Preimplantation Genetic Testing

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Abstract The application of genetic technologies to the field of reproductive medicine has ushered in a new era of medicine that is likely to greatly expand in the coming years. Concurrent with an in vitro fertilization (IVF) cycle, it is now possible to obtain a cellular biopsy from a developing embryo and genetically evaluate this sample with increasing sophistication and detail. Information obtained from this analyzed sample may then guide which embryos are selected for uterine transfer. Applications for this technology are currently being employed to avoid the propagation of certain genetically based diseases and to increase the efficiency of assisted reproductive technologies in general. The way such technologies are implemented, however, has caused a continuous and ever evolving debate among reproductive medicine physicians and geneticists. This article will define the current uses of preimplantation genetic testing and discuss the benefits and limitations of this technology.

Keywords Preimplantation genetic screening · Preimplantation genetic diagnosis · Embryo · Recurrent pregnancy loss · Microarray · FISH

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Introduction

In 1953, when DNA was first described by James D. Watson and Francis Crick, the manner in which human beings view themselves fundamentally changed [1]. From this point onward, human biology could be understood on a profoundly deeper level and a paradigm shift within medicine occurred. Since this discovery, continuous and exponential advances have been made in the field of genetics. Today, sequencing an entire individual's genome is available and in many cases can be performed in a day (rather than years) for thousands (rather than millions) of dollars [2]. Once thought of a separate discipline of science, genetics now is an integral aspect of essentially all aspects of medicine. There is an increasing focus within medicine to identify the genetic root of disease both from a diagnostic and therapeutic vantage point.

Perhaps in no other field is this emphasis on genetics more profound than in reproductive medicine. Specifically, preimplantation genetic testing has dramatically expanded over the past several decades both in volume and types of clinical applications available. Broadly speaking, preimplantation genetic testing is the practice of obtaining embryos through in vitro fertilization (IVF) procedure. A cellular biopsy from these embryos is obtained and genetic testing performed on these samples. The results from this genetic analysis helps guide clinical decision making about which embryos would be optimally chosen for subsequent transfer to the maternal uterus in an attempt for a resultant pregnancy. This type of testing was first performed by Dr. Alan Handyside in 1990 [3–5].

The manner and type of testing that is utilized varies greatly. When first introduced, preimplantation genetic testing was only applied to determine if embryos harbored specific genetic mutations that were known to exist from parental DNA analysis, a procedure called preimplantation genetic diagnosis (PGD) [3–5]. As the technology continued to advance, the concept of preimplantation genetic screening (PGS) was born. PGS evaluates a sample for aneuploidy (the presence of too many or too few chromosomes) in genetically normal parents in an attempt to maximize pregnancy outcomes in conjunction with assisted reproductive technologies. There is continual debate about the optimal patient populations served by preimplantation genetic testing. The following article attempts to encapsulate the current thinking regarding the various forms of preimplantation genetic testing and current appropriate applications of this technology.

The Mechanics of Performing Preimplantation Genetic Testing

To understand preimplantation genetic testing, one must first understand the clinical context in which such technology is utilized. An IVF cycle consists of administrating injectable gonadotropins to women. This process induces controlled ovarian hyperstimulation in which more than the usual number of ovarian follicles is recruited and matured [6•, 7•]. The oocytes within these follicles are then surgically harvested and inseminated with sperm. In IVF cycles not utilizing genetic testing, the resultant embryos are grown in vitro until either 3 or 5 days of development at which time the one or two "best" embryos as determined by their physical appearance are placed into the uterus and the remaining surviving embryos are cryopreserved.

Preimplantation genetic testing is employed by genetically evaluating cells obtained from biopsy of these developing embryos with the aid of specialized microsurgical tools and microscopes removes one or more cells from a developing embryo. These cells are then sent for genetic evaluation by a genetics laboratory and a genetic report is generated, which helps to guide which embryos may be optimal for uterine transfer. This process is complex and customized to each patient's situation. Therefore, the type of genetic evaluation performed and even the stage of embryonic development at which the biopsy is performed is not constant from patient to patient.

By 3 days after fertilization (at what is called the cleavage stage), embryos are comprised of approximately 8 cells and by 5 days many embryos are comprised of approximately 100 cells (which is called the blastocyst stage; Fig. 1). Traditionally, embryo biopsy is performed at the cleavage stage. At the cleavage stage, embryos are generally comprised of 6-8 cells that are totipotent, meaning that none of the cells making up the embryo are dedicated to a certain differentiation path. A totipotent cell is incredibly plastic and may differentiate into any part of an embryo or even placenta. Many stem cells used today are derived from these early embryonic totipotent cells.

