

Preimplantation genetic testing: a Practice Committee opinion

The Practice Committee of the Society for Assisted Reproductive Technology and the Practice Committee of the American Society for Reproductive Medicine

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This Practice Committee Opinion reviews the techniques for embryo biopsy and genetic analysis and addresses issues relating to safety, accuracy, and overall efficacy of preimplantation genetic diagnosis (PGD) and preimplantation genetic screening (PGS). (Fertil Steril® 2008;90:S136–43. ©2008 by American Society for Reproductive Medicine.)

In vitro fertilization (IVF) offers unique access to the oocyte and preimplantation embryo. The term “preimplantation genetic testing” describes procedures involving the removal of one or more nuclei from oocytes (polar bodies) or embryos (blastomeres or trophoctoderm cells) to test for mutations in gene sequence or aneuploidy before transfer (1). The term “preimplantation genetic diagnosis” (PGD) applies when one or both genetic parents carry a gene mutation or a balanced chromosomal rearrangement and testing is performed to determine whether that specific mutation or an unbalanced chromosomal complement has been transmitted to the oocyte or embryo. The term “preimplantation genetic screening” (PGS) applies when the genetic parents are known or presumed to be chromosomally normal and their embryos are screened for aneuploidy.

This document reviews the techniques for embryo biopsy and genetic analysis and addresses issues relating to the safety, accuracy and overall efficacy of PGD and PGS. Although the techniques used to collect DNA specimens from oocytes and embryos are similar, PGD and PGS are sufficiently distinct to merit separate discussion.

PREIMPLANTATION GENETIC DIAGNOSIS

Many diseases are the result of specific gene mutations. Tests to determine whether an embryo contains a normal or a mutated gene can be performed after removing one or more of its nucleated cells. Alternatively, when the genetic mother carries a detectable mutation, the first and second polar bodies can be removed from her oocytes for analysis. PGD can be used to detect the presence of specific autosomal recessive, autosomal dominant, and X-linked disorders as well as unbalanced chromosomal translocations in efforts to reduce or eliminate the otherwise expected 25–50% risk for having an affected child (Table 1). Because the genetic material that can be removed from an oocyte

or embryo is limited, PGD necessitates prior knowledge of the genetic mutation carried by the genetic parent(s) and searches for that specific mutation in each oocyte or embryo. Although PGD requires that couples who may not be infertile undergo IVF, some couples at risk nonetheless prefer to pursue IVF with PGD to reduce the risk for conceiving an affected child.

Indications

PGD is indicated for couples at risk for transmitting a specific genetic disease or abnormality to their offspring. For carriers of autosomal dominant disorders, the risk that any given embryo may be affected is 50%, and for carriers of autosomal recessive disorders, the risk is 25%. For female carriers of X-linked disorders, the risk of having an affected embryo is 25% (half of male embryos) (Table 1). PGD also can be performed and may be elected by patients who carry mutations such as BRCA-1 that do not cause a specific disease but are thought to confer significantly increased risk for a disease. In some cases, there may be more than one indication for PGD, such as when human leukocyte antigen (HLA) matching is performed in conjunction with testing for a specific genetic mutation (Table 2).

For individuals who carry a balanced chromosomal translocation, inversion, or other structural chromosomal rearrangement, there is increased risk that their gametes will have an unbalanced genetic composition due to excess or missing genetic material. An embryo derived from the union of such an unbalanced gamete with a partner’s normal gamete also will have an unbalanced genetic composition and may be identified using telomeric probes specific for the loci of interest that must be selected for individual patients, according to their unique abnormality.

Counseling

Due to the risk for conceiving a child with a genetic disease or other abnormality, counseling for couples considering PGD is required and, at a minimum, must include information relating to the following key points:

1. The risks associated with IVF procedures.

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TABLE 1

Percentage of embryos that will be affected, normal, and carriers of single gene mutations having different patterns of inheritance*.

Mutation	Affected	Normal	Carrier	Example
Autosomal dominant	50%	50%	—	Marfan syndrome
Autosomal recessive	25%	25%	50%	Cystic fibrosis
X-linked (Female carrier)	25% (Male)	50%	25% (Female)	Hemophilia A

* For each type of mutation, the sum of normal and carrier embryos equals the total percentage of all embryos potentially available for transfer.

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- The option of choosing not to proceed with IVF and PGD.
- The risks associated with embryo biopsy and extended culture.
- For carriers of autosomal and X-linked disorders, the relevant patterns of inheritance and the impact of the disorder on the quality of life for an affected child.
- For carriers of balanced chromosomal translocations or other structural chromosomal abnormalities, a review of the possible patterns of segregation during meiosis and the increased risk for conceiving offspring having an unbalanced chromosomal composition.
- The technical limitations and pitfalls of PGD, including the risk for misdiagnosis, (the actual probability, when known) and the need for subsequent prenatal diagnostic testing via chorionic villus sampling (CVS) or amniocentesis to confirm the results obtained with PGD.
- Options relating to prenatal diagnostic testing and their associated risks:
 - Chorionic villus sampling.
 - Amniocentesis.
 - Ultrasonography with or without additional blood tests.
 - No prenatal testing.

