Refresher Course

Preimplantation Genetic Diagnosis

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INTRODUCTION

Preimplantation genetic diagnosis (PGD) has been presented as a recently developed diagnostic procedure that offers an alternative to prenatal diagnosis (PND) for detecting single gene defects and aneuploidy as well as structural chromosomal abnormalities.[1-3] Early diagnosis of genetic defects in the embryos used in in vitro fertilisation allows the selection and transfer of unaffected embryos to the couples at risk, leading to hopefully normal pregnancies and avoiding termination of affected pregnancies.[4] Advanced molecular cytogenetic techniques used in PGD, which are polymerase chain reaction (PCR) and fluorescent in situ hybridisation (FISH), have been performed for diagnosis of embryo gender[5,6], age-related aneuploidy[7], specific gene defects, e.g. cystic fibrosis[8,9], Tay-Sachs disease[10], thalassaemia[11]; X-linked diseases, e.g. Duchenne muscular dystrophy[12]; inherited dominant diseases, e.g. Marfan's syndrome[13]; inherited cancers, e.g. familial adenomatous polyposis coli[14]; and chromosomal abnormalities.[15,16]

PGD consists of three important processes, which are; in vitro fertilisation (IVF), isolation of genetic material from preconception oocytes or embryos at various stages of development, and genetic diagnosis based on PCR or FISH.

In vitro fertilisation for preimplantation genetic diagnosis

In vitro fertilisation has offered the opportunity to manipulate the embryo(s) and provided enough embryos for PGD. IVF involves the administration of gonadotrophin-releasing hormone (GnRH) for inducing hypothalamopituitary down regulation.[17] Human menopausal gonadotrophin (HMG), highly purified follicle-stimulating hormone (HP-FSH) and recombinant FSH are introduced for stimulating the follicular growth.[18-21] Follicular growth is monitored by transvaginal ultrasonography. Human chorionic gonadotrophin (HCG) is administered to induce ovulation when the follicles reach the optimal size. Oocytes are retrieved 36 hours after HCG administration and incubated under culture condition. Intracytoplasmic sperm injection (ICSI) is performed to achieve fertilisation. The embryos are biopsied at various stages in order to obtain genetic materials for PGD.

Isolation of genetic materials from preconception oocytes or preimplantation embryos

The isolation of the first polar body from an oocyte was first reported in 1990.[22] This technique has been used for age-related aneuploidy[23-25], translocation abnormalities[26] and maternal single gene defects.[27] Since some aneuploidies can arise in meiosis II, analysis of the second polar body has been suggested together with the first polar body.[22] Nevertheless, the disadvantages of this technique are that it provides only maternal genetic information.[28] It is labour intensive and may fail to diagnose the possibility of recombination and post-zygotic events.[29]

Cleavage stage embryo biopsy has been the preferred technique for most groups performing PGD.[30] The 8-cell stage has been suggested to be the most suitable stage for embryo biopsy because of its

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higher mitotic index and totipotency.[31] Since at the cleavage stage there are a limited number of blastomeres to be biopsied, the occurrence of mosaicism if found in the embryos might lead to a misdiagnosis in PGD.[7,23,32] Dual blastomere analyses can reduce the likelihood of misdiagnosis and has no effect on blastocyst formation.[15,29,33]

In human, the number of cells in blastocysts increases from an average of 58 to 126 cells between day 5 to 7 post-insemination and the majority are trophectoderm cells.[34] Blastocyst stage biopsy offers a greater number of cells, available for genetic analysis.[35,36] A greater number of cells available for analysis may decrease the likelihood of misdiagnosis, which can occur when the number of cells obtained from the biopsy is limited, and may allow more diagnostic techniques to be performed.[35,36] It is thought that removal of trophectoderm cells from blastocyst is unlikely to have an effect on the development of the embryo proper,[28] and avoids ethical concerns over removal of totipotent cells.[37] However, the disadvantage of blastocyst biopsy is that a limited number of embryos reach the blastocyst stage.[38] Although using co-culture or appropriate culture medium may overcome developmental arrest and result in higher pregnancy rates for IVF, blastocyst culture may not provide enough blastocysts for PGD and intrauterine transfer. The possibility of confined placental mosaicism (CPM), where the trophectoderm may possess different genetic material from the inner cell mass, has been considered to be another disadvantage.[39]