By 5 or 6 days of development following fertilization, most viable embryos have entered the blastocyst stage of development. At the blastocyst stage, embryos are comprised of approximately 100 cells and have clearly differentiated into either the inner cell mass (ICM), which is destined to form the fetus, or the trophectoderm (TE), which is destined to form the placenta. These cells, because they have been committed down particular cellular paths, are referred to as pluripotent.

Until relatively recently, the act of embryo biopsy was performed at the cleavage stage. However, trophectoderm biopsy is increasingly being utilized currently. Additionally, the technologies used to perform genetic analysis are constantly evolving and are very specific depending upon the exact type of information desired based on the clinical situation.

Preimplantation Genetic Diagnosis

The practice of evaluating embryos for a known parental genetic defect is known as preimplantation genetic diagnosis (PGD). The indications for genetic assessment of embryos include the presence, in one or both parents, of single gene mutations or structural chromosome aberrations, !such as balanced reciprocal/Robertsonian translocation or chromosomal inversion in one or both parents. PGD, therefore, is a targeted evaluation designed to identify or exclude the presence of an undesirable genetic aberration, known to exist in one or both parents, in embryos before uterine transfer. By placing back only embryos not affected by the disorder in question, the chances of passing on the disorder are dramatically decreased.

Single-Gene Disorders

Single-gene disorders are DNA sequence variations in a gene that cause a specific type of genetic phenotype (i.e., delta F508 and cystic fibrosis). Single-gene disorders are characterized by their patterns of transmission in families. To establish the pattern of transmission, the first step is to obtain information about the family history of the patient and to summarize the details in the form of a pedigree.

Single-gene inheritance patterns include autosomal dominant (AD) inheritance, autosomal recessive (AR) inheritance, X-linked dominant inheritance, or X-linked recessive inheritance. Using the information obtained from this pedigree, a targeted search for certain genetic disorders can be performed. For example, in women with a strong family history of breast and/or ovarian cancer, testing for *BRCA* gene mutations may be indicated.
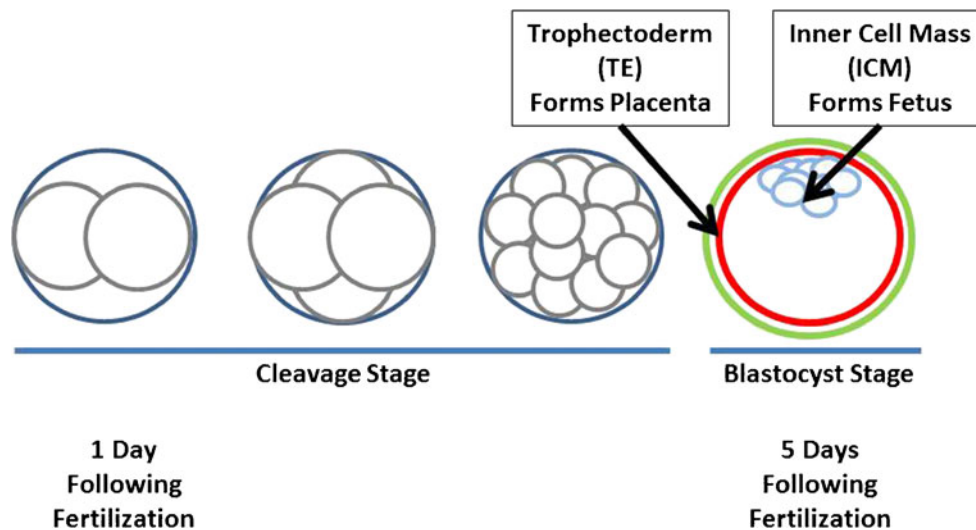
Fig. 1 **Embryo Development** This figure outlines the stages of embryo development from fertilization to the blastocyst stages

Testing for Single-Gene Disorders

The most common method for testing single-gene disorders is by genotyping or direct sequencing. Although not the only method for performing PGD, the most common method used to obtain cells for PGD analysis is by embryo biopsy at the cleavage stage [8, 9]. However, trophectoderm biopsy of blastocyst stage embryos is becoming increasingly common.

