# Particular Qualities of Diagnostic Materials and Tests Used in Preimplantation Genetic Diagnosis: Scientific Letter

## Preimplantasyon Genetik Tanıda Kullanılan Tanı Materyali ve Testlerin Önemli Özellikleri

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Geliş Tarihi/*Received:* 09.09.2008
Kabul Tarihi/*Accepted:* 26.11.2008

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**ABSTRACT** Preimplantation genetic diagnosis (PGD) is a technique used to identify genetic defects in diagnostic materials created through in vitro fertilization (IVF) prior to transfer of embryos to the uterus. This enables couples to significantly improve their chances of having a healthy child. Since the early 1990s, PGD has been expanding in scope and applications. PGD was first accomplished by Alan Handyside in 1990 to diagnose sex-linked genetic disorders. Also, PGD provides an alternative to current postconception diagnostic procedures, ie, amniocentesis, chorionic villus sampling, and chord blood. These procedures are usually followed by pregnancy termination if results are unfavorable. There are two main stages in PGD application. The first main step contains the collection of diagnostic material for diagnosis. This is generally carried out in a clinical IVF laboratory under sterile conditions. Different diagnostic materials such as first polar body from metaphase II (MII) oocyte and second polar body from zygote, blastomere from cleavage-stage embryo and trophoblast cells from blastocyst have been used in PGD applications. The second main stage involves diagnostic tests in this technique. PGD has largely focused on two methods: fluorescent in situ hybridization (FISH) and polymerase chain reaction (PCR) based techniques [comparative genomic hybridization (CGH), different PCR types]. Decision on the diagnostic materials and tests used in PGD is very important to determine genetic status of the embryo correctly. In this review, advantages, disadvantages and particular characteristic qualities of the diagnostic materials and tests were discussed.

**Key Words:** Preimplantation diagnosis; blastocyst; polymerase chain reaction; in situ hybridization, fluorescence

**ÖZET** Preimplantasyon genetik tanı (PGD), in vitro fertilizasyon (IVF) yöntemi ile elde edilen tanı materyallerindeki genetik bozuklukların embriyonun uterusa transferi öncesinde belirlendiği bir tekniktir. Bu, çiftlerin sağlıklı bir çocuk sahibi olma şanslarını önemli derecede artırmaktadır. 1990'lı yılların başlarından bu yana, PGD'nin faaliyet ve uygulama alanları genişlemektedir. PGD, ilk defa 1990 yılında Alan Handyside tarafından cinsiyetle ilişkili genetik bozuklukların tanımlanmasında başarıyla kullanılmıştır. Ayrıca, PGD gebelikte uygulanan amniyosentez, koryon villus biyopsisi ve kordon kanı incelemesi gibi tanı yöntemlerine güncel bir alternatif sağlamaktadır. Genellikle, bu uygulamalar, sonuçların olumsuz olması durumunda gebeliğin sonlandırılması ile sonuçlanmaktadır. PGD uygulamasında iki ana aşama vardır. İlk ana aşama, tanı için materyal eldesini içerir. Bu, genellikle steril koşullar altında klinik in vitro fertilizasyon laboratuvarında gerçekleştirilir. PGD uygulamalarında, metafaz II (MII) oositten birinci polar cisimcik, zigottan ikinci polar cisimcik, bölünme aşamasındaki embriyoda blastomer ve blastosistten trofoblast hücreleri gibi farklı tanı materyalleri kullanılmaktadır. Bu teknikte ikinci ana aşama tanı testlerini içerir. PGD, çoğunlukla iki metoda odaklanmaktadır: Floresan in situ hibridizasyon (FISH) ve polimeraz zincir reaksiyonu (PZR) temelli teknikler [karşılaştırmalı genomik hibridizasyon (KGH), farklı PZR tipleri]. Embriyonun genetik durumunu doğru bir şekilde belirlemek için, uygun tanı materyali ve testinin seçilmesi çok önemlidir. Bu derlemede, tanı materyalleri ve testlerinin avantajları, dezavantajları ve belirli karakteristik özellikleri tartışılmıştır.

**Anahtar Kelimeler:** Preimplantasyon tanı; blastosist; polimeraz zincir reaksiyonu; floresan in situ hibridizasyon

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**Turkiye Klinikleri J Med Sci 2009;29(1):236-45**

Prior to the advent of the PGD, couples at risk for pregnancies with genetic defects were offered prenatal tests such as amniocentesis or chorionic villus sampling. While these methods do allow for diagnosis of genetic abnormalities during pregnancy, couples are faced with difficult decisions following an abnormal result. PGD enables couples to avoid issues including pregnancy termination and giving birth to an affected child by initiating pregnancies with unaffected embryos.[1-7] Furthermore, IVF procedures may increase the risk of genetic abnormalities affecting the number and structure of the chromosomes and changing the expression levels of certain genes for patients applying to IVF centers. Such genetic defects originating from IVF procedures can be determined by PGD testing.[8-11]

PGD is a technique that allows polar bodies, cleavage-stage embryos and trophoblast cells to be tested for genetic abnormalities.[1,7] The first clinical application of PGD by Handyside et al used a PCR method for gender determination to avoid the transfer of male embryos.[2] In this application, there is a 50% probability of being affected by an X-linked recessive disorder. Gender was investigated in a single blastomere by a single round of PCR using primers for Y chromosome-specific repetitive DNA sequences.

