Review

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Preimplantation genetic screening: Pitfalls and opportunities

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Abstract

Introduction

Preimplantation genetic screening is an increasingly utilized as a tool to optimize the efficiency of IVF in a variety of clinical situations. Initial data from PGS was discouraging using florescence in situ hybridization (FISH) and cleavage stage biopsy. However, more recently the trophectoderm biopsy at the blastocyst stage of embryonic development coupled with evaluation of all 23 chromosome pairs has yielded encouraging clinical results.

Conclusion

Current data suggests that appropriate application of PGS evaluating all 23 chromosome pairs with blastocyst stage biopsy may significantly improve the efficiency and efficacy of IVF for many patients.

Introduction

Preimplantation genetic screening (PGS) has emerged as a powerful tool to optimize pregnancy outcomes within the context of Assisted Reproductive Technologies. This procedure is performed in the context of an in vitro fertilization (IVF) cycle in which oocytes are surgically obtained from stimulated ovaries and fertilized with sperm resulting in embryos. PGS is the practice of performing a biopsy on each of these resulting embryos and then testing the DNA obtained from this biopsy for aneuploidy. Following this, euploid embryos are then selected for either transfer to the uterus or cryopreservation for future transfer attempts.

Aneuploidy is common among developing embryos. The majority of fertilized embryos that fail to develop fail to implant into the maternal uterus, or result in early miscarriage are aneuploid. Therefore, the purpose of PGS is to increase the efficiency of IVF per embryo transfer by of identifying euploid embryos. Theoretically, this should increase the pregnancy rate and decrease the miscarriage rate per embryo undergoing transfer.

Recent clinical data shows a significant benefit to PGS in certain populations when the proper techniques and technologic diagnostic platforms are utilized. However, failure to utilize these techniques and platforms leads to suboptimal results. In this review we seek to define the limitations associated with PGS and identify strategies to minimize these pitfalls and increase the efficiency of the procedure.

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Determining the Optimal Embryonic Stage to Perform Embryo Biopsy

Determining exactly where to obtain cells for PG testing has been a subject of some debate since the inception of the procedure.[1] One method is to biopsy the polar body (PB) obtained from an oocyte.[1,2,3] Other methods rely on taking cell(s) from the developing embryo at either the cleavage stage or blastocyst stage of development. [1,4] All three of these techniques have been described to perform PGS.[1, 2, 4, 5]

To understand how these biopsy methods differ, a basic understanding of early human embryology is necessary. Human embryonic development follows a relatively predictable timeline in most cases. The human oocyte is comprised of a diploid set of chromosomes that undergo a series of meiotic divisions, once at the time of ovulation and once again at the time of fertilization. This process results in unused DNA that is relegated to 2 PBs that exist within the zona pellucida.[1,6] Three days following fertilization, most human embryos have reached the cleavage stage at which point they are comprised of about 6-8 totipotent cells. [1,6] (Figure 1) Totipotent cells are thought not to be committed to any specific cellular fate. Therefore, any of the 6-8 cells within a cleavage stage embryo may differentiate down any cell line, either fetal or placental. [1,6] By 5-6 days following fertilization, most embryos have reached the blastocyst stage. Blastocyst embryos are comprised of around 100 cells and have a clearly defined fetal component, the Inner Cell Mass (ICM), and a placental component, the Trophectoderm (TE)[1,6] (Figure 1), Unlike cells in the cleavage stage embryo, the cells in the blastocyst embryo are pluripotent. Pluripotency means that cells have now been committed down specific cell lines.[1,,6] TE cells may only follow a placental path and ICM cells may only follow a fetal path. [1, 6]

PB biopsy has the advantage of evaluating a part of the oocyte that will become neither the placenta nor embryo. Subsequently, concerns regarding a possible deleterious impact on embryogenesis from direct embryo biopsy are in theory minimized. [7,8] A significant disadvantage of PB biopsy, however, is that this approach evaluates only maternal, not paternal, DNA prior to the development of the embryo. [1, 9,10,11] Therefore, any errors that occur after the meiotic division that forms the PB or any errors associated with the paternal contribution are, by definition, not identified by PB biopsy. Recent prospective and retrospective data suggest that PB biopsy may be less accurate in PGD and PGS compared to embryo biopsy. [12,13,14] The utilization of PB biopsy has significantly declined in recent years. [1] However, PB is still utilized,

