PRENATAL COUNSELING

Here are recommendations, and cautions, when you are considering a patient's request for preimplantation genetic diagnosis or screening

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Population-based screening for carriers of genetic diseases and advances in neonatal and pediatric genetic testing have resulted in more and more couples identified as at-risk for inherited disorders. Increasingly, women in these couples ask their ObGyn about their options for future pregnancies.

For some women, genetic testing of a pregnancy as early as possible—even before implantation—is desirable. In vitro fertilization affords such direct access to the genetic material of either gametes before fertilization (i.e., **polar-body biopsy**) or blastomeres once fertilization has occurred (**blastomere biopsy**). Complex genetic analysis of these single cells is now possible. Because polar-body biopsy is restricted to testing for maternal disease, blastomere biopsy has gained favor as the method of choice for genetic testing of preimplantation pregnancies.

The duality of genetic testing

Regardless of what genetic material is tested, preimplantation genetic testing encompasses two distinct categories: **preimplantation genetic diagnosis**, or PGD, and **preimplantation genetic screening**, or PGS.

What is PGD?

Here, testing is confined to **women at risk of an offspring with an identified genetic abnormality.** These women, or their partner, typically carry a gene mutation that, alone or in combination with another mutation in the same gene, would result in an identifiable outcome in their child (for example, autosomal-recessive, autosomal-dominant, and X-linked disorders).

PGD, by definition, also includes testing of **women, or their partner, who possess a balanced chromosome rearrangement** (translocation, inversion). Offspring of carriers of balanced chromosome rearrangements are at increased risk of particular genetic abnormalities, as a result of unbalanced segregation of chromosomes involved in their rearrangement.

How does PGS differ from PGD?

Screening, in contrast, focuses analysis on **offspring of women who are theoretically at increased risk of a genetic abnormality based on their age or reproductive history, not on their genetic makeup.** PGS looks specifically for chromosomal content, and is based on the premise that decreasing the rate of aneuploidy among the conceptions of women **1**) of advanced maternal age, **2**) who experience habitual miscarriage, or **3**) who have failed multiple cycles of in vitro fertilization (IVF) would increase the rate of implantation and, ultimately, the live birth rate.

The articles below, beginning with a committee opinion from the American Society for Reproductive Medicine (ASRM), address the following:

- **evidence in support of PGD** for genetic disease
- **caution about using PGS**, in its current format, for aneuploidy screening.

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PGD can reduce the risk of a child with a specific genetic abnormality carried by one or both parents

Practice Committee of the Society for Assisted Reproductive Technology; Practice Committee of the American Society for Reproductive Medicine. Preimplantation genetic testing: a Practice Committee opinion. Fertil Steril. 2007;88:1497–1504.

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PGD has been extended to women who possess a gene mutation placing them at higher risk of cancer, and who don't want to transmit that gene to offspring

A gene mutation carried by one or both parents can increase the risk that their offspring will be affected with an inherited condition. Common examples include autosomal-recessive disorders such as cystic fibrosis; autosomal-dominant disorders such as neurofibromatosis; and X-linked disorders such as hemophilia A.

Recently, human leukocyte antigens (HLA) have been assessed in conjunction with testing for specific genetic diseases, such as Fanconi anemia. In these settings, the intent is to recognize not only the blastomeres that are free of Fanconi anemia, but also those that are potential HLA matches and, therefore, potential donors for an (older) affected sibling.

PGD has been extended to women, or their partner, who possess a gene mutation that places them at increased risk of cancer (such as BRCA-1) and who wish to avoid transmitting that risk-conferring gene to their offspring.

For these diseases, and for many others, knowledge of the specific genetic mutation enables similar molecular testing to be accomplished on a single cell, such as a blastomere.

Technical concerns of testing must be part of the physician–patient discussion

Typically, PGD analysis is initiated by polymerase chain reaction (PCR) of DNA content extracted from the single cell. This is followed by application of mutation-appropriate molecular technology. Given **1**) the short time in which these PGD results are needed (often, 24 to 48 hours) and **2**) the limited amount of genetic material available for analysis, technical restraints on testing are recognized:

- Extraneous DNA contamination remains a problem with molecular technology, despite application of intracytoplasmic sperm injection
- Only partial amplification of the allele may occur, or allele "drop-out" may be present; both of these phenomena can cause false-negative results
- Error can occur dually: **1**) Presumably unaffected embryos that are, indeed, affected are transferred and **2**) actually normal embryos that have been interpreted incorrectly as abnormal are discarded
- The rate of misdiagnosis (false-negative results) ranges from 2% (with autosomal-recessive disorders) to 10% (with autosomal-dominant disorders), although this rate can be lessened with the use of linked markers.