- The possibilities that no embryos may be transferred if all are affected and that unaffected embryos which carry the recessive or X-linked disorder may be transferred.
- The disposition of embryos for which testing yields no conclusive result.
- The disposition of embryos not transferred (e.g., discard, cryopreservation, research, or donation) as and when appropriate.
- Alternative methods for avoiding risk of disease (e.g., use of donor gametes).

Biopsy of Cells for Analysis

To remove material from an oocyte or embryo, an opening in the zona pellucida is created using a laser, acid Tyrode's solution, or a sharpened glass needle. The polar body or blastomere(s) then may be extracted using a small suction pipette or by gently compressing the oocyte or embryo to extrude material through the opening. For maternally inherited mutations, genetic analysis can be performed on the oocyte by removing the first and, sometimes, the second polar body and by inferring the genetic composition of the oocyte from the result (2, 3). Genetic analysis usually is performed on one or two nucleated blastomeres removed three days after fertilization. The decision to remove one or two blastomeres

TABLE 2

Percentage of embryos that will be a human leukocyte antigen (HLA) match to an existing child of the same parents.

Match	Non-Match	Example
25%	75%	Bone marrow transplant for child with leukemia

Percentage of embryos that will be a human leukocyte antigen (HLA) match to an existing child of the same parents and unaffected by an autosomal recessive disorder

HLA match	Unaffected	HLA match and unaffected	Example
25%	75%	19%	Bone marrow transplant for child with Fanconi's Anemia

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TABLE 3**Applications and methods for preimplantation genetic testing.****Polymerase chain reaction (PCR)**

Autosomal single gene mutations
 X-linked single gene mutations
 Gender selection (X-linked mutations)^a
 HLA matching

Fluorescence in situ hybridization (FISH)

Aneuploidy screening
 Structural chromosomal abnormalities
 Gender selection (X-linked mutations)^a

^a Preferably by FISH because PCR has risk for misdiagnosis due to contamination.

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depends on the quality of the embryo and the disease of interest. The embryo then may remain in culture up to the blastocyst stage or be cryopreserved until such time as the results of the genetic analysis can be completed. Alternatively, mural trophectoderm cells can be removed from the embryo for analysis at the blastocyst stage.

Methods of Genetic Analysis

To identify specific gene mutations, PGD employs techniques involving the polymerase chain reaction (PCR) to amplify a segment of the genome that contains the specific gene of interest (Table 3). Many of the relevant genetic disorders involve single-gene defects and mutations in a single base pair. PCR provides the means to obtain sufficient genetic material for evaluation using mutation-specific primers, digestion with restriction enzymes, or heteroduplex analysis. Heteroduplex analysis is a technique in which amplified DNA fragments from both test and known normal samples are hybridized and subsequently subjected to double-stranded DNA gel electrophoresis; a mismatch due to a genetic deletion or substitution involving even one base pair will result in differential migration on the gel thus permitting diagnosis.

Fluorescence in situ hybridization (FISH) is an analytic technique that employs DNA probes labeled with distinctly colored fluorochromes that bind to specific DNA sequences unique to each chromosome. FISH is used to detect missing or excess chromosomal material in oocytes (when the female partner is the carrier) or embryos (when either partner is the carrier) of individuals known to harbor a balanced chromosomal translocation or other structural rearrangement (Table 3). After removal, the cells are fixed on a glass slide, the cytoplasm is dispersed, and fluorescent DNA probes are allowed to hybridize to the chromosomes. The number of fluorescent signals of a particular color reflects the number of copies of each of the chromosomal segments of interest.

Technical Challenges

The major challenges of PGD relate primarily to the relatively short interval of time available for analysis and the fact that only one or two cells can be analyzed (compared

with the hundreds of cells obtained via amniocentesis or CVS). When PCR-based methods are employed, misdiagnoses may result from use of an anucleate cell, from failure of amplification of the targeted DNA segment, or from external contamination. Errors can result when one allele for the gene of interest fails to amplify ("allele drop-out") or amplifies poorly ("partial amplification"). Both types of technical failure have been described and have resulted in misdiagnoses after single-cell PCR, leading to transfer of an affected embryo believed to be normal or to discard of a normal embryo thought to be affected. The risk for misdiagnosis due to allele drop-out or partial amplification relates directly to the type of genetic disorder for which testing is performed (4, 5, 6). The estimated risk of transferring an affected embryo mistakenly identified as normal by PGD is approximately 2% for recessive disorders and 11% for dominant disorders (7). Such errors can be reduced significantly if linked markers also are analyzed. The true risk for obtaining a clinically relevant false negative result can be determined only by further genetic testing of all conceptions after PGD, but no such studies have been conducted. Laboratories must take specific precautions to avoid other errors that may result from inadvertent amplification of contaminating extraneous DNA.