Several methods for opening the zona pellucida of oocytes and embryos have been developed. Mechanical biopsy methods, which are partial zona dissection[40-42] and piercing[42], have been reported previously. Chemical dissolution by acid Tyrode's solution was eventually introduced for embryo biopsy.[43,44] The toxicity and cytoplasmic acidification of acid Tyrode's solution is thought to change the intracellular pH, leading to cytoplasmic degeneration.[45] However, Cui et al.[46,47] and Viville et al[48] reported that no significant adverse effect was found in the embryos which were biopsied by acid Tyrode's solution. Recently, embryo biopsy by laser has been developed for use in PGD.[36,49,50] Laser assisted blastocyst biopsy has been suggested as an alternative tool in PGD because of its reliability and the possibility that diagnosis can be offered because of the greater number of cells obtained from the biopsy. However, this technique has not been used clinically to date.[30]

Removal of genetic material can be divided into four methods, which are; displacement, stitch and pull, push, and aspiration. The aspiration method has been used for clinical PGD by the majority of centres.[30] Using this method, the blastomere being genetically analysed in cleavage stage biopsy, is sucked into the biopsy micropipette that is in direct contact with the blastomere through the opening in the zona pellucida, as in Figure 1.

Molecular genetic diagnosis techniques

The genetic materials, which are isolated from the various stage embryos, are further analysed by molecular genetic diagnostic techniques based on polymerase chain reaction (PCR) and in situ hybridisation (FISH).

Polymerase chain reaction (PCR)

PCR has been developed in order to rapidly amplify fragments of deoxyribonucleic acid (DNA) from a small number of cells for further analysis.[51] This approach has been recommended for genetic diagnosis because of its capability of amplifying DNA from as few as one or two embryonic cells. PCR was first used for the diagnosis of embryo gender.[52] Despite this technical improvement, FISH has been used for embryo sexing instead of PCR. Currently PCR techniques have been applied to the diagnosis of specific gene defects, e.g. cystic fibrosis[9], Tay-Sachs disease[10], β-thalassaemia[11]; X-linked diseases, e.g. Duchenne muscular dystrophy[12]; autosomal dominant diseases, e.g. Marfan's syndrome[13]; and inherited cancers, e.g. familial adenomatous polyposis coli.[14]

Contamination by other DNA material and the occurrence of allele drop out (ADO) have been reported when using this technique.[29,53,54] Intracytoplasmic sperm injection (ICSI) and careful laboratory procedures have been recommended to avoid possible contamination. Nested PCR has also been introduced to reduce the risk of contamination.[55] Fluorescent PCR, with high sensitivity detection has been