Since PGD is performed before a pregnancy starts, it may be more acceptable for couples: (1) whose children are at increased risk for a specific genetic defect, such as, a) carriers of single gene disorders, b) carriers of structural chromosome abnormalities; (2) who may have affected family members or family ancestry that puts them at high risk for transmitting a particular disorder to their offspring; (3) who had termination of pregnancy/pregnancies because of a genetic defect or objections to termination of pregnancy; (4) where the women are of advanced maternal age, to avoid having chromosomally abnormal offspring; (5) with repeated implantation failure following assisted reproduction treatments (ART); and (6) with repeated miscarriages.[1,12-14]

Particularly, PGD has been used to test for a variety of aneuploidies, autosomal disorders, chromosomal rearrangements, X-linked diseases, nonmedical sex selection, human leukocyte antigen (HLA) typing, and single gene disorders.[15,16] Currently, it is accepted that at least 50% of human embryos are affected by aneuploidy and other chromosomal abnormalities. Although the complex origins of aneuploidy remain ambiguous, the fact remains that aneuploidy frequency is extraordinarily high in human IVF embryos.[17-19]

## DIAGNOSTIC TESTS USED IN PGD

There are two main stages in PGD application. The first main step contains the collection of diagnostic material for investigation. This is generally carried out in a clinical IVF laboratory under sterile conditions. Theoretically, diagnostic material can be collected from MII oocyte (first polar body), zygote (first or second polar body), cleavage-stage embryo and blastocyst (Figure 1a, b, c, d). The different technical applications required for obtaining the material itself influence the success of the procedure.[1,20] The second main stage involves diagnostic tests in this technique. PGD has largely focused on two methods: FISH (Figure 2) and PCR (Figure 3).[1,21,22]

FISH can be used on genetic material (metaphase chromosomes from polar bodies or nuclei from blastomeres and trophoblast cells) spread onto slides. After dehydration, probe mixes are appli-

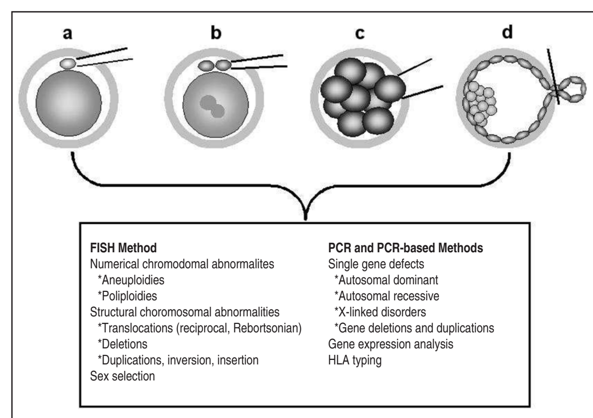


**FIGURE 1:** Illustration shows the resources of diagnostic materials used in PGD; (a) 1PB from MII oocyte, (b) 1PB or 2PB from zygote, (c) blastomere from cleavage-stage embryo, (d) trophoblast cells from blastocyst. The rectangular box summarizes the indications of utilization of PCR and FISH techniques in PGD applications.

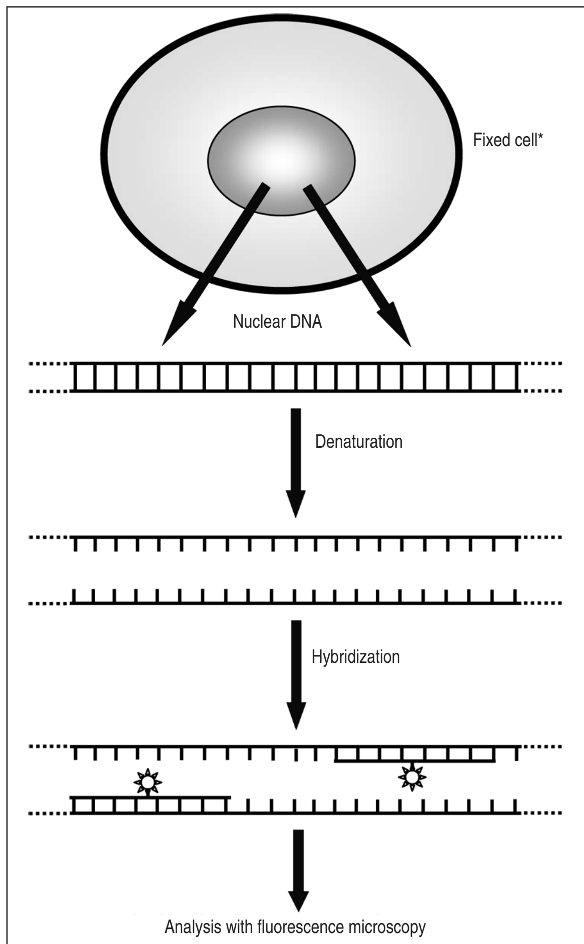


**FIGURE 2:** Schematic diagram of FISH used to determine chromosomal abnormalities in PGD.
*: The cells such as polar body, blastomere or trophoblast cells are fixed onto microscopy slides,
✲ : Probes signed with fluorescence.

ed and analyzed under fluorescence microscope (Figure 2).[23,24] FISH protocols have been used to address the problem of low success rates for some patient groups. They are older women (over 37 years), couples with recurrent implantation failure following IVF, and couples with recurrent miscarriages.[3] The majority of the embryo sexing is achieved using FISH that is less prone to contamination and which tests the copy number for each sex chromosome thereby avoiding the transfer of common chromosome abnormalities such as trisomies and X-monosomy.[1,3] However, FISH has several limitations, the most important of which being the number of chromosomes that can be analyzed, simultaneously. Although the current panel of nine probes used in a number of PGD laboratories cover the most frequent abnormalities detected in cleavage-stage embryos or oocytes, some studies indicated that 25-30% of chromosomal abnormalities would remain undetected using FISH with nine chromosome-spesific probes (13, 15, 16, 17, 18, 21, 22, X and Y), leading to the transfer of aneuploid embryos incorrectly diagnosed as normal.[25-30] Cur-

