of 111 cycles (78%) for AS, and 9 of 12 cycles (75%) for TS. Table 1 lists the number of PGD referrals and their outcomes. Implantation rate (IR) is the number of gestational sacs divided by the number of embryos transferred. The IRs for SGD, AS, and TS were 24%, 27%, and 47%, respectively. Clinical pregnancy rate (CPR) is the presence of a fetal heartbeat divided by the number of cycles resulting in an embryo transfer. The CPRs for SGD, AS, and TS were 35%, 37%, and 67%, respectively.

Clinical histories were reviewed for the 92 patients referred for SGD. Many (35%) had a prior affected pregnancy, and half (50%) underwent a second-trimester termination. The additional patients had a maternal, paternal, and/or family history of a disorder. In most cases, both parents were known carriers of cystic fibrosis, predominantly the delta F508 mutation.

Embryos were not transferred in 23 cycles for SGD: in seven cycles with no HLA match, nine cycles with no unaffected embryo, and two cycles with unaffected embryos that did not divide properly. In five cycles, genetic analysis failed, because no PCR signal was obtained.

When HLA matching was performed for Diamond-Black Fanconi anemia, adrenoleukodystrophy, and Bloom's disease for four patients, 8 of 16 cycles (50%) resulted in an embryo transfer, and one eventuated in a full-term delivery of an unaffected child that was not an HLA match. In seven cycles, no HLA match was obtained. In one cycle, an HLA match was found but the embryo stopped dividing before transfer.

Eighty-eight patients underwent 111 cycles of PGD for AS: 44 for recurrent ( $\geq 2$ ) miscarriage, 37 for advanced maternal age (generally  $\geq 38$  years, all with a history of elevated FSH), 5 for repeated IVF failures, 7 for couples with an aneuploid fetus/child, and 18 for a combination of the above indications. Of note, 78 cycles (70%) were performed

**TABLE 1**

**PGD outcome 1995–2005 at New York University Fertility Center.**

	Referrals	Cycles	Age, yrs $\pm$ SD (range)	IR%	CPR %	Singleton birth	Twin Birth	Miscarriage 1st, 2nd trimester	Ongoing pregnancy
SGD	92	181	33 $\pm$ 3.9 (22–44)	23.8	35.0	28	20	8, 2	2
AS	88	111	38 $\pm$ 4.2 (25–47)	26.9	36.8	14	4	6, 1	10
TS	10	12	34 $\pm$ 6.8 (23–48)	46.7	66.7	3	1	1, 0	1

*Note:* AS = aneuploidy screening; CPR = clinical pregnancy rate; IR = implantation rate; PGD = preimplantation genetic diagnosis; SGD = single gene defects; STD = standard deviation; TS = translocation screening.

*Grifo. PGD at NYU Fertility Center. Fertil Steril 2007.*



in 2005, indicating a recent increase in demand for PGD for this indication. There was no embryo transfer in 24 cycles of PGD for AS; in all cases, no euploid embryos were identified. Twelve cycles (in ten patients) used PGD for TS; three did not have an embryo transfer, because of a lack of unaffected embryos.

Miscarriage rates, defined as a proportion of gestational sacs not resulting in live births, were 22% for SGD, 29% for AS, and 14% for TS. When calculated as the proportion of fetal heartbeats aborted per fetal heartbeats detected, then rates were much lower: 12% for SGD, 12.5% for AS, and 14% for TS.

Congenital anomalies were rare: a female child with bilateral clubfoot after PGD for SGD and an aortic arch anomaly in a premature twin born after PGD for AS for advanced maternal age. Two cycles resulted in newborn carriers of genetic disorders: twin carriers of Tay Sachs disease and a singleton carrier of cystic fibrosis. However, in both cases the carrier status of the embryos was revealed by PGD and the patients elected to continue with the transfer. Two cycles resulted in newborns who were affected by disease (one with hemophilia and one with familial dysautonomia) presumably owing to misdiagnoses by PCR.

Twenty cycles of PGD for SGD and two cycles for AS resulted in cryopreservation of unaffected embryos. Five frozen embryo transfer cycles in the SGD group resulted in an unaffected live birth; a patient who did not get pregnant from the PGD-AS cycle did get pregnant and delivered full term from her frozen cycle.

Our ten-year experience performing PGD illustrates that this procedure successfully provides couples with healthy children unaffected by either genetic diseases or chromosomal abnormalities. Over 33% of those who underwent PGD for SGD had an earlier affected pregnancy and of these, 50% terminated an earlier pregnancy for this reason. Performing PGD allowed these patients to initiate a pregnancy with a reduced potential of undergoing a second-trimester termination.

Verlinsky et al. (19) recently published a multicenter report on PGD, citing a pregnancy rate of 25.2% in 4,748 cycles (23.3% for AS, 30.5% for SGD, and 34.6% for TS). The present data in conjunction with those published in this large multicenter study support the use of PGD for appropriate cases.

Data from 2005 indicate that more PGD cycles are being conducted for AS in high-risk patients. Whether PGD for AS increases the implantation and clinical pregnancy rates for IVF patients is currently controversial. In a retrospective blinded control study, higher ongoing pregnancies and delivered babies were reported in patients who underwent PGD for AS compared with those who underwent routine IVF (20). Another randomized controlled study reported similar clinical pregnancy rates but higher ongoing preg-

nancy rates in older patients ( $\geq 36$  years) who underwent PGD for AS compared with IVF alone (21). Another prospectively randomized controlled trial did not find any statistically significant difference in positive pregnancy tests or implantation rates in older patients ( $\geq 37$  years) who underwent PGD for AS compared with patients who underwent IVF alone (22). However, in that study, two blastomeres were biopsied on average, which may compromise embryo quality.

The present data reflect several explanations of why pregnancy rates are not higher when PGD is added to IVF in poor-prognosis patients. Approximately 20% of the patients who underwent PGD for AS failed to have genetically normal and viable embryos for transfer. Moreover, nearly 20% of patients who sought PGD for AS were canceled before retrieval owing to an insufficient number of follicles. Several investigators have clearly demonstrated that once pregnancy is initiated with embryos identified as chromosomally normal, the incidence of miscarriage is significantly lower (23–26). As a result, the live birth rate per transfer appears to be increased after PGD.

While there are potential pitfalls with PGD, as in the case of the two misdiagnoses, all efforts are made to avoid them, and patients should be counseled about the risks involved. Confirmatory prenatal diagnosis with chorionic villus sampling or amniocentesis should be encouraged.

We anticipate that more PGD cycles will be performed to select embryos that are not only morphologically attractive but also free of potentially harmful genetic mutations and chromosomal aberrations. Although embryo biopsy adds to the cost of an IVF cycle, potentially improved ongoing pregnancy rates will likely offset the cost of repeatedly failed IVF cycles in patients at risk for AS. At the minimum, performing PGD for AS in poor-prognosis patients, by revealing the high number of abnormal embryos, may identify the cause of their failed cycles.

To date we have performed PGD for over 30 different genetic disorders, for chromosomal aberrations, and for HLA matching, resulting in the birth of over 90 healthy children. Our clinical pregnancy rates for PGD cycles are consistent with those we have reported to the national CDC registry (27). We foresee a global expansion in the use of PGD as its application is realized to its fullest potential and more physicians and embryologists are trained to perform this procedure.

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