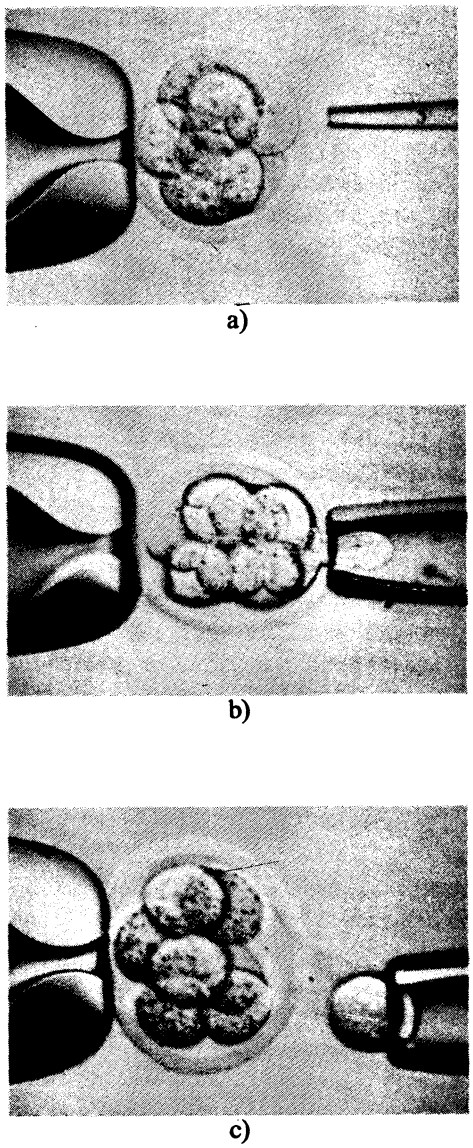


Figure 1. Cleavage stage embryo biopsy with acid Tyrode's solution
a) The cleavage stage embryo was immobilised with holding pipette (left). Acid Tyrode's solution was expelled from the drilling pipette (right) and lysed the zona pellucida of the embryo.
b) The biopsy pipette (right) was contacted a blastomere through the opening created in the zona pellucida.
c) The blastomere was aspirated and removed from the embryo by the biopsy pipette.

developed to reduce the need for nested PCR. Multiplex PCR and the polymorphic nature of short tandem repeat (STR) have been used to detect contamination by linkage analysis.[55,56] Increasing the denaturation temperature during the first cycles of amplification, lysing a single cell with sodium dodecyl sulphate/proteinase K (SDS/PK), and multiplex fluorescent PCR have been suggested to overcome allele drop out.[57-59] Simultaneous amplification of a disease-causing mutation and an informative polymorphism offers two chances for the detection of a chromosome carrying a mutant gene and the probability of ADO affecting both amplified fragments is low.[60] Whole genome amplification (WGA) that maximises the information obtained from a single cell has been developed.[61,62] Primer extension preamplification (PEP) and degenerate oligonucleotide primed PCR (DOP-PCR) are the two main methods of WGA. PEP has been used for single cell analysis of Tay-Sachs disease, cystic fibrosis, haemophilia A, Duchenne muscular dystrophy and familial adenomatous polyposis coli[14,63,64]; meanwhile DOP-PCR has been successfully used to amplify efficient DNA fragments for additional procedures such as comparative genomic hybridisation (CGH).[62] However, most centres within the ESHRE PGD Consortium are still using nested PCR and conventional methods for mutation detection and approximately half of them currently use fluorescence PCR for mutation detection and/or linkage analysis.[30]

Fluorescent in situ hybridisation (FISH)

FISH has offered the possibility of direct visualisation of chromosomes in both metaphase and interphase nuclei.[65,66] FISH was first used for embryo sexing in couples carrying X-linked diseases.[5] Most centres within the ESHRE PGD Consortium are using FISH primarily for identification of sex in X-linked disease.[30] FISH has also been used for aneuploidy detection. More than half of all PGD cycles performed world-wide have been done for screening of age-related aneuploidy.[67] Approximately 95 % of all postnatal chromosomal abnormalities can be diagnosed, using FISH probes to detect chromosome X, Y, 13, 18 and 21.[67] However, general screening for age-related aneuploidy is still controversial.[68-70] FISH has been successfully developed to detect chromosomal structural abnormalities such as translocations and microdeletions.[15,16,26]

FISH has also been used to analyse chromosomal abnormalities in preimplantation embryos in human.[7,23,32] Using two autosomal probes, as many as 46 % of human preimplantation embryos are found to contain chromosomal abnormalities.[32] Two recent techniques related to FISH, which are comparative genomic hybridisation (CGH) and DNA chips, have been developed. CGH allows the copy number of every chromosome region to be assessed in only a single hybridisation.[71] DNA chips provide the capability of simultaneously detecting thousands of possible sequence variants in previously defined genes. These techniques might be applied to PGD in the future.

CONCLUSION

Many couples at risk of inherited diseases have not risked an affected pregnancy and have chosen not to have further pregnancies. PGD might be a promising way of providing the opportunity for an unaffected pregnancy. However, PGD has been developed for only a decade and consists of many complicated and sensitive processes. More extensive research to design and develop biopsy techniques as well as the test specific FISH and PCR protocols are required due to the diversity of chromosomal and single gene disorders. Ethical issues, moreover, have to be carefully considered in individuals prior to embarking on such procedures clinically.

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