You should counsel patients about these technical concerns *before* PGD is considered. You should also discuss the option of performing prenatal diagnostic testing during the pregnancy.

PGD for investigating balanced chromosome rearrangements

These rearrangements represent another type of genetic abnormality in which PGD can reduce the likelihood of a conception that carries a specific genetic abnormality.

When one parent carries a balanced chromosome translocation, fluorescence in-situ hybridization (FISH) can be applied to assess the segregation of at-risk chromosomes in a single blastomere cell. In this technique, fluorescence-labeled DNA probes, selected for specificity to the translocation in question, are applied to the single cell fixed on a glass slide. Copies of the DNA segment and, by inference, the chromosomal segment in

question are assessed by quantification of the sites of positive fluorescence.

Because translocation carriers are, theoretically, at high risk of transmission of an unbalanced segregant to the blastomere, as many as 10 blastomeres will often be screened until one or two are deemed normal for the FISH probes in question. When implantation does succeed after FISH analysis for a chromosome rearrangement, however, the pregnancy loss rate is lower and the likelihood of a live birth is higher.

Again, in-depth consultation is needed before PGD

Whether PGD is planned for investigating a single-gene disorder or a chromosome translocation, detailed consultation with the woman or the couple is important. This effort should include not only genetic counseling about inheritance, the natural history of the disorder in question, and other options for avoiding the transmission of the disorder—in addition, additional time should be spent describing:

- risks associated with IVF procedures and embryo biopsy (and with extended culture, if needed)
- technical limitations of the particular testing that is being considered
- options for prenatal testing during a pregnancy
- the possibility that embryos suitable for transfer will *not* be found (and that, potentially, erroneously tested normal embryos will not be transferred)
- disposition of embryos in which test results are inconclusive.

PGS for women at increased risk of aneuploidy isn't supported by evidence; consider it investigational

Mastenbroek S, Twisk M, van Echten-Arends J, et al. In vitro fertilization with preimplantation genetic screening. N Engl J Med. 2007;357:9–17.

Mersereau JE, Pergament E, Zhang X, Milad MP. Preimplantation genetic screening to improve in vitro fertilization pregnancy rates: a prospective randomized controlled trial. Fertil Steril. 2008;90:1287–1289.

Aneuploidy contributes to pregnancy loss among women as they become older. Theoretically, avoiding aneuploid pregnancy among embryos transferred during IVF cycles—in older women and in women experiencing multiple pregnancy losses and failed IVF cycles—was expected to increase the implantation rate and decrease the rate of pregnancy loss.

This hypothesis was supported, at first, by observational trials. But at least one randomized study, by Staessen and colleagues,[1] failed to demonstrate that PGS is beneficial in women of advanced maternal age.

Now, a large multicenter, randomized, double-blind, controlled trial conducted by Mastenbroek and co-workers provides further evidence that **PGS does not increase the rate of pregnancy and, in fact, significantly reduces that rate among women of advanced maternal age.**

The Mastenbroek study compared outcomes among 206 women who had PGS and 202 women who did not. Both groups were matched for maternal age older than 35 years. Blastomeres were analyzed for eight chromosomes, including those known to be highly associated with miscarriage (1, 16, 17, 13, 18, and 21; X and Y).

Among women who underwent PGS, 25% had an ongoing pregnancy of at least 12 weeks' gestation, compared with 37% of un-

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PGS does not increase the rate of pregnancy. In fact, it significantly reduces the rate of pregnancy among women of advanced maternal age

screened women. A similar higher rate of live birth was seen among unscreened women (35%, versus 24% in the PGS group).

