Looking to the future: developments in preimplantation genetic diagnosis

Since the first clinical preimplantation genetic diagnosis (PGD) cycles carried out in 1989, continuous technical improvements have supported progression from what was initially perceived to be an experimental procedure to a widely acceptable alternative to conventional prenatal diagnosis. PGD requires the use of assisted reproductive technology (ART) to create the preimplantation-stage embryo, followed by biopsy to obtain cell(s) for genetic analysis and, finally, transfer of selected embryos to the womb to establish a pregnancy. PGD is an important reproductive option for parents at high risk of transmitting a single-gene or specific chromosomal abnormality to their children (high-risk PGD), supporting the establishment of a healthy pregnancy while precluding possible pregnancy termination. Alternatively, embryos may be tested for ploidy status, a test widely known as preimplantation genetic screening (PGS). PGS is considered to be a low-risk form of PGD, offered to women of advanced maternal age or couples with poor reproductive history, which aims to select euploid embryos for transfer to improve the implantation and live birth rates after ART. However, low-risk PGD is controversial and constitutes one of the most highly debated topics in reproductive medicine over the last decade, chiefly because it was introduced into routine clinical practice before its clinical benefit was clarified. Reports to date evaluating ART outcomes following PGS have shown contradicting evidence, mainly complicated by the numerous parameters involved in PGS procedures, many of which may introduce bias. It is paramount to resolve this issue and the only way is through large multicenter randomized controlled studies, such as one currently being organized with the support of the European Society of Human Reproduction and Embryology (ESHRE) [1].

Today, both high-risk and low-risk PGD cycles are widely performed, and the number of indications has increased steadily. Based on the latest data published by both ESHRE and the American Society for Assisted Reproductive Technology, we can estimate that approximately 10,000 cycles are completed each year worldwide [2,3]. ART and PGD/PGS are closely linked, aiming to achieve the birth of healthy babies, and both have undergone significant developments over the last 23 years [4].

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Where are we now?

The steps of ART in PGD are the same as those for fertility treatments, except for certain aspects of embryology. These include intracytoplasmic sperm injection (ICSI) for oocyte fertilization, recommended to preclude contamination by excess sperm at biopsy, and the embryo biopsy itself. Biopsy procedures have evolved, relative to both the techniques for zona breach and to the stage of embryo development at which biopsy is performed. To date, biopsy of cleavage-stage embryos is most commonly used. Polar body biopsy is considered more ethically and biologically acceptable (the material removed is superfluous to the formation of the embryo), and represents the only choice for countries where embryo testing is prohibited. It is, however, a technically laborious option, providing information only for the maternal genetic contribution, thus leaving the full embryo status unknown. Alternatively, blastocyst-stage biopsy allows the testing of multiple cells versus a single cell. However, time to complete a genetic analysis is limited, potentially introducing the need for embryo freezing (vitrification), technologies for which have lately vastly improved, demonstrating high embryo survival rates [5].

Other developments on the embryology front involve the technology and media for in vitro maturation and improved embryo culture. Of course, higher numbers of mature oocytes have a direct impact on PGD treatment success, while the possibility of culture to the blastocyst stage facilitates selection of potentially the most viable preimplantation embryos for transfer.

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Genotyping protocols for PGD need to have the highest diagnostic efficiency and accuracy. Single-gene PGD protocols have mostly been based on PCR and are continuously evolving. The most robust diagnostic strategies are multiplex, and investigate the disease-associated locus both directly and indirectly (the latter through linked markers), confirming the genotype with multiple results. To address the limitations of single-cell analysis, whole-genome amplification (WGA) has been introduced as the initial step for single-cell DNA amplification, followed by PCR-based protocols. Compared with PCR alone, WGA can support the application of more generic, highly multiplexed haplotype analysis, precluding the development of case-specific tests and the use of single-cell conditions. In addition, WGA provides more material, allowing reanalysis, or even analysis for further indications [6].

Methods for the chromosomal analysis of preimplantation embryos have included single-cell FISH, metaphase comparative genomic hybridization (CGH) and array CGH. Low-resolution array CGH, requiring a preanalytical step of WGA, is currently the most widely used technique for both high-risk (parental translocations) and low-risk PGD. It enables a rapid, relatively low-cost analysis of the complete chromosomal complement of a cell. Its main shortfalls are the inability to detect polyploidies, gains or losses in regions not covered by the array. In addition, for high-risk PGD (translocations), PGS detects abnormalities additional to those being investigated (important clinically) but which means that couples may have no embryos for transfer, an issue that should be addressed during counseling [7,8].