Because only one or a few cells are biopsied for single gene testing, a DNA amplification step must be included in the analysis [10, 11]. Additionally, something known as a modified linkage analysis assay also often is used to increase diagnostic accuracy [12•]. There is a risk of misdiagnosis with PGD that is introduced by failure to amplify genetic material (allele drop out), achieving only incomplete amplification (partial amplification), or contamination [13–15]. By incorporating a modified linkage analysis assay in the single gene mutation analysis, one greatly reduces the risk of allele dropout which is the leading cause of a single-gene misdiagnosis [16–19].

PGD for HLA Typing

Another application for PGD is human leukocyte antigen (HLA) typing [20, 21]. This technology is generally employed by parents who have a child affected by a particular disorder that could benefit from some sort of human tissue transplant: for example, a child with leukemia who requires a bone marrow transplant. In these cases, PGD has been employed as a modality to ensure that the next child that the couple conceives will be HLA compatible with their existing child with the given illness. This practice is relatively uncommon but has generated considerable debate regarding the ethics of HLA typing PGD [22].

Structural Chromosome Aberrations

PGD to detect structural chromosome imbalances in embryos are due to balanced parental chromosome rearrangements [23]. Parental chromosome rearrangements include reciprocal translocations, Robertsonian translocations, pericentric inversions, or paracentric inversions. Chromosomal rearrangements do not usually have a phenotypic effect if they are balanced, because all of the chromosomal material is present even though it is packaged differently. Even when structural rearrangements in parents are truly balanced, they can pose a threat to their subsequent generation because carriers are likely to produce a high frequency of unbalanced gametes and therefore have an increased risk of having a miscarriage or an abnormal offspring with a genetic syndrome related to the unbalanced karyotype [24, 25]. The degree and severity of the phenotype observed in the offspring with the unbalanced karyotype depends upon the chromosomes (genes) involved in the structural chromosome imbalance [26].

One can test for structural chromosome aberrations using fluorescence in situ hybridization or microarrays (discussed in later section) [23]. FISH uses telomere specific probes for chromosomes involved in the structural chromosome imbalance along with centromeric markers of the appropriate chromosomes. Increasingly, microarrays are being utilized instead of FISH for evaluation of embryos in patients with documented translocations or inversions. While arrays are not able to detect embryos with balanced translocations, arrays are capable of detecting chromosomal imbalances both related to the parental chromosome aberration and other aneuploidies in all 23 pairs of chromosomes. This approach has resulted in a significant improvements in pregnancy rates with array based, compared with FISH, technology in this patient population.

Preimplantation Genetic Screening

The Background of PGS

In standard IVF cycles the one or two "best" embryos as determined by their physical appearance are placed into the uterus and the remaining surviving embryos are cryopreserved. Determining which embryos are the "best" has been a subject of much debate since the birth of the technology in the late 1970s. Traditionally, the use of morphology, the visual appearance of embryos, has been the principal modality of choosing optimal embryos for uterine transfer [7•].

However, the implantation rate per transferred embryo in most clinics rarely exceeds 40 % [27]. Therefore, many investigators have for some time been searching to establish other diagnostic methods capable of more accurately determining embryo quality than morphology alone. This effort has produced several promising technologies, including metabolomics, real-time videography, and preimplantation genetic screening.

The Evolution of PGS

Certain patient populations, including couples with advanced maternal age, recurrent miscarriage, repeated implantation failure, and severe male factor, are thought to have a predisposition for producing aneuploid embryos [7•, 27, 28]. Many have suggested that these patient populations may be benefit from PGS [7•, 29]. However, the indications for using PGS in many centers are constantly expanding [7•]. As reported by the European Society of Human Reproduction and Embryology (ESHRE), over the past 10 years, 61 % of all preimplantation genetic testing cycles were performed for PGS [30•].