Results

For couples known to be at risk for having children with a heritable and debilitating genetic disease, IVF with PGD represents a major scientific advance. For couples who harbor a balanced chromosomal translocation, PGD using telomeric probes to identify and exclude embryos having an unbalanced genetic complement appears to decrease significantly the risk of spontaneous abortion (to <20%) and to increase significantly the likelihood of achieving a live birth (8, 9). Generally, because the birth of a healthy child validates the efficacy of PGD, randomized controlled trials are not necessary. However, the possibility that other abnormalities presenting later as a consequence of the procedure cannot be excluded. Patients considering IVF with PGD also should be informed that pregnancy rates may be lower than those achieved with IVF in age-matched patients when PGD is not performed. Pregnancy rates after PGD may be reduced because genetic testing will decrease the number of embryos

suitable for transfer. Abnormal or affected embryos will be specifically excluded. Others may yield inconclusive results, fail to develop to the blastocyst stage, or have decreased viability due to the biopsy procedure itself.

PREIMPLANTATION GENETIC SCREENING (PGS)

Aneuploidy is the most common cause for early pregnancy failure. The prevalence of oocyte and embryo aneuploidy increases with maternal age and also may be increased in chromosomally normal couples with recurrent early pregnancy loss or repeated failed IVF cycles despite the transfer of high-quality embryos (based on morphology). PGS has been advocated for use in conjunction with IVF in efforts to identify and transfer only euploid embryos and thereby to improve the likelihood for a successful pregnancy.

Proposed Indications

There are no specific indications for PGS since, by definition, it is performed in patients having no known chromosomal anomaly, mutation or other genetic abnormality. PGS has been proposed for patients at risk for having an increased prevalence of aneuploid embryos, including women of advanced maternal age and those with a history of recurrent early pregnancy loss, repeated IVF failure, or severe male factor infertility.

Counseling

Because patients considering IVF with PGS have no specific identifiable genetic abnormality and PGS is intended to detect aneuploidies which, in most cases, will result in preclinical loss, counseling is challenging but nonetheless extremely important. At a minimum, counseling before PGS must include the following key points:

1. The risks associated with IVF.
2. The option of choosing not to proceed with IVF and PGS.
3. The risks associated with embryo biopsy and extended culture.
4. The possibility of a false positive result that may lead to the discard of a normal embryo.
5. The possibility of a false negative result that may lead to the transfer of an abnormal embryo.
6. The possibility that testing may yield inconclusive results.
7. The possibility that no embryos may be transferred if all appear abnormal.
8. Options relating to prenatal diagnostic testing and their associated risks:
 - a. Chorionic villus sampling.
 - b. Amniocentesis.
 - c. Ultrasonography with or without additional blood tests.
 - d. No prenatal testing.

9. The nature and quality of the available evidence with regard to live-birth rates after IVF with PGS.
10. The disposition of embryos not transferred (e.g., discard, cryopreservation, research, or donation) as and when appropriate.

Genetic Analysis for PGS

Aneuploidy screening can be performed on the polar body removed from an oocyte or on one or more cells removed from an embryo. The nuclei of polar bodies or isolated blastomeres usually are analyzed using FISH to identify the copy number of selected chromosomes. Only one or two blastomeres can be removed from an embryo without adversely affecting its developmental potential and the number of chromosome pairs from each nucleus that can be evaluated by FISH is limited (usually 9-11 at present). The specific chromosomes evaluated are chosen according to the patient's prior history and include those involved in the most common aneuploidies identified in spontaneous miscarriages. Alternatively, the entire genome (all 23 chromosome pairs) can be amplified using random primers for analysis by comparative genomic hybridization (CGH) (10). CGH is a technique in which test and reference samples are amplified simultaneously using red (test sample) and green (reference sample) fluorochromes and products are allowed to hybridize with a normal male metaphase chromosome spread for two to three days. Image-processing software is used to analyze the relative amounts of red and green signal to determine the chromosome numbers and identify any structural chromosomal abnormalities. Newer methods of whole-genome analysis using multiple displacement amplification (MDA), gene chips (11) or pyrosequencing (12) may offer greater accuracy and more rapid through-put in the future.