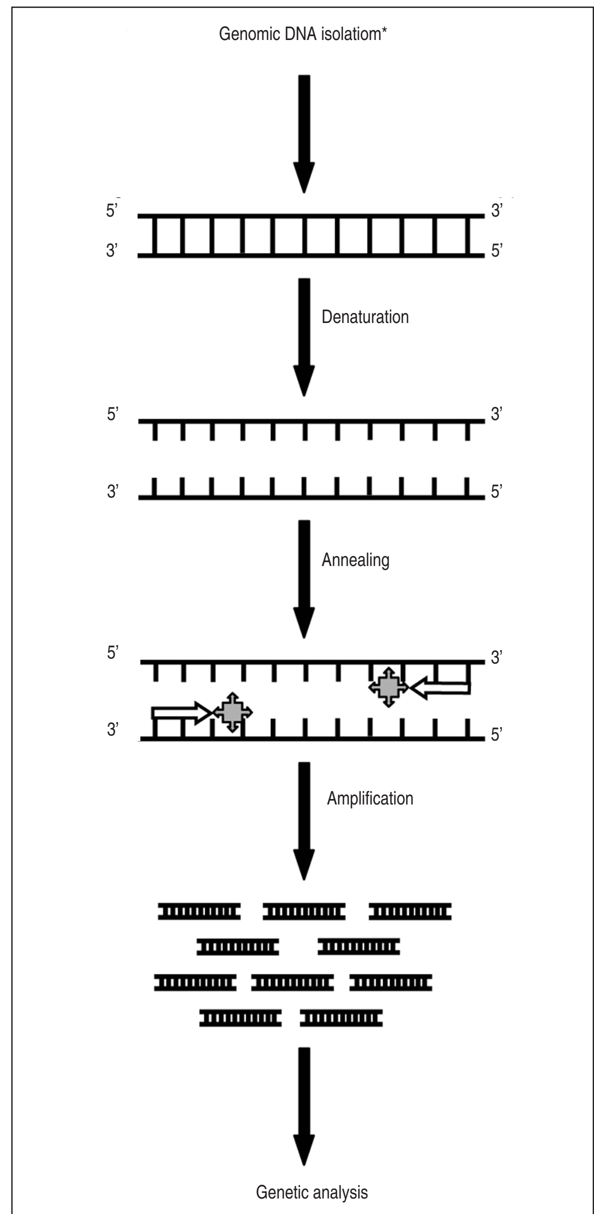


**FIGURE 3:** Schematic diagram of polymerase chain reaction usually used to determine gene mutations or other genetic abnormalities in PGD.
✥: taq polymerase, → ←: primers,
* : Genomic DNA can be isolated from polar body, blastomere or trophoblast cells.

rent FISH protocols have used probes for up to 13 chromosomes but this represents only half of the whole karyotype and accuracy per probe is reduced when large numbers of probes are combined because of signal overlaps.[25,31] Moreover, other problems related to FISH technique contain insufficient fixation of the materials, having only five spectrally distinct fluorochromes for probe labeling, and probe target polymorphisms.[1,3,12,17] FISH probes should always be tested on blood samples from both partners before being used for PGD to prevent misdiagnosis because of target polymorphisms.[13]

PCR is a technique widely used in molecular biology and also derives its name from one of its key components, a DNA polymerase used to amplify a piece of DNA by in vitro enzymatic replication (Figure 3). [5,32] PCR-based methodologies are often used to diagnose single gene disorders in single cells.[1] The numbers of diseases currently diagnosed via PGD-PCR technique are approximately 200.[12] Generally, PGD-PCR evaluation can be finished within one day. Healthy embryos are then transferred to the uterus on day 4 or 5. Additionally, it is strongly recommended that the normality is approved by chorionic villus sampling (CVS) or amniocentesis.[4]

PCR-based methods used in PGD have some limitations. Only one or two copies of DNA molecules are present per haploid (second polar body) or diploid (blastomere, trophoblast cell and first polar body) cell, respectively.[1] The limited amount of template DNA (approximately 7 pg) present in a single diploid cell (1PB and blastomere) causes a number of problems including an increased incidence of detectable contamination, amplification failure and allele drop out (ADO) in heterozygotes. Amplification efficiencies at the single cell level are usually lower than the routine PCR of DNA samples where there is a much higher amount of template DNA (approximately 10 ng). Reduced amplification efficiency can be a result of cell loss during the delicate process of cell transfer to the tube, spontaneous cell lysis before entering the tube or degradation of the DNA. In addition, contamination is an important threat in the molecular diagnostic analyses for PGD, which may result from the large number of PCR cycles, extraneous DNA, DNA in the form of aerosol, inadvertent amplification of PCR products and cellular DNA from sperm or cumulus cells.[1,33,34] The use of ICSI instead of IVF eliminates the risk of sperm or cumulus cell contamination and is routinely used for all PGD-PCR cases. Denuding the oocyte of cumulus cells is also a standard practice for PCR-based PGD. An additional problem which is common to all single-cell based PCR tests, is a phenomenon known as ADO. ADO can be defined as amplification failure affecting only one of the parental alleles present in the single cell. The frequency of ADO was reported up to 25% in a clinical PGD case. Reports suggest that blastomeres usually have a greater ADO rate than polar bodies, lymphocytes, or fibroblasts.[1,12]

PCR-based new techniques have started to be used in clinical PGD applications. One of them, CGH, is a molecular cytogenetic test that allows the analysis of the full set of chromosomes, which has been applied to detect aneuploidy/aneuploidies in single cells. This method is based on the hybridization of fluorescently labeled DNA (frequently rhodamine or texas red) to normal human metaphase preparations.[35] Afterwards, the average red/green fluorescent ratio for each chromosome is determined by the CGH software. The total time required to perform the CGH would be about 60 h (13 h for the DOP-PCR-CGH experiment, plus 45-48 h of hybridization).[4,25,36] Whole genome amplification followed by CGH detects genomic imbalances and has been used in PGD cycles with blastomere biopsy, with successful results.[3,37-39] PGD using CGH to detect aneuploidy for almost all chromosomes might increase IVF pregnancy rates by detecting abnormalities.[25]