Where are we going?

Even for fertile couples undergoing PGD, pregnancy rates rarely surpass 30–35% [3,9]. Thus the selection of the best quality embryo(s) for transfer is a key objective, also driven by the trend towards single-embryo transfer, to avoid maternal and neonatal risks associated with multiple pregnancies. Besides unreliable subjective morphological embryo assessment (e.g., fragmentation and cleavage rate), determining the euploid status of an embryo by PGS has so far been the main—although controversial—method of predicting potential embryo implantation and live birth. However, the invasive biopsy procedure involved introduces risk of embryo damage, while additionally the cells biopsied and tested may not represent the remaining embryo owing to embryo mosaicism [10].

Methodologies for noninvasive embryo assessment are under intense investigation, with ‘omics’ approaches including transcriptomics, proteomics and metabolomics. The transcriptome of cumulus cells (with a known role in oocyte maturation and competence) is a main focus of investigations to identify potential biomarkers for noninvasively predicting oocyte competence, embryo quality and pregnancy outcome, and, more recently, oocyte aneuploidy [11,12]. miRNAs may also represent a new category of biomarkers, and preliminary studies have already indicated their importance in oocyte maturation and embryo development, as well as their association with human infertility [13,14].

Other noninvasive approaches include investigation of embryo metabolic activity, embryo proteome and secreted factors in culture media, potentially identifying important characteristics of good-quality embryos [15]. However, these approaches are limited by the tiny quantities of material for analysis, and the use of specialized and often costly instrumentation.

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The future of ART is likely to involve the standardization and automation (robotics) of procedures in gamete assessment, embryo growth and monitoring. The production of gametes from stem cells may also become an option, although likely controversial. Overall, the aim is to support improved outcomes through ensuring optimal sample quality for PGD analyses and the selection of healthy embryos with the highest chance of implantation [16,17].

The fast-emerging technologies of arrays and next-generation sequencing for genome analysis potentially offer generic approaches for simultaneous ploidy analysis (PGS) and high-risk PGD. In 2009, a seminal approach was described, named ‘karyomapping’, based on the use of single-nucleotide polymorphism (SNP) arrays for embryo fingerprinting, enabling – through family linkage (or ‘parental support’) – analysis of single-gene
disorders along with aneuploidy testing. Karyomapping and analogous SNP array approaches can also potentially distinguish normal from balanced embryos in translocation cases, and identify uniparental disomy and the parental origin of abnormalities. However, SNP array analysis of single cells involves long, complex laboratory protocols, specific instrumentation, and for data analysis, specialized algorithms and software. The complexity of data generated and the high cost of procedures are currently the main obstacles to the routine introduction of SNP arrays in PGD. Efforts are underway to address these limitations, and some progress is evident. However, this technology awaits thorough validation and more knowledge to support interpretation of complex genetic information produced [18,19].

Next-generation sequencing in PGD may facilitate multiple-gene testing, the detection of SNPs, copy-number variants and chromosomal aneuploidies, as well as epigenetic profiling. Its use at a single-cell level has been described in cancer and for PGD is currently being explored. Besides providing a more holistic approach to PGD, next-generation sequencing in patients undergoing ART may additionally support more ‘personalized’ procedures.

Other genetic technologies are also expected to revolutionize PGD applications. Techniques such as transfer of ooplasm or nuclei may, in the future, provide an alternative solution to avoid the transmission of disorders that are currently difficult to diagnose by PGD, such as mitochondrial disorders [20].

The ultimate outcome of PGD (necessarily involving ART) is the birth of a healthy child. Although the future of PGD will likely incorporate many of the aforementioned approaches, it is imperative that safety and clear clinical benefit are established prior to their introduction within clinical practice. The innate biology of human reproduction will always limit the positive outcome of ART and thus PGD, while additional complex issues, such as the impact of epigenetics on embryos, remain unresolved. Ideally, all developments in PGD should be based on hypothesis-driven research and the exchange of expertise and knowledge through international forums, ensuring that PGD is always applied with the highest standards of laboratory, clinical and ethical conduct.

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References