Technical limitations

Accurate screening of a single cell for a number of potential abnormalities requires a high degree of technical proficiency. Currently, FISH technology permits evaluation of fewer than half of the 23 chromosome pairs in a single cell. Studies comparing results obtained with FISH and CGH have revealed that up to 25% of aneuploid embryos are judged normal by FISH because the abnormal chromosome pair(s) were not among those included in the analysis (13). Unfortunately, CGH analysis currently cannot be completed in the short interval between biopsy and transfer, and therefore requires embryo cryopreservation.

Approximately 10% of cells removed for screening yield no results or results not confirmed by analysis of the remaining cells in embryos not transferred. The likelihood of obtaining no result or an incorrect result with FISH depends on the number of blastomeres and the number of chromosomes that are analyzed (14, 15, 16). No result may be obtained if the labeled probes fail to hybridize with the denatured chromosomes. Erroneous results may be obtained when the

orientation or overlapping of chromosomes yields split or dif-fused signals that are misinterpreted.

Aneuploidy may arise in several ways. When non-disjunction occurs during meiosis, all of the cells in the embryo typ-ically are aneuploid. In contrast, mitotic non-disjunction yields two or more distinct cell lines and results in an embryo that contains both normal and abnormal cells; the actual pro-portions of normal and abnormal cells will vary, depending on the point at which the abnormal segregation occurred. A mosaic embryo can be identified only if two or more cells are removed and analyzed. Even then, the true proportions of normal and abnormal cells cannot be determined unless all of the cells are analyzed, thus destroying the embryo. One study, in which the two cells removed from each embryo yielded discordant results (one normal and one abnormal), found that half of the embryos were euploid after all cells were analyzed (17). The observation suggests that, in some cases, embryo biopsy actually may “correct” an abnormality. However, because the actual proportion of euploid cells re-quired for normal development is unknown, mosaic embryos must be considered potentially abnormal and therefore be re-analyzed at the blastocyst stage or discarded. Evidence sug-gests that up to half of all embryos identified as aneuploid at the cleavage stage and that survive to the blastocyst stage will “self correct” (18, 19, 20). Theories proposed to explain the phenomenon include embryonic mosaicism in which ab-normal cell lines fail to proliferate, self-correction of the ab-normal cell line, and a false positive or incorrect result from the earlier analysis (19, 20). In summary, an abnormal result from FISH analysis of a single blastomere removed from a day 3 embryo does not necessarily indicate that the embryo is abnormal and ill-fated.

PGS Results

Advanced maternal age The majority of spontaneous mis-carriages result from aneuploidy. The risk for miscarriage and the prevalence of aneuploidy increase with maternal age in both naturally conceived pregnancies and those result-ing from IVF (21, 22). In women of advanced reproductive age, the majority of day 3 embryos analyzed by FISH (up to 10 chromosomes) are aneuploid (23, 24). The prevalence of aneuploidy among embryos increases with the number of chromosome pairs that are examined. In theory, PGS should increase the likelihood that embryos selected for transfer will be euploid and thus result in improved implantation, pregnancy, and live birth rates. However, the results achieved with PGS for advanced maternal age have been mixed.

One prospective study observed a significantly higher im-plantation rate (26%) in a group of 73 women who had PGS, compared with that in a group of 84 “controls” (14%) who chose not to have PGS but did undergo assisted hatching (25). In a trial involving 400 women age 37 years or older who were randomized to receive PGS or blastocyst transfer without PGS (controls) after ICSI, 148/200 women in the PGS group (74%) and 141/200 (71%) of controls went to

oocyte retrieval (26). In the PGS group, 130/148 (88%) had embryos suitable for biopsy; one blastomere was removed from embryos having five cells and two were removed from those containing six or more cells on day 3 after fertil-ization. Among 685 embryo biopsies analyzed by FISH (7 chromosomes; X, Y, 13, 16, 18, 21, 22), 653 (95%) yielded a result. Only 240/653 embryos (37%) were normally dip-loid; 353/653 (54%) exhibited a variety of abnormalities, and the remaining 60 embryos (9%) contained one normal and one abnormal cell. In the PGS group, 81/148 (55% of re-trievals) received an embryo transfer, compared with 121/141 (86% of retrievals) in the control group; up to three blasto-cysts were transferred in women ages 37-39 years and up to six blastocysts were transferred in women age 40 and older. In the control group, 15/141 women (11%) had no blas-tocysts available for transfer. In the PGS group, 11/130 women with analyzed embryos (8%) had no morula or blas-tocysts derived from genetically normal embryos and 38/130 (29%) had no genetically normal embryos. Among 67/240 normal embryos not developmentally or morphologically suitable for transfer or cryopreservation (28%), reanalysis by FISH was successful in 43/67 (64%) and all contained a majority of normal cells (0% false negative). Among 285/413 abnormal embryos reanalyzed successfully (69%), 211/285 (74%) were uniformly abnormal, 50/285 (18%) con-tained both abnormal and normal cells, and 24/285 (8%) were normal (false positive). Among the 49/130 women in the PGS group having only genetically abnormal embryos (38/49; 78%) or no morula or blastocyst derived from normal embryos (11/49; 22%), reanalysis identified normal embryos in 2/38 (5.3%) and 1/11 (9.1%), respectively. Thus, in 3/49 cycles (6.1%), no transfer was performed due to a false positive result. In the PGS group, the implantation rate (fetal hearts/embryos transferred) was 17% (28/164), compared with 11% (39/338) among controls (Not Significant [NS]). In the PGS group, 27/164 transferred embryos (16.5%) resulted in an ongoing implantation, compared to 39/338 (10%) among controls (NS). There were 22 ongoing preg-nancies in the PGS group (15% per retrieval, 27% per trans-fer), compared with 29 among controls (21% per retrieval, 24% per transfer) (NS). Overall, the pregnancy loss rates (including preclinical and clinical abortions) in the PGS (7/29, 24%) and control (10/39, 26%) groups also were not different.