Mastenbroek's results are comparable to what was reported from an earlier randomized trial of PGS,[1] in which the implantation rate as the primary outcome among women who had PGS and among controls was not significantly different. Contributors to **1**) the lack of success of PGS and **2**) the apparent detriment of PGS to the ongoing pregnancy rate include:

- potential for damage to the embryo at biopsy
- limitations imposed by FISH technology on the number of probes that can be accurately assessed technically
- a growing knowledge that a significant percentage of embryos are chromosomal mosaics at this stage—a phenomenon that likely results in nontransfer of embryos that have the potential for developing karyotypically normally.

Does PGS improve outcomes?

More recently, Mersereau and colleagues reported pilot results from a prospective, randomized, controlled trial that assessed whether PGS could improve pregnancy outcomes. Here, selection of infertile women for the study was not restricted to poor prognosis categories, such as advanced maternal age and recurrent pregnancy loss.

Using the live birth rate as the outcome measure, PGS for seven chromosomes was determined not to be associated with a significantly increased live birth rate among screened pregnancies. Sample sizes had been calculated to establish, with significance, a 50% increase in live births—from 30% in the control (unscreened) population to 45% in the screened population. Secondary endpoints, such as the implantation rate and pregnancy loss, also did not differ significantly between the PGS cases and controls.

Again, technical difficulties of two-blastomere biopsy, with its potential for embryo damage, and the presence of underlying embryo mosaicism represent possible barriers to improving the live birth rate when utilizing PGS.

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We've learned that embryos have the potential to self-correct early mosaicism. With PGS, therefore, random biopsy solely of an abnormal cell would relegate a potentially euploid embryo to nontransfer

Technical limitations may be one of the largest obstacles to applying PGS

Practice Committee of the Society for Assisted Reproductive Technology; Practice Committee of the American Society for Reproductive Medicine. Preimplantation genetic testing: a Practice Committee opinion. Fertil Steril. 2007;88:1497–1504 .

FISH probes can be chosen to reflect the nature of a given patient's risk (advanced maternal age, recurrent pregnancy loss) when performing PGS, but the technique itself is limited by the number of probe sites that can be interpreted accurately at one time. Typically, analysis of more than five chromosomes requires two cycles of hybridization, with their associated time requirement and potential for degradation of the single cell.

Alternatively, advances in the analysis of all 23 chromosomes through **comparative genomic hybridization** may, ultimately, provide an avenue for applying PGS. At the moment, time limitations prohibit comparative genomic hybridization without embryo cryopreservation. Further investigation of other technical limitations, such as the high rate of mosaicism, has revealed that, when two cells are examined and found to be karyotypically discordant, further analysis of the entire embryo will reveal that more than 50% of embryos are, in fact, euploid—that is, chromosomally normal. Random biopsy of the abnormal cell solely would relegate the embryo to nontransfer, despite the predominance of an underlying euploid state.

Understanding of the potential that embryos have to self-correct early mosaicism is

growing; we now know that **almost one half of embryos identified as aneuploid** at cleavage stage correct to euploid if they survive to blastocyst stage. A karyotypic abnormality in a single cell from a day-3 embryo does not always signal an abnormal embryo.

ASRM does not support PGS to improve the live birth rate

This determination by ASRM is based on available evidence about advanced maternal age, recurrent pregnancy loss, recurrent implantation failure, and recurrent aneuploidy loss:

- In women of advanced maternal age, many day-3 embryos display aneuploidy when studied by FISH. In *theory*, exclusion of these embryos for transfer should improve implantation and live birth rates, but *evidence* does not support that premise.
- Because almost 70% of spontaneous pregnancy loss is caused by a karyotypic abnormality, and women with karyotypically recurrent pregnancy loss are more likely to experience subsequent loss with karyotype abnormalities, the premise of preimplantation screening for aneuploidy also appeared to be well founded. Studies at this time are limited to retrospective series, without randomized controlled trials published.
- Among women who experience repeated implantation failure, a finding of more than 50% abnormal embryos isn't uncommon, yet several studies have not supported an increased implantation rate or live birth rate after PGS.

A literature review of PGS calls its introduction "premature"

Gleicher N, Weghofer A, Barad D. Preimplantation genetic screening: "established" and ready for prime time? Fertil Steril. 2008;89:780–788.

After ASRM recognized PGD as an established technique in a 2001 committee opinion, extension of this status to PGS was inadvertently assumed. But PGS is a different testing modality—with different indications, risk/benefit profiles, and efficacy than PGD.