As CGH is a labor intensive technique that requires up to 3-4 days to obtain results, two different strategies were proposed to apply CGH to PGD. The first was the use of CGH for PGD by blastomere analysis. In this case, embryo freezing was required to provide time enough to perform the CGH analysis. Although this approach has recently proved higher implantation and pregnancy

rates than FISH, some researchers found that 46% of the embryos had not survived the freezing-thawing process.[25,39,40] The second strategy was to perform CGH for PGD by first polar body analysis. Since polar body biopsy is performed on the same day, CGH analysis is compatible with embryo replacement on day 4, without embryo freezing.[25] In addition, CGH test has some limitations. One of the main limitations is its inability to detect alterations such as balanced predivision of chromatids, which predisposes to aneuploidy, but does not result in an immediate gain or loss of chromosomal material.[25] Additionally, CGH is incapable of detecting changes in ploidy (e.g. diploid oocytes) and heterochromatic, telomeric and centromeric regions have to be excluded from the analysis because they usually show a deviation in the CGH pattern. Some studies have already reported the difficulty in interpreting the CGH profile of chromosomes 17, 19 and 22 (CG-rich areas) in CGH applied to the analysis of single cells.[25,29,41] Despite the high levels of mitochondrial DNA in MII oocytes, there is no interference with CGH profiles, as mitochondrial DNA does not hybridize to the template chromosomes. However, mitochondrial DNA may compete with genomic DNA in the amplification and nick translation procedure. This could explain why MII oocytes, which contain many mitochondria, usually give a weaker hybridization than the polar bodies.[25,42]

A new technique that may be used for PGD in the future is microarray analysis. Gene-expression profiling using DNA microarrays can identify certain gene expression levels and aneuploidies in blastomere. With the invention of highly sensitive molecular detectors, single RNA molecules can be analyzed directly, without amplification. This technique could be used for analysis of gene transcription in single embryonic cells.[43-45]

## POLAR BODY BIOPSY

One of the diagnostic materials used in PGD is the polar body that contains haploid genetic material and small amount of cytoplasm. PGD investigates the genetic material within the first and second polar bodies, by-products of meiosis I and meiosis II, respectively. First polar body (1PB) is characterized by the presence of a complement of 23 bivalent maternal chromosomes. On fertilization, a second polar body (2PB) containing a complement of 23 maternal chromatids, is extruded from the oocyte and can also be tested to provide further confirmation (Figure 1a, b).[25,46] The PBs are similar to the female pronucleus that combines with the male pronucleus to become the zygote. By establishing whether there is an abnormal gene or chromosome arrangement in the PBs, it is possible to determine the maternal genetic contribution to the embryo. This method can be used in cases of maternally derived dominant mutations, translocations, and aneuploidies. It can not be used when paternally derived genetic information is critical to the diagnosis. Unlike blastomere biopsy, in which two cells can be studied to replicate data, PB biopsy data can not be replicated unless the PB biopsy is followed by blastomere biopsy.[3,4,43] In clinical application, PB has been used primarily for aneuploidy screening and less commonly for the determination of maternal transmission of single gene disorders such as β-thalassaemia.[7]

PB has the advantage of sampling extraembryonic material and is therefore less likely to affect detrimentally subsequent embryonic development, and it might be considered ethically preferable by some investigators. However, PB can only provide information about the maternal genotype. In addition, where predivision of chromatids or undetected recombination between markers has taken place, a reliable diagnosis may not always be possible.[13] In female meiosis I, a set of chromosomes, with two chromatids each, segregate to the 1PB while the MII oocyte retains the reciprocal chromosome complement. Since the 1PB is thought to have no biological role once it has been extruded, the analysis of 1PB allows the indirect characterization of chromosome constitution of the MII oocyte. This means that if a segregation error occurs during this first meiotic division, and for instance, an extra chromosome is present in the MII oocyte, then the 1PB will show the complementary loss. Most embryo aneuploidies as well as most first trimester aneuploi-

dies were classified as originating in female meiosis I. However, FISH analysis results of first and second PBs indicated that a sizable part of aneuploidy occured in meiosis II.[25,46-48]

An alternative approach is the investigation of the 1PBs, which are available for analysis three days earlier than the blastomeres, providing sufficient time to perform CGH, and avoiding embryo cryopreservation. However, further FISH analysis of biopsied blastomeres should occur, as chromatid anomalies detected in meiosis I have only a 50% chance of leading to an aneuploid embryo.[12,49] The use of 1PBs has inherent limitations in itself, since second meiotic, paternally derived and post-zygotic chromosome errors (i.e. embryonic mosaicism, which was detected in 30% of cleavage-stage embryos) can not be detected.[25]

## CLEAVAGE-STAGE EMBRYO BIOPSY

Most of the centers obtain genetic material for the PGD by cleavage-stage biopsy on the third day following insemination when the embryo is between 5 and 10 cells (Figure 1c). At this stage, blastomeres are believed to be totipotent and embryo survival and metabolism seems to be unaffected by biopsy.[1,3] Individual cells of the cleaving embryo are distinct and discernible until around the 8-16-cell stage (day 3) when the embryo begins to undergo the process of compaction. From 16-cell stage, tight junctions begin to form and cellular apposition becomes too great to separate individual cells.[13,50] Biopsy at the two- or four-cell stage involves removal of a large proportion of the cellular mass of the embryo, with detrimental effects on further development potential.[13,51,52] The decision as to whether one or two cells should be removed is controversial. Removing two cells reduces the cellular mass of the embryo and therefore might reduce its developmental capacity. The accuracy of the diagnosis, however, is likely to be enhanced if embryos are replaced only when the results from both cells are concordant. Discordant results between cells could also reduce the number of the embryos deemed suitable for replacement, and might decrease pregnancy rates. After genetic diagnosis, suitable embryos are usually transferred to the uterus on day four or five (the blastocyst stage) of development.[13,53,54]