Another double-blind controlled trial randomized 408 women ages 35-41 having no previous IVF failures to undergo up to three cycles of IVF with or without PGS (27). Neither the patients nor the physicians were aware of group assign-ment or the number or quality of embryos transferred on day 4 after fertilization. A maximum of two embryos was trans-ferred, any extra embryos of sufficient quality were cryopre-served, and if pregnancy did not result, thawed embryos were transferred before a new cycle was initiated. In the PGS group, a single blastomere was removed from all embryos containing at least four cells for analysis by FISH (8 chromo-somes; X, Y, 1, 13, 16, 17, 18, 21). If no chromosomally

normal embryos with adequate morphology were available for transfer to women assigned to PGS, embryos of undetermined chromosomal composition (failed biopsy, absent or incomplete nucleus after fixation, failed FISH) were transferred. In the control group, the selection of embryos for transfer was based solely on morphologic features. Overall, the study compared outcomes observed in 434 cycles involving PGS with those in 402 cycles that did not. In the PGS group, the implantation rate (gestational sacs at 7 weeks/embryos transferred) was 12% (75/642), compared with 15% (99/673) among controls (NS). In the PGS group, the implantation rate was 17% (53/316) for cycles in which two normal embryos (by FISH) were transferred, compared with 6% (6/100) for cycles in which undetermined embryos were transferred. Overall, the cumulative ongoing pregnancy and live birth rates for the group of women assigned to PGS (25% and 24%, respectively) were significantly lower than those observed among controls (37% and 35%, respectively).

Thus, available evidence does not support the use of PGS (aneuploidy screening) to increase live birth rates in women of advanced maternal age.

Recurrent pregnancy loss Miscarriage is very common and the large majority of concepti that abort spontaneously are aneuploid (21, 28). Recurrent pregnancy loss (RPL) classically is defined as three or more pregnancy losses under 500 g (before 20 weeks' gestation). When thorough evaluation reveals no specific cause or predisposing factor, the probability for a subsequent successful pregnancy is approximately 70%, depending on the number of previous miscarriages and the karyotype of prior abortuses (29, 30, 31). Because women with a history of RPL are more likely to have a chromosomally abnormal abortus, PGS for aneuploidy has been proposed for decreasing the risk for conceiving another aneuploid pregnancy. Indeed, PGS of embryos from women with RPL has demonstrated a high prevalence of aneuploidy, independent of the age of the female partner (32, 33). At least in theory, patients with RPL who likely would not benefit from PGS are those with a history of chromosomally normal miscarriage(s). Subsequent losses in such patients are more often again chromosomally normal and the prognosis for live birth is generally poor even with IVF and PGS (29).

There have been no randomized controlled trials to evaluate the clinical utility and efficacy of PGS in patients with RPL. In one prospective study that compared outcomes in a group of 71 patients with RPL with those in a group of 28 others having PGD performed for X-linked disease (controls), ongoing pregnancy rates and miscarriage rates in the two groups were similar (32). The ongoing/delivered pregnancy rate was 26% per cycle and 33% per transfer in the RPL group, compared with 29% per cycle and 32% per transfer among controls. In the RPL group, three miscarriages (12%) occurred and 71% of embryos were aneuploid, compared with 45% among controls. Among the embryos of women in the RPL group, euploid embryos were significantly

more likely to develop to the blastocyst stage (62%) than those that were aneuploid (25%), an observation suggesting that extended culture itself might help in selection of euploid embryos for transfer. In another prospective study (34) that compared outcomes in both younger (age less than 37; 35 cycles) and older (age 37 and older; 34 cycles) women with a history of RPL, a high proportion of embryos was aneuploid in both younger (44%) and older patients (67%). The ongoing pregnancy rates/cycle in both the younger women (26%, mean age 32.5 ± 2.7 years) and the older patients (2.9%, mean age 40.2 ± 2.5 years) did not differ significantly from those achieved in the general IVF population. Approximately 20% of patients with RPL in both studies did not have an embryo transfer because they had no normal embryos. The control group in the latter study does not allow a valid assessment of the benefit of PGS for RPL.