Today, FISH probes are utilized for PGS; the false-negative rate of FISH appears to be driven by the technical constraints of the technology. Potentially increasing the false-negative rate are inadequate hybridization and the use of increasing numbers of probes and hybridization cycles.

Conversely, the false-positive rate—the number of embryos not transferred that are, in fact, chromosomally normal—varies markedly from one study to another, and may be as high as 20% when discarded embryos are more completely assessed.

Similarly, laboratories utilize different methods of obtaining the genetic material. These methods range from biopsy of polar bodies to single-cell blastomere and routine two-cell blastomere biopsy—and, more recently, to blastocyst biopsy. **The impact of these various embryo manipulations has yet to be fully considered.** Whether biopsy affects the embryo has received little attention.

In fact, embryos that are of poor quality before biopsy—such as those found in women of advanced maternal age—may be more susceptible to the effects of biopsy. The outcome with such embryos may be of even greater detriment to the implantation rate (as discussed in regard to the Mastenbroek study earlier in this article).

The logic of performing PGS for aneuploidy in women of advanced maternal age was reasonable. But this group of women—in whom ovarian reserve is diminished, who respond poorly to ovulation induction, thereby limiting the total number of embryos for analysis and the poorer quality embryos possibly further impaired by the biopsy itself—represent the population that may be least amenable to PGS.

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prolonged E/M services. Guidelines for these codes mandate **cumulative time** rather than **continuous time,** and using the add-on codes is contingent on the additional time spent being **30 or more minutes above the typical time** allotted for the basic E/M service that you are billing.

Here's a case that exemplifies how coding works in these circumstances:

CASE

You evaluate a patient for severe uterine bleeding, and report a level-4 visit (99214), which has a typical time of 25 minutes. At the same visit, you determine that endometrial biopsy is required, and you perform it during the visit. But the patient faints during the procedure—and you spend an additional 35 minutes (cumulative time) with her before you send her home.

Because the typical time of 25 minutes was exceeded by at least 30 minutes, you should report **99354** (*prolonged physician service in the office or other outpatient setting requiring direct [face-to-face] patient contact beyond the usual service; first hour [list separately in addition to code for office or other outpatient Evaluation and Management service]*) in addition to **99214**.

Guidelines for correct use of these codes are also being revised to emphasize that only **outpatient** prolonged services codes are intended to be used to report **total duration of face-to-face time;** on the other hand, **inpatient** codes are intended to report the total duration of the time spent (whether continuous or noncontinuous) by the physician on the unit **actively involved in caring** for the patient.

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A further observation about PGS in women who have experienced recurrent pregnancy loss or IVF failure: Any impairment of embryos that is a consequence of the method of biopsy may further undermine the generally unsupportive results of PGS that have been documented in these patients.

Consensus on performing PGS

An assessment of European studies and practices reveals similar concerns voiced by the European Society for Human Reproduction and Embryology (ESHRE) PGD Consortium Steering Committee. The committee recently asserted a comparable opinion about "the insufficient data that demonstrate PGS is indeed a cost-effective alternative for standard IVF."[2] Gleicher and colleagues, in their review of the literature, conclude that **the indications for PGS are currently undefined** and, as such, screening should be considered experimental.

Gleicher's sentiments echo the recommendations of ASRM that, when PGS is considered,

- patients undergo counseling about its limitations, risk of error, and lack of evidence that it improves the live-birth rate
- available evidence does not support improvement in the live birth rate in women of advanced maternal age, who have failed previous implantation, who have experienced recurrent pregnancy loss, or who have experienced recurrent pregnancy loss specifically related to aneuploidy
- decisions about management should not be based on aneuploidy results of prior PGS cycles for a woman who has experienced recurrent implantation failure.

References

1. Staessen C, Platteau P, Van Assche E, et al. Comparison of blastocyst transfer with and without preimplantation genetic diagnosis for aneuploidy screening in couples with advanced maternal age: a prospective randomized controlled trial. Hum Reprod. 2004;19:2849–2858.

2. Sermon KD, Michiels A, Harton G, et al. ESHRE PGD Consortium data collection VI: cycles from January to December 2003 with pregnancy follow-up to October 2004. Hum Reprod. 2007;22:323–336.

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A 2008 literature review concludes that:
1) the indications for preimplantation genetic screening are, at this time, undefined and
2) PGS should therefore be considered experimental