Cleavage-stage embryos obtained by IVF undergo a biopsy procedure in which one or two blastomers are removed and tested for a specific disorder. Embryos are transferred to calcium-free medium to disassemble tight junctions and then stabilized by a holding pipette. The zona pellucida is breached by laser or acid Tyrode's solution; finally one or two cells are removed by a biopsy pipette.[3,4] Also, according to European Society of Human Reproduction and Embryology (ESHRE) PGD Consortium data collections, cleavage-stage biopsy, usually containing aspiration of one or two blastomeres, is the most common practice for determining embryonic genetic material for PGD analysis.[7] If the blastomer is unaffected, the embryo from which it is taken is interpreted to be free of the abnormality. After that, the embryo can be transferred to the uterus to initiate pregnancy.[1]

Embryonic blastomeres, however, in their majority are found to be in interphase. To overcome this problem, PGD protocols commonly employ a molecular cytogenetic method termed FISH. This method is rapid to determine the numerical or structural chromosomal disorders and performs equally well regardless of whether applied to metaphase or interphase nuclei.[12,55]

Much of current knowledge is based on an experiment that was conducted by Hardy et al who showed that cells that were removed initially by biopsy demonstrated no adverse effects on preimplantation development to the blastocyst stage other than a proportional loss of biomass.[17,52] Later studies revealed that biopsy of one-fourth of an embryo might reduce the ratio of inner cell mass to trophectoderm cells.[17,56,57] This would likely diminish viability beyond just a proportional decrease in biomass. The assumption that biopsy has little effect on the embryo simply remains unproven. Cohen et al stated that an implantation potential of 20% without biopsy would diminish to 17.0% and 14.0% after a single- or 2-cell biopsy, respectively.[17] Therefore, this analysis revealed that 2-cell biopsy significantly impeded embryo development and was

inadvisable in cases in which PGD was used to increase the IVF success rates. The decision to remove 1 or 2 cells should be based not only on reducing error rates and increasing the efficiency of the assay but also on premise that embryo viability is diminished at least proportionally with each cell that is removed.[13,17] On the other hand, Bick et al stated that evaluation of embryo survival after biopsy suggesting the removal of one or two cells did not have a significant impact on embryo viability, the course of pregnancy or the baby's characteristics (birth weight, length, gestational age at delivery).[43]

## BLASTOCYST STAGE BIOPSY

The third and latest method is blastocyst-stage biopsy that is performed approximately 5 to 6 days after insemination. The embryo at this stage has differentiated into the trophectoderm, which gives rise to the placenta, and the inner cell mass, which gives rise to the fetus.[43] Laser assisted biopsy of the human blastocyst using noncontact infrared laser for drilling of the zona pellucida enables removal of several cells from the trophectoderm layer (Figure 1d).[58] It does not expose the embryos to chemicals and does not invade the inner cell mass destined for fetal development. Because several cells can be removed from the trophectoderm for analysis, the accuracy and reliability of PGD is improved.[43,59] As blastocyst culture and cryopreservation improve, it is expected that this technique supplants cleavage-stage biopsy and polar body biopsy.[43]

A major problem with polar body and/or cleavage-stage biopsy is the paucity of material that might lead to an accurate and unreliable genetic diagnosis. Biopsy of the embryo at the blastocyst stage obviates many of these problems as the embryo can contain up to 300 cells.[13] In addition, because blastocyst biopsy involves the preferential removal of the more accessible trophectoderm cells, the inner cell mass that is destined to become the fetus proper is unlikely to be damaged, thereby reducing possible ethical concerns.[13,60] In blastocyst biopsy, a larger amount of material is obtained for testing than either polar body or blastomer biopsy. When diagnosing monogenic defects in single cells using PCR-based protocols, there is a high risk of PCR failure and allele dropout, potentially resulting in a decreased number of unaffected embryos available for transfer. Consequently, increasing the amount of starting template DNA should in principle increase the sensitivity and reliability of genetic diagnosis.[1,7] The main disadvantages of this application are that culture conditions may not permit all embryos to progress to the blastocyst stage in vitro. Also, as the blastocyst stage embryo must be transferred to the uterus before hatching on day 6, the time available for diagnosis is very limited.[3]

Blastocyst biopsy normally takes place on day five or six after fertilization and involves making a hole in the zona pellucida before the removal of cells. The cells are biopsied either by gentle teasing using needles or by induced herniation of a trophectodermal vesicle which can then be separated by physical means using needles or by a laser.[13,61,74] So far, it has not been extensively used in humans because of the difficulty in culturing embryos to the blastocyst stage. However, the development of sequential media that have been specifically designed for the long-term culture of embryos and the recent report of a human live birth after blastocyst biopsy might encourage the increased use of this promising technique.[13,15,62]