Although evidence suggests that PGS does not improve live-birth rates in patients with RPL, some have argued that PGS has prognostic value in such patients. Because the prevalence of aneuploid embryos generally is consistent across cycles (35, 32), advocates have suggested that patients having few or no normal embryos might be best advised to pursue oocyte donation. However, because the prevalence of aneuploidy in the embryos of women with RPL is already increased, the threshold level that would justify such a recommendation is difficult to determine.

Overall, available evidence currently does not support the use of PGS for patients with RPL because it does not improve ongoing pregnancy or live-birth rates and does not decrease miscarriage rates in such women. However, couples in whom RPL can be attributed to a balanced translocation may indeed benefit from specific genetic testing (PGD, discussed earlier) to detect excess or missing genetic material in their embryos.

Repeated implantation failure Repeated implantation failure has been defined by the number of failed IVF attempts (usually three or more) or by the failure of implantation after a specific total number of embryos has been transferred. There have been no randomized controlled trials to evaluate critically the clinical utility of PGS in women with repeated implantation failure. One study observed a 34% pregnancy rate after IVF with PGS in a group of patients (mean age 36.3 ± 2.5 yr) with an average of 4.2 previous failed IVF cycles, compared with a 33% pregnancy rate in a group of women who had IVF with PGS for X-linked diseases (mean age mean age of 31.6 ± 2.5 yr) (36). Approximately half of the embryos from patients with recurrent implantation failure were chromosomally abnormal. A second study observed no differences in the implantation or pregnancy rates in patients with three or more previous failed IVF cycles who self-selected either PGS or assisted hatching with day 5 transfer (25); across the group, 54% of embryos analyzed by PGS were aneuploid. A third study observed a 14% implantation rate in 54 cycles in patients age 35 or older with recurrent implantation failure (two or more failed IVF cycles),

compared to 12% in a group of controls matched retrospectively (NS) (37).

Overall, at present, available evidence does not support the use of PGS for patients with repeated implantation failure.

Male factor infertility An abnormal karyotype may be expected in approximately 10-15% of azoospermic men, in 5% of oligospermic men, and in less than 1% of men with normal semen quality (38). Up to another 15% of infertile men with azoospermia or severe oligospermia harbor a microdeletion in the Y chromosome too small to detect in a standard karyotype (38). Ejaculated or testicular sperm obtained from such men typically exhibit a high prevalence of chromosomal abnormalities which may predispose to an increased risk for embryo aneuploidy (39). With the exception of screening for mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene and PGD for couples in which the male partner has congenital bilateral absence of the vas deferens (CBVAD), no studies have been performed to evaluate the clinical utility of PGS for couples with male factor infertility. PGD also may be indicated for specific genetic anomalies that cause male factor infertility, such as Klinefelter syndrome or autosomal rearrangements or Y-microdeletions associated with azoospermia or oligospermia.

Available evidence does not support the use of PGS for couples receiving IVF/ICSI for male factor indications at this time.

RECOMMENDATIONS: PGD

- Before PGD is performed, genetic counseling must be provided to ensure that patients fully understand the risk for having an affected child, the impact of the disease on an affected child, and the limitations of available options that may help to avoid the birth of an affected child.
- PGD can reduce the risk for conceiving a child with a genetic abnormality carried by one or both parents if that abnormality can be identified with tests performed on a single cell.
- Prenatal diagnostic testing to confirm the results of PGD is encouraged strongly because the methods used for PGD have technical limitations that include the possibility for a false negative result.

RECOMMENDATIONS: PGS

- Before PGS is performed, thorough education and counseling must be provided to ensure that patients fully understand the limitations of the technique, the risk of error, and the lack of evidence that PGS improves live-birth rates.
- Available evidence does not support the use of PGS as currently performed to improve live-birth rates in patients with advanced maternal age.
- Available evidence does not support the use of PGS as currently performed to improve live-birth rates in patients with previous implantation failure.

- Because the prevalence of aneuploidy is high in the embryos of patients with recurrent implantation failure, decisions concerning future treatment should not be based on the results of PGS in one or more cycles.
- Available evidence does not support the use of PGS as currently performed to improve live-birth rates in patients with recurrent pregnancy loss.
- Available evidence does not support the use of PGS as currently performed to reduce miscarriage rates in patients with recurrent pregnancy loss related to aneuploidy.