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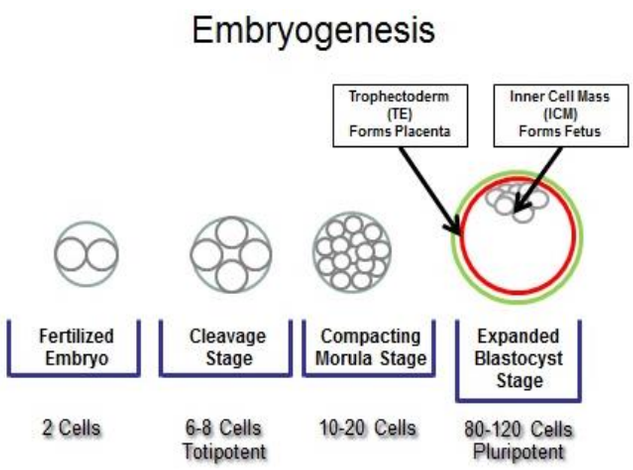

Figure 1: This figure depicts the course of early embryo development. The figure on the far left depicts a 2 cell embryo the day follow fertilization. The figure immediately right of the labeled "cleavage stage" depicts an embryo 3 days following fertilization. The figure to the right of this labeled "compacting morula stage" depicts an embryo between the cleavage and blastocyst stage. The figure on the far right depicts an embryo that has achieved the expanded blastocyst stage with a clear distinction between the trophectoderm and ICM cell populations.

especially when legislation curtails or even outlaws embryo biopsy for PGD or PGS. [9,10,15,16]

Historically, cleavage stage biopsy has been the most commonly utilized method of performing PG testing. [1,11,17] A clear advantage of cleavage stage biopsy is that cells obtained at this stage, unlike PB biopsy, contain DNA from the actual embryo. Several disadvantages, however, do exist. Firstly, removal of a cohort of totipotent cells found within the cleavage stage embryo is now thought not to be entirely benign. Intuitively, this makes sense as removing just a single cell from an 8 cell embryo is removing 1/8th of the embryonic cellular mass. Prospective randomized data indicates that just the act of embryo biopsy at the cleavage stage confers a significant deleterious effect upon subsequent embryonic development, both in terms of developmental pace and overall embryo survival. [18,19]

The phenomenon of embryonic mosaicism introduces another significant drawback to performing cleavage stage embryo biopsy. Many, and perhaps most, embryos at the cleavage stage have more than one karyotypic cell line. [1,9,11,20,21] This phenomenon of mixed cell lines within a single developing embryo is termed mosaicism.[22] Because of mosaicism, the cell selected for biopsy within a developing cleavage stage embryo may not represent the cell line within the embryo that will eventually emerge as dominant.[23] Therefore, there is a strong biologic mechanism that may introduce error with cleavage stage biopsy even in the presence of accurate genetic analysis.

Because of these limitations, obtaining cellular biopsy from the TE of blastocyst stage embryos has recently emerged as the preferred modality of PG testing in many centers. [1,11,23] Like cleavage stage biopsy, but unlike PB biopsy, TE biopsy obtains embryonic DNA. The ICM, however, is untouched with this technique and the developmental lag and increased mortality seen with cleavage stage biopsy is thought not to be less significant. [19,24] Karyotypic discordance still may exist between the ICM and TE cell lines. [1,22] However, the rate of mosaicism at the blastocyst stage is thought to be significantly reduced compared to rates observed in cleavage stage embryos. [1,22,25] Current retrospective and prospective clinical PG testing data generally supports the utilization of TE biopsy at the blastocyst stage over cleavage stage biopsy. [14,19,24,26]