## CONCLUSION

The clinical perspectives for PGD have recently broadened to include diagnoses for more than 100 different genetic disorders as well as for chromosomal aneuploidies and translocations. As a result, over 1.000 healthy children have been born worldwide from PGD-IVF cycles.[30,63,64] Even more controversial uses are contemplated, including PGD to select "cosmetic" traits, such as skin or eye color, and to select genetic traits that are usually considered disabilities, such as deafness.[63] In recent years, late-onset inherited disorders and highly penetrant cancer predisposition mutations have also been approached using PGD. PGD testing has been applied for a variety of late-onset inherited genetic disorders including familial adenomatous coli, BRCA1 and BRCA2 gene mutations associated with breast and ovarian cancers, autosomal do-

minant polycystic kidney disease (PKD1 mutations), and amyloid precursor protein (APP) gene mutations associated with early-onset Alzheimer disease.[43,65-68] Another novel indication for PGD involves blood group incompatibility such as Kell or Rhesus (Rh) alloimmunization.[69] Although these disorders can be detected by prenatal diagnosis and treated with intrauterine blood transfusion, the potential complication for the fetus can not be completely eliminated even after transfusion. PGD for HLA matching was provided for families that have children affected with inherited genetic disorders such as Fanconi anemia, Thalassemia, Wiscott-Aldrich syndrome, X-linked hipohydrotic ectodermal dysplasia with immune deficiency, as well as sporadic diseases such as aplastic anemia and leukemia.[43,70,71]

Some consortiums were constituted worldwide to undertake systematic assessment of PGD outcome. One of them is ESHRE PGD Consortium. This consortium was established in 1997 to collect data concerning reasons for referrals. The membership has increased steadily, since the first report including data from sixteen centers (ESHRE PGD Consortium Committee, 1999).[43,72] In 1997, the ESHRE PGD Consortium was formed as part of the ESHRE Special Interest Group on Reproductive Genetics, in order to undertake a long-term study on efficacy and clinical outcome of PGD. In December 1999, the first PGD Consortium report was published discussing referrals of 323 couples, 392 PGD cycles and 82 pregnancies and 79 children born. The aims of the ESHRE PGD Consortium are to, i) survey availability of PGD for different conditions; ii) initiate follow-up studies of pregnancies and children born, iii) produce guidelines and recommended PGD protocols to promote best practice; and iv) formulate a consensus on the use of PGD. The ESHRE PGD Consortium aids to increase the success of PGD applications.[73]

In conclusion, application lands of PGD broaden rapidly. Also, diagnostic tests and biopsy methods are improving effectively to determine the genetic defects in the embryo in a short time and correctly. Diagnostic materials from polar bodies, cleavage-stage embryo and trophectoderm cells can be taken at distinct developmental times. Selection of the diagnostic material should be specific for the conditions of couples applying to IVF centers. Further studies should aim to find what kind of applications might increase the success of PGD testing and the rate of healthy pregnancies.

***Acknowledgement***

*The present study was supported by Akdeniz University Research Fund. The authors thank Ahter Şanlıoğlu, PhD and Gamze Tanrıöver, MSc for their comments and help with the manuscript.*

## REFERENCES

1. Thornhill AR, Snow K. Molecular diagnostics in preimplantation genetic diagnosis. J Mol Diagn 2002;4(1):11-29.
2. Handyside AH, Kontogianni EH, Hardy K, Winston RM. Pregnancies from biopsied human preimplantation embryos sexed by Y-specific DNA amplification. Nature 1990;344(6268):768-70.
3. Ogilvie CM, Braude PR, Scriven PN. Preimplantation genetic diagnosis--an overview. J Histochem Cytochem 2005;53(3):255-60.
4. The Practice Committee of the American Society for Reproductive Medicine and the Practice Committee of the Society for Assisted Reproductive Technology. Preimplantation genetic diagnosis. Fertil Steril 2006;86(5 Suppl):S257-8.
5. Sermon K. Current concepts in preimplantation genetic diagnosis (PGD): a molecular biologist's view. Hum Reprod Update 2002; 8(1):11-20.
6. Swanson A, Strawn E, Lau E, Bick D. Preimplantation genetic diagnosis: technology and clinical applications. WMJ 2007;106(3):145-51.
7. Kokkali G, Traeger-Synodinos J, Vrettou C, Stavrou D, Jones GM, Cram DS, et al. Blastocyst biopsy versus cleavage stage biopsy and blastocyst transfer for preimplantation genetic diagnosis of beta-thalassaemia: a pilot study. Hum Reprod 2007;22(5):1443-9.
8. Niemann H, Wrenzycki C. Alterations of expression of developmentally important genes in preimplantation bovine embryos by in vitro culture conditions: implications for subsequent development. Theriogenology 2000;53(1):21-34.
9. Kobayashi H, Sato A, Otsu E, Hiura H, Tomatsu C, Utsunomiya T, et al. Aberrant DNA methylation of imprinted loci in sperm from oligospermic patients. Hum Mol Genet 2007;16(21):2542-51.
10. Wang S, Cowan CA, Chipperfield H, Powers RD. Gene expression in the preimplantation embryo: in-vitro developmental changes. Reprod Biomed Online 2005;10(5):607-16.
11. Lonergan P, Pedersen HG, Rizos D, Greve T, Thomsen PD, Fair T, et al. Aberrant DNA methylation of imprinted loci in sperm from oligospermic patients. Hum Mol Genet 2007;16(21):2542-51.