PGS, unlike PGD, has been and continues to be a controversial technology. Recent studies indicate that greater than 60-90 % of all first-trimester miscarriages may be the result of chromosomal aneuploidy [7•]. Because so many early miscarriages are due to aneuploidy, PGS seems to be a reasonable intervention to improve the efficiency with which euploid (chromosomally normal) embryos are selected for uterine transfer in IVF cycles. Classic studies have reported that miscarriages that are caused by aneuploidy are disproportionally concentrated on select chromosomes [31]. These data are based on karyotype analysis of failed pregnancies that developed far enough along to have tissue available for genetic analysis [31, 32]. Consequently, clinics performing PGS in the early days of the technology focused on detecting aneuploidy on only select chromosomes using fluorescence in situ hybridization (FISH), which typically evaluates between 5-14 chromosome pairs rather than all 23 chromosome pairs [33, 34]. Traditionally, PGS biopsy was exclusively performed at approximately 3 days of embryonic development following fertilization [33, 34]. Initial data using PGS in the context of cleavage stage biopsy with

FISH showed promising results and generated much excitement for this new technology [7•, 35–37]. Unfortunately the results from this approach failed to result in improvements in clinical pregnancy rates and this lack of efficacy was widely referenced following a landmark paper by Mastenbroek in the *New England Journal of Medicine* [38•]. Subsequently, similar papers cast further doubt on the benefits of PGS and position statements from major medical societies formally discouraged its use [3, 39, 40].

Further research, however, elucidated several biologic limitations that could explain the prior shortcomings of clinically applied PGS. The practice of polar body biopsy to determine the genetic composition of a fertilized oocyte is a commonly utilized modality for performing preimplantation genetic testing [7•, 8, 41]. A critical component of oocyte development is meiotic division in which a haploid set of unused maternal DNA is marginalized into what is termed a polar body [7•, 8]. Genetic evaluation of this polar body was initially quite popular as this process obtained a diagnosis without disturbing the developing embryo and could be performed before fertilization [7•]. However, this approach is incapable of detecting paternally derived genetic errors or any errors introduced after or during fertilization. Due to these limitations, polar body biopsy is now principally performed in countries where strict legislation limit the practice of embryo biopsy [7•, 42].

However, PGS using biopsied cells from developing embryos also presents challenges. Studies have repeatedly documented that embryos at day 3 of development have high levels of mosaicism [43, 44]. Mosaicism is a condition in which a single developing embryo is comprised of more than one distinct genetic cell line. In other words, mosaic embryos may have euploid (normal) and aneuploid (abnormal) cell lines within a single embryo. Studies evaluating this phenomenon have concluded the majority of all embryos may be mosaic at day 3 of development [43–45]. Consequently, a biopsy performed at day 3 of development may produce a result that is not representative of the entire embryo [7•]. Mosaicism has been shown to also exist at day 5 of embryo development [46]. However, recent data suggest that mosaicism may be much reduced by day 5 of development [7•, 47].

Another limitation of traditionally performed PGS was the use of FISH for determination of chromosomal abnormalities. FISH typically evaluates between 5-14 rather than all 23 chromosome pairs [48]. Recent studies have indicated that embryonic aneuploidy occurs in clinically significant amounts in all 23 chromosome pairs [49•]. Therefore, FISH is incapable of diagnosing many of the chromosomal abnormalities commonly found in developing embryos.

Realization of these two principal limitations have led many genetic laboratories to offer PGS using technologies evaluating the chromosomal status of all 23 chromosome pairs using an embryonic biopsy performed at the blastocyst stage, typically reached by day 5 or 6 of development. The clinical

pregnancy rates using this approach have been reported to be markedly superior to the traditional approach of performing PGS [50, 51]. For example, a recent study evaluating more than 4,500 embryos using 23 chromosome pair determination found clinical pregnancy rates in women suffering from recurrent pregnancy loss (RPL) to be significantly improved above similar studies using FISH PGS [50]. Additionally, pregnancy rates were further improved when 23 chromosome evaluation PGS was performed on blastocyst stage embryos (day 5/6 of development) compared with when the biopsy was performed on embryos at day 3 of development [33, 50, 52]. Similar results have been reported consistently by many clinics in the United States and around the world [33, 52]. This has caused a renewed interest in PGS, although it still remains to be determined if PGS is an efficacious technology and which patient populations are best served by PGS.

Evaluation of all 23 chromosomes in the context of PGS possesses inherent complexities that potentially may compromise the integrity of data if not properly performed. There are multiple approaches that are used to perform 23 chromosome pair evaluation. The two modalities that are most commonly utilized today both utilize microarray technology, either using single nucleotide polymorphism (SNP) or comparative genomic hybridization (CGH) technology [7•]. Both of these technologies rely on obtaining embryonic DNA, fragmenting and then amplifying this DNA, and evaluating this amplified product using microarrays. This amplification process is a potential source of error as failure to amplify the entire embryonic DNA product could produce a false result. Additionally, because the DNA product being initially amplified is taken from only one to several cells, any external DNA contamination can produce a spurious result. Both CGH and SNP microarray platforms are appropriate platforms to use while performing PGS, each with its own advantages and disadvantages [7•].