Determining the Optimal Platform for Genetic Analysis

FISH

The first reported cases of PGS utilized florescence in situ hybridization (FISH) as the genetic diagnostic platform. Clinical data was initially reported to be encouraging for optimized pregnancy outcome using PGS with FISH. However, a series of prospective trials failed to demonstrate a benefit to the use of PGS with FISH and cleavage stage biopsy.[27,28] Consequently, major professional societies do not currently recommend the route use of clinical PGS. [29,30,31,32,33]

FISH has a number of significant limitations that hinder its effectiveness in the setting of PGS. Several studies have questioned the ability of the FISH platform to consistently determine aneuploidy in the setting of single cell evaluation. [34] FISH is capable of generating a result in a relatively short time frame (4-10 hours). [26] However, the accuracy is potentially compromised by a number of factors including hybridization errors. [10,35] Additionally, FISH is technically demanding, and operator error is common when the individual performing the procedure is not experienced.

However, the most significant limitation of FISH when used for the purposes of PGS is its general inability to detect aneuploidy in all 23 chromosome pairs.[10,26,36] Aneuploidy in developing embryos can be seen in any of the 23 chromosomes.[36] FISH generally only evaluates a select number of chromosomes (5-12 chromosome pairs). The inability to provide comprehensive chromosomal screening places FISH at an inherent disadvantage for PGS as many embryos harboring aneuploidy cannot be diagnosed.

Diagnostic Platforms Evaluating All 23 chromosome Pairs

The genetic diagnostic platforms available to perform PGS have greatly increased in the past decade. One of the first technologies capable of evaluating all 23 chromosome pairs for aneuploidy was comparative genomic hybridization (CGH) on metaphase chromosomes. While accurate, this approach was laborious and required a highly skilled operator to perform well. [1, 26 ,37,38,39,40]

More recently, microarrays have proven to be excellent platforms with which to perform PGS testing. Microarrays may use either a single nucleotide microarray (SNP) or CGH platform. Each of these approaches has various

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advantages and disadvantages. For example, CGH allows for a more rapid testing time but SNP mircorarrays are capable of detecting loss of heterozygosity and trisomy. [41,42] In addition to CGH and SNP arrays, other technologies such as quantitative polymerase chain reaction (qPCR) have been successfully utilized to perform 23 chromosome pair PGS. qPCR is able to evaluate aneuploiody in all 23 chromosome pairs in a relatively short period of time (4-6 hours) but has a relatively low density of probes and can fail to detect chromosomal deletions or duplications. [26,43]

Perhaps one of the more promising technologies available currently to perform 23 chromosome pair PGS analysis is next generation sequencing (NGS). NGS has recently been applied to PGS with encouraging results. Unlike other forms of PGS testing, NGS has the ability to scan areas of the genome for specific DNA sequences (A, T, C, or G), lending itself to a host of other applications in addition to PGS testing for aneuploidy alone. [44,45,46,47] For example, the ability to perform sequencing may be utilized to test for specific genetic diseases (a procedure known as preimplantation genetic diagnosis, or PGD) while concurrently searching for aneuploidy on the same platform. [44,45,46,47]

Validations by CGH or SNP microarray platforms and real-time PCR analysis have demonstrated that each technology provides accurate PGS diagnostic results.[43,44,48,49,50,51,52,53] Therefore, for the purposes of 23 chromosome pair evaluation PGS, any of these technologies is acceptable and results in comparable clinical outcomes. However, in the opinion of the authors, NGS is superior to many other available modalities because of its additional power to diagnose genetic sequences in addition to aneuploidy. Therefore, the increased utilization of NGS platforms at the expense of other technologies in the future is likely.

Conclusion

PGS is a technology that is increasingly utilized as a strategy to maximize the efficiency of IVF. Genetic diagnostic platforms utilizing FISH are now thought to be inefficient and inferior to other technologies for performing PGS. Similarly, cleavage stage biopsy is now thought to be inferior to biopsy of the trophectoderm at the blastocyst stage. Currently, available data encourages PGS to be performed in the setting of a trophectoderm biopsy at the blastocyst stage with genetic analysis using a platform evaluating aneuploidy in all 23 chromosome pairs. NGS is an emerging technology likely to become dominant for the application of PGS.

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