12. Lonergan P, Pedersen HG, Rizos D, Greve T, Thomsen PD, Fair T, Evans A, et al. Effect of the post-fertilization culture environment on the incidence of chromosome aberrations in bovine blastocysts. Biol Reprod 2004;71(4): 1096-100.
13. Braude P, Pickering S, Flinter F, Ogilvie CM. Preimplantation genetic diagnosis. Nat Rev Genet. 2002;3(12):941-53.
14. Goossens V, De Rycke M, De Vos A, Staessen C, Michiels A, Verpoest W, et al. Diagnostic efficiency, embryonic development and clinical outcome after the biopsy of one or two blastomeres for preimplantation genetic diagnosis. Hum Reprod 2008;23(3):481-92.
15. Braude P, Flinter F. Use and misuse of preimplantation genetic testing. BMJ 2007;335 (7623):752-4.
16. Baruch S, Kaufman D, Hudson KL. Genetic testing of embryos: practices and perspectives of US in vitro fertilization clinics. Fertil Steril 2008;89(5):1053-8.
17. Cohen J, Wells D, Munné S. Removal of 2 cells from cleavage stage embryos is likely to reduce the efficacy of chromosomal tests that are used to enhance implantation rates. Fertil Steril 2007;87(3):496-503.
18. Munné 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(2): 382-91.
19. Márquez C, Sandalinas M, Bahçe M, Alikani M, Munné S. Chromosome abnormalities in 1255 cleavage-stage human embryos. Reprod Biomed Online 2000;1(1):17-26.
20. Harper J. Preimplantation Genetic Diagnosis. In: Harper, JC; Delahant, JDA; Handyside AH, eds. UK: John Wiley and Sons; 2001. p.141-63.
21. Griffin DK, Wilton LJ, Handyside AH, Atkinson GH, Winston RM, Delhanty JD. Diagnosis of sex in preimplantation embryos by fluorescent in situ hybridisation. BMJ 1993;306(6889): 1382.
22. Grifo JA, Tang YX, Munné S, Alikani M, Cohen J, Rosenwaks Z. Healthy deliveries from biopsied human embryos. Hum Reprod 1994;9(5):912-6.
23. Kuo HC, Ogilvie CM, Handyside AH. Chromosomal mosaicism in cleavage-stage human embryos and the accuracy of single-cell genetic analysis. J Assist Reprod Genet 1998;15(5):276-80.
24. Verlinsky Y, Cieslak J, Freidine M, Ivakhnenko V, Wolf G, Kovalinskaya L, et al. Polar body diagnosis of common aneuploidies by FISH. J Assist Reprod Genet 1996;13(2):157-62.
25. Gutiérrez-Mateo C, Wells D, Benet J, Sánchez-García JF, Bermúdez MG, Belil I, et al. Reliability of comparative genomic hybridization to detect chromosome abnormalities in first polar bodies and metaphase II oocytes. Hum Reprod 2004;19(9):2118-25.
26. Pujol A, Boiso I, Benet J, Veiga A, Durban M, Campillo M, et al. Analysis of nine chromosome probes in first polar bodies and metaphase II oocytes for the detection of aneuploidies. Eur J Hum Genet 2003;11(4):325-36.
27. Munné S, Bahçe M, Sandalinas M, Escudero T, Márquez C, Velilla E, et al. Differences in chromosome susceptibility to aneuploidy and survival to first trimester. Reprod Biomed Online 2004;8(1):81-90.
28. Boué A, Boué J, Gropp A. Cytogenetics of pregnancy wastage. Adv Hum Genet 1985; 14:1-57.
29. Voullaire L, Wilton L, McBain J, Callaghan T, Williamson R. Chromosome abnormalities identified by comparative genomic hybridization in embryos from women with repeated implantation failure. Mol Hum Reprod 2002;8 (11):1035-41.
30. Grifo J, Talebian S, Keegan D, Krey L, Adler A, Berkeley A. Ten-year experience with preimplantation genetic diagnosis (PGD) at the New York University School of Medicine Fertility Center. Fertil Steril 2007;88(4):978-81.
31. Abdelhadi I, Colls P, Sandalinas M, Escudero T, Munné S. Preimplantation genetic diagnosis of numerical abnormalities for 13 chromosomes. Reprod Biomed Online 2003;6(2): 226-31.
32. Cheng S, Fockler C, Barnes WM, Higuchi R. Effective amplification of long targets from cloned inserts and human genomic DNA. Proc Natl Acad Sci U S A 19947;91(12):5695-9.
33. Cui KH, Matthews CD. Nuclear structural conditions and PCR amplification in human preimplantation diagnosis. Mol Hum Reprod 1996;2(1):63-71.
34. Ray PF, Ao A, Taylor DM, Winston RM, Handyside AH. Assessment of the reliability of single blastomere analysis for preimplantation diagnosis of the delta F508 deletion causing cystic fibrosis in clinical practice. Prenat Diagn 1998;18(13):1402-12.
35. Kallioniemi A, Kallioniemi OP, Sudar D, Rutovitz D, Gray JW, Waldman F, et al. Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. Science 1992 ;258(5083):818-21.
36. Coskun S, Alsmadi O. Whole genome amplification from a single cell: a new era for preimplantation genetic diagnosis. Prenat Diagn 2007;27(4):297-302.
37. Voullaire L, Wilton L, Slater H, Williamson R. Detection of aneuploidy in single cells using comparative genomic hybridization. Prenat Diagn 1999;19(9):846-51.
38. Wells D, Delhanty JD. Comprehensive chromosomal analysis of human preimplantation embryos using whole genome amplification and single cell comparative genomic hybridization. Mol Hum Reprod 2000;6(11): 1055-62.
39. Hill DL. Aneuploidy screening of preimplantation embryos using comparative genomic hybridization versus fluorescence in situ hybridization techniques. Fertil Steril 2003;80 (4):873-4.
40. Munné S, Wells D. Questions concerning the suitability of comparative genomic hybridization for preimplantation genetic diagnosis. Fertil Steril 2003;80(4):871-2.
41. Moore DH 2nd, Pallavicini M, Cher ML, Gray JW. A t-statistic for objective interpretation of comparative genomic hybridization (CGH) profiles. Cytometry 1997;28(3):183-90.
42. Voullaire L, Slater H, Williamson R, Wilton L. Chromosome analysis of blastomeres from human embryos by using comparative genomic hybridization. Hum Genet 2000;106(2): 210-7.
43. Bick DP, Lau EC. Preimplantation genetic diagnosis. Pediatr Clin North Am 2006;53(4): 559-77.
44. Hughes TR, Roberts CJ, Dai H, Jones AR, Meyer MR, Slade D, et al. Widespread aneuploidy revealed by DNA microarray expression profiling. Nat Genet 2000;25(3):333-7.
45. Neely LA, Patel S, Garver J, Gallo M, Hackett M, McLaughlin S, et al. A single-molecule method for the quantitation of microRNA gene expression. Nat Methods 2006;3(1):41-6.
46. Gitlin SA, Gibbons WE, Gosden RG. Oocyte biology and genetics revelations from polar bodies. Reprod Biomed Online 2003;6(4):403-9.
47. Nicolaidis P, Petersen MB. Origin and mechanisms of non-disjunction in human autosomal trisomies. Hum Reprod 1998;13(2):313-9.
48. Hassold T, Hunt P. To err (meiotically) is human: the genesis of human aneuploidy. Nat Rev Genet 2001;2(4):280-91.
49. Wells D, Escudero T, Levy B, Hirschhorn K, Delhanty JD, Munné S. First clinical application of comparative genomic hybridization and polar body testing for preimplantation genetic diagnosis of aneuploidy. Fertil Steril 2002;78(3):543-9.
50. Fleming TP, McConnell J, Johnson MH, Stevenson BR. Development of tight junctions de novo in the mouse early embryo: control of assembly of the tight junction-specific protein, ZO-1. J Cell Biol 1989;108(4):1407-18.
51. Liu J, Van den Abbeel E, Van Steirteghem A. The in-vitro and in-vivo developmental potential of frozen and non-frozen biopsied 8-cell mouse embryos. Hum Reprod 1993;8(9): 1481-6.