Evidence for the Clinical Application of PGS

Studies from centers using PGS on day 5 and 6 blastocysts show promising results with transferred embryos generating pregnancy rates greater than 75 % [33, 50, 52]. However, a central criticism of the widespread use of PGS is a lack of randomized, controlled trials that conclusively show the procedure to be beneficial. To our knowledge, at the time of the writing of this article, there is only one randomized trial to show a pregnancy benefit using PGS versus IVF using morphology alone in the setting of exclusive single embryo uterine transfer [53]. This study was relatively small and had several significant limitations. Therefore, further larger and more rigorously studies are required before PGS will be more broadly accepted [7•]. However, several large, randomized, controlled clinical trials are currently underway that will hopefully provide such data in the near future.

Despite the lack of support from professional societies and lack of large, randomized, controlled trials definitively demonstrating the benefits of the technology, PGS comprises the majority of all preimplantation genetic testing internationally and is being increasingly utilized [8]. However, the patient population in which PGS may be appropriate is unclear at the present [7•]. Many PGS clinics have traditionally recommended PGS for couples with risk factors believed to be associated with embryonic aneuploidy such as unexplained RPL, severe male factor, and advanced maternal age. However, in recent years many clinics have liberally expanded the use of PGS to many women without such risk factors. In fact, some clinics broadly recommend PGS to virtually all IVF patients as a strategy to improve pregnancy rates in all couples battling infertility. The debate surrounding the appropriate patient populations for PGS is currently in flux and will likely be a source of debate for years to come.

Limitations of PGS

Despite the positive data that are emerging within the field of PGS, there are tangible technical and biological limitations to the technology. The limitations of FISH PGS evaluation and the use of biopsy taken from day 3 embryos are significant and have been discussed previously. Additionally, technical limitations surrounding the use of both SNP and CGH arrays may produce spurious results if not properly executed [7•]. Furthermore, while automated results are generated, the raw data also are interpreted by a trained geneticist. Therefore, the interpretation of results may be subjectively interpreted and this leaves inherent room for human error in addition to inaccurate automated resulting.

Perhaps the most significant source of error from PGS using 23 chromosome pair evaluation and blastocyst biopsy (day 5/6 of development) is the presence of cellular discordance within the developing embryo. A blastocyst embryo is comprised of two components, an inner cell mass (ICM) and trophectoderm (TE) [7•]. The ICM possesses cells that are destined to form fetal tissue and the TE possesses cells that will form the placenta. Blastocyst biopsy utilizes cells taken from the TE to minimize potential deleterious effects that may be caused from biopsy of the ICM, cells destined to form the fetus. Although there is clearly a high degree of correspondence between the genetic composition of the ICM and the TE, some data suggest that in up to 10 % of developing blastocysts, there may be aneuploidy present in the TE but not ICM or vice versa [47]. Therefore, TE biopsy taken at the blastocyst stage, from a biological standpoint, may not be universally predictive of the chromosomal status of the developing embryo even if no technical error exists in the performance of genetic analysis [3, 7•]. Mosaicism also may exist within a given TE cell population. Array technology, however,

is capable of detecting all but very low levels of mosaicism within TE samples analyzed provided [47, 54]. The above limitations of PGS demand that patients be adequately counseled on the risks and limitations of PGS, preferably with the aid of a specialized physician, geneticist, or genetic counselor. Furthermore, antenatal genetic testing is still recommended in all patients undergoing PGS [7•].

Conclusions

New technologies associated with both PGD and PGS are producing encouraging data suggesting that these procedures will continue to be valuable adjuncts to assisted reproductive technologies in the future to optimize outcomes for many patients. While PGD is a well-accepted technique in selected patients, the optimal patient population that may benefit from PGS is still controversial. However, Both PGS and PGD are increasingly being applied to patients with ever expanding clinical indications.

Compliance with Ethics Guidelines

Conflict of Interest Paul R. Brezina, Patrick Jaeger, Michael A. Kutteh, and William G. Kearns declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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