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REFERENCES

1. Handyside A, Kontogianni EH, Hardy K, Winston RM. Pregnancies from biopsied human preimplantation embryos sexed by Y-specific DNA amplification. *Nature* 1990;344:768-70.
2. Verlinsky Y, Rechitsky S, Verlinsky O, Ivakhnenko V, Lifchez A, Kaplan B, et al. Prepregnancy testing for single-gene disorders by polar body analysis. *Genet Test* 1999;3:185-90.
3. Verlinsky Y, Cieslak J, Ivakhnenko V, Evsikov S, Wolf G, White M, et al. Prepregnancy genetic testing for age-related aneuploidies by polar body analysis. *Genet Test* 1997-1998;1:231-235.
4. Navidi W, Arnheim N. Using PCR in preimplantation genetic disease diagnosis. *Hum Reprod* 1991;6:836-49.
5. Rechitsky S, Verlinsky O, Amet T, Rechitsky M, Kouliev T, Strom C. Reliability of preimplantation diagnosis for single gene disorders. *Mol Cell Endocrinol* 2001;183(Suppl 1):S65-8.
6. Hussey ND, Davis T, Hall JR, Barry MF, Draper R, Norman RJ. Preimplantation genetic diagnosis for beta-thalassaemia using sequencing of single cell PCR products to detect mutations and polymorphic loci. *Mol Hum Reprod* 2002;8:1136-43.
7. Lewis CM, Pinel T, Whittaker JC, Handyside AH. Controlling misdiagnosis errors in preimplantation genetic diagnosis: a comprehensive model encompassing extrinsic and intrinsic sources of error. *Hum Reprod* 2001;16:43-50.
8. Munne S, Sandalinas M, Escudero T, Fung J, Gianaroli L, Cohen J. Outcome of preimplantation genetic diagnosis of translocations. *Fertil Steril* 2000;73:1209-18.
9. Verlinsky Y, Tur-Kaspa I, Cieslak J, Bernal A, Morris R, Taranissi M, et al. Preimplantation testing for chromosomal disorders improves reproductive outcome of poor-prognosis patients. *Reprod Biomed Online* 2005;11:219-25.
10. Wilton L, Williamson R, McBain J, Edgar D, Voullaire L. Birth of a healthy infant after preimplantation confirmation of euploidy by comparative genomic hybridization. *N Engl J Med* 2001;345:1537-41.
11. Sohni YR, Burke JP, Dyck PJ, O'Kane DJ. Microfluidic chip-based method for genotyping microsatellites, VNTRs and insertion/deletion polymorphisms. *Clin Biochem* 2003;36:35-40.
12. Okada Y, Nakamura K, Wada M, Nakamura T, Tsukamoto N, Nojima Y, et al. Genotyping of thiopurine methyltransferase using pyrosequencing. *Biol Pharm Bull* 2005;28:677-81.
13. Wilton L, Voullaire L, Sargeant P, Williamson R, McBain J. Preimplantation aneuploidy screening using comparative genomic hybridization or