52. Hardy K, Martin KL, Leese HJ, Winston RM, Handyside AH. Human preimplantation development in vitro is not adversely affected by biopsy at the 8-cell stage. Hum Reprod 1990; 5(6):708-14.

53. Lewis CM, Pinêl 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(1):43-50.

54. Van de Velde H, De Vos A, Sermon K, Staessen C, De Rycke M, Van Assche E, et al. Embryo implantation after biopsy of one or two cells from cleavage-stage embryos with a view to preimplantation genetic diagnosis. Prenat Diagn 2000;20(13):1030-7.

55. Munné S. Chromosome abnormalities and their relationship to morphology and development of human embryos. Reprod Biomed Online 2006;12(2):234-53.

56. Somers GR, Trounson AO, Wilton LJ. Allocation of cells to the inner cell mass and trophectoderm of 3/4 mouse embryos. Reprod Fertil Dev 1990;2(1):51-9.

57. Tarín JJ, Conaghan J, Winston RM, Handyside AH. Human embryo biopsy on the 2nd day after insemination for preimplantation diagnosis: removal of a quarter of embryo retards cleavage. Fertil Steril 1992;58(5):970-6.

58. Kokkali G, Vrettou C, Traeger-Synodinos J, Jones GM, Cram DS, Stavrou D, et al. Birth of a healthy infant following trophectoderm biopsy from blastocysts for PGD of beta-thalassaemia major. Hum Reprod 2005;20(7): 1855-9.

59. McArthur SJ, Leigh D, Marshall JT, de Boer KA, Jansen RP. Pregnancies and live births after trophectoderm biopsy and preimplantation genetic testing of human blastocysts. Fertil Steril 2005;84(6):1628-36.

60. Dokras A, Sargent IL, Ross C, Gardner RL, Barlow DH. Trophectoderm biopsy in human blastocysts. Hum Reprod 1990;5(7):821-5.

61. Veiga A, Sandalinas M, Benkhalifa M, Boada M, Carrera M, Santaló J, et al. Laser blastocyst biopsy for preimplantation diagnosis in the human. Zygote 1997;5(4):351-4.

62. Gardner DK, Schoolcraft WB, Wagley L, Schlenker T, Stevens J, Hesla J. A prospective randomized trial of blastocyst culture and transfer in in-vitro fertilization. Hum Reprod 1998;13(12):3434-40.

63. Rao R. Preimplantation genetic diagnosis and reproductive equality. Gend Med 2004;1(2): 64-9.

64. ESHRE Preimplantation Genetic Diagnosis Consortium: data collection III (May 2001). Hum Reprod 2002;17(1):233-46.

65. Wagner JE. Practical and ethical issues with genetic screening. Hematology Am Soc Hematol Educ Program 2005:498-502.

66. Ao A, Wells D, Handyside AH, Winston RM, Delhanty JD. Preimplantation genetic diagnosis of inherited cancer: familial adenomatous polyposis coli. J Assist Reprod Genet 1998;15(3):140-4.

67. De Rycke M, Georgiou I, Sermon K, Lissens W, Henderix P, Joris H, et al. PGD for autosomal dominant polycystic kidney disease type 1. Mol Hum Reprod 2005;11(1):65-71.

68. Verlinsky Y, Rechitsky S, Verlinsky O, Masciangelo C, Lederer K, Kuliev A. Preimplantation diagnosis for early-onset Alzheimer disease caused by V717L mutation. JAMA 2002 ;287(8):1018-21.

69. Seeho SK, Burton G, Leigh D, Marshall JT, Persson JW, Morris JM. The role of preimplantation genetic diagnosis in the management of severe rhesus alloimmunization: first unaffected pregnancy: case report. Hum Reprod 200520(3):697-701.

70. Rechitsky S, Kuliev A, Tur-Kaspa I, Morris R, Verlinsky Y. Preimplantation genetic diagnosis with HLA matching. Reprod Biomed Online 2004;9(2):210-21.

71. Kuliev A, Verlinsky Y. Preimplantation HLA typing and stem cell transplantation: report of International Meeting, Cyprus, 27-8 March, 2004. Reprod Biomed Online 2004;9(2):205-9.

72. Sermon KD, Michiels A, Harton G, Moutou C, Repping S, Scriven PN, 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(2):323-36.