- fluorescence in situ hybridization of embryos from patients with recurrent implantation failure. *Fertil Steril* 2003;80:860–8.
14. Munne S, Magli C, Bahce M, Fung J, Legator M, Morrison L, et al. Preimplantation diagnosis of the aneuploidies most commonly found in spontaneous abortions and live births: XY, 13, 14, 15, 16, 18, 21, 22. *Prenat Diagn* 1998;18:1459–66.
 15. Munne S, Magli C, Cohen J, Morton P, Sadowy S, Gianaroli L, et al. Positive outcome after preimplantation diagnosis of aneuploidy in human embryos. *Hum Reprod* 1999;14:2191–9.
 16. Michiels A, Van Assche E, Liebaers I, Van Steirteghem A, Staessen C. The analysis of one or two blastomeres for PGD using fluorescence in situ hybridization. *Hum Reprod* 2006;21:2396–402.
 17. Baart EB, Martini E, van den Berg I, Macklon NS, Galjaard RJ, Fauser BC, et al. Preimplantation genetic screening reveals a high incidence of aneuploidy and mosaicism in embryos from young women undergoing IVF. *Hum Reprod* 2006;21:223–33.
 18. Magli MC, Jones GM, Gras L, Gianaroli L, Korman I, Trounson AO. Chromosome mosaicism in day 3 aneuploid embryos that develop to morphologically normal blastocysts in vitro. *Hum Reprod* 2000;15:1781–6.
 19. Li M, DeUgarte C, Surrey M, Danzer H, DeCherney A, Hill D. Fluorescence in situ hybridization reanalysis of day-6 human blastocysts diagnosed with aneuploidy on day 3. *Fertil Steril* 2005;84:1395–400.
 20. Munne S, Velilla E, Colls P, Garcia Bermudez M, Vemuri MC, Steuerwald N, et al. Self-correction of chromosomally abnormal embryos in culture and implications for stem cell production. *Fertil Steril* 2005;84:1328–34.
 21. Simpson JL. Genes, chromosomes and reproductive failure. *Fertil Steril* 1980;33:107–16.
 22. Warburton D, Stein Z, Kline J, Susser M. Chromosome abnormalities in spontaneous abortions: data from the New York City study. In: Porter LH, Hook EB, eds. *Human embryonic and fetal death*. New York: Academic Press, 1980:261–7.
 23. Platteau P, Staessen C, Michiels A, Van Steirteghem A, Liebaers I, Devroey P. Preimplantation genetic diagnosis for aneuploidy screening in women older than 37 years. *Fertil Steril* 2005;84:319–24.
 24. Munne S, Alikani M, Tomkin G, Grifo J, Cohen J. Embryo morphology, developmental rates, and maternal age are correlated with chromosome abnormalities. *Fertil Steril* 1995;64:382–91.
 25. Gianaroli L, Magli MC, Ferraretti AP. Preimplantation diagnosis for aneuploidies in patients undergoing in vitro fertilization with a poor prognosis: identification of the categories for which it should be proposed. *Fertil Steril* 1999;72:837–44.
 26. Staessen C, Platteau P, Van Assche E, Michiels A, Tournaye H, Camus M, et al. Comparison of blastocyst transfer with or without preimplantation genetic diagnosis for aneuploidy screening in couples with advanced maternal age: a prospective randomized controlled trial. *Hum Reprod* 2004;19:2849–58.
 27. Mastenbroek S, Twisk M, Echten-Arends J, Sikkema-Raddatz B, Korevaar JC, Verhoeve HR, et al. In vitro fertilization with preimplantation genetic screening. *N Engl J Med* 2007;357:9–17.
 28. Eiben B, Bartels I, Bahr-Porsch S, Borgmann S, Gatz G, Gellert G, et al. Cytogenetic analysis of 750 spontaneous abortions with the direct-preparation method of chorionic villi and its implications for studying genetic causes of pregnancy wastage. *Am J Hum Genet* 1990;47:656–63.
 29. Ogasawara M, Aoki K, Okada S, Suzumori K. Embryonic karyotype of abortuses in relation to the number of previous miscarriages. *Fertil Steril* 2000;73:300–4.
 30. Balasch J, Creus M, Fabregues F, Civico S, Carmona F, Martorell J, et al. In-vitro fertilization treatment for unexplained recurrent abortion: a pilot study. *Hum Reprod* 1996;11:1579–82.
 31. Clifford K, Rai R, Regan L. Future pregnancy outcome in unexplained recurrent first trimester miscarriage. *Hum Reprod* 1997;12:387–9.
 32. Rubio C, Simon C, Vidal F, Rodrigo L, Pehlivan T, Remohi J, et al. Chromosomal abnormalities and embryo development in recurrent miscarriage couples. *Hum Reprod* 2003;18:182–8.
 33. Kahraman S, Benkhalifa M, Donmez E, Biricik A, Sertyel S, Findikli N, et al. The results of aneuploidy screening in 276 couples undergoing assisted reproductive techniques. *Prenat Diagn* 2004;24:307–11.
 34. Platteau P, Staessen C, Michiels A, Van Steirteghem A, Liebaers I, Devroey P. Preimplantation genetic diagnosis for aneuploidy screening in patients with unexplained recurrent miscarriages. *Fertil Steril* 2005;83:393–7.
 35. Ferraretti AP, Magli MC, Kopcow L, Gianaroli L. Prognostic role of preimplantation genetic diagnosis for aneuploidy in assisted reproductive technology outcome. *Hum Reprod* 2004;19:694–9.
 36. Pehlivan T, Rubio C, Rodrigo L, Romero J, Remohi J, Simon C, Pellicer A. Impact of preimplantation genetic diagnosis on IVF outcome in implantation failure patients. *Reprod Biomed Online* 2003;6:232–7.
 37. Munne S, Sandalinas M, Escudero T, Velilla E, Walmsley R, Sadowy S, et al. Improved implantation after preimplantation genetic diagnosis of aneuploidy. *Reprod Biomed Online* 2003;7:91–7.
 38. Male Infertility Best Practice Policy Committee of the American Urological Association; Practice Committee of the American Society for Reproductive Medicine. Report on optimal evaluation of the infertile male. *Fertil Steril* 2004;82(Suppl 1):S123–30.
 39. Calogero AE, De Palma A, Grazioso C, Barone N, Romeo R, Rappazzo G, et al. Aneuploidy rate in spermatozoa of selected men with abnormal semen parameters. *Hum Reprod* 2001;16:1172–9.