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Pre-implantation genetic diagnosis in Hong Kong

香港胚胎植入前的基因診斷

This paper presents the first two successful cases of pre-implantation genetic diagnosis in Hong Kong and discusses the indications and the advantages over prenatal diagnosis. Patients should be informed about the procedure and extensively counselled about the possibility of misdiagnosis and the need for conventional prenatal diagnosis during pregnancy.

本文介紹了香港首兩宗胚胎植入前作基因診斷的成功病例，並討論此種診斷相對於出生前診斷的意義和優點。應為病人提供有關程序的資料，並就誤診的可能性，以及在懷孕期間需要進行常規的出生前診斷方面作全面討論。

Introduction

Pre-implantation genetic diagnosis (PGD) uses molecular biological techniques to select genetically normal embryos for replacement to the uterine cavity of a woman during an assisted reproduction cycle. Such a procedure is indicated for couples at risk of having babies with serious genetic disorders. The first baby to undergo PGD[1] was born more than 10 years ago and, since then, PGD has shown an exponential growth in many countries. Currently, more than 50 centres worldwide are offering PGD for a variety of genetic disorders, including single gene defects, chromosomal imbalances, and sexing for X-linked diseases.[2]

The Assisted Reproduction Unit at Queen Mary Hospital (QMH) is the first unit in Hong Kong to provide a PGD programme. This paper reports the first two successful PGD cases out of seven treatment cycles performed in 2001 and to discuss the indications and advantages over prenatal diagnosis. The procedure is described and the counselling aspect is highlighted.

Case 1

A 28-year-old woman and her 37-year-old husband were referred to the Subfertility Clinic in October 1998 because of primary infertility due to severe oligoasthenoteratozoospermia (OAT) for 2 years. Repeated semen analyses during subfertility examination revealed only one to two motile spermatozoa at microscopic examination at low magnification. The husband was in good health and he neither smoked nor drank. There was no previous history of mumps orchitis, genital trauma, or surgical operation. Clinical examination of the husband at the Male Infertility Clinic showed slightly atrophic testes of approximately 10 mL on both sides. The epididymis and vas were all normal. His serum follicle stimulating hormone (FSH) level at that time was 25 mIU/mL (normal range, 1-12 mIU/mL). His wife also enjoyed good health and had a regular menstrual cycle.

This couple were enrolled into the in vitro fertilisation/embryo transfer (IVF/ET) programme at QMH and intracytoplasmic sperm injection (ICSI) was required to assist fertilisation because of the poor semen quality. The first and second IVF/ICSI treatment cycles were done in December 1999 and August 2000. The long protocol of ovarian stimulation, gamete handling, and ICSI has been previously described.[3] In the first cycle, 15 of 17 oocytes at metaphase II were fertilised after ICSI. Only two good quality embryos were available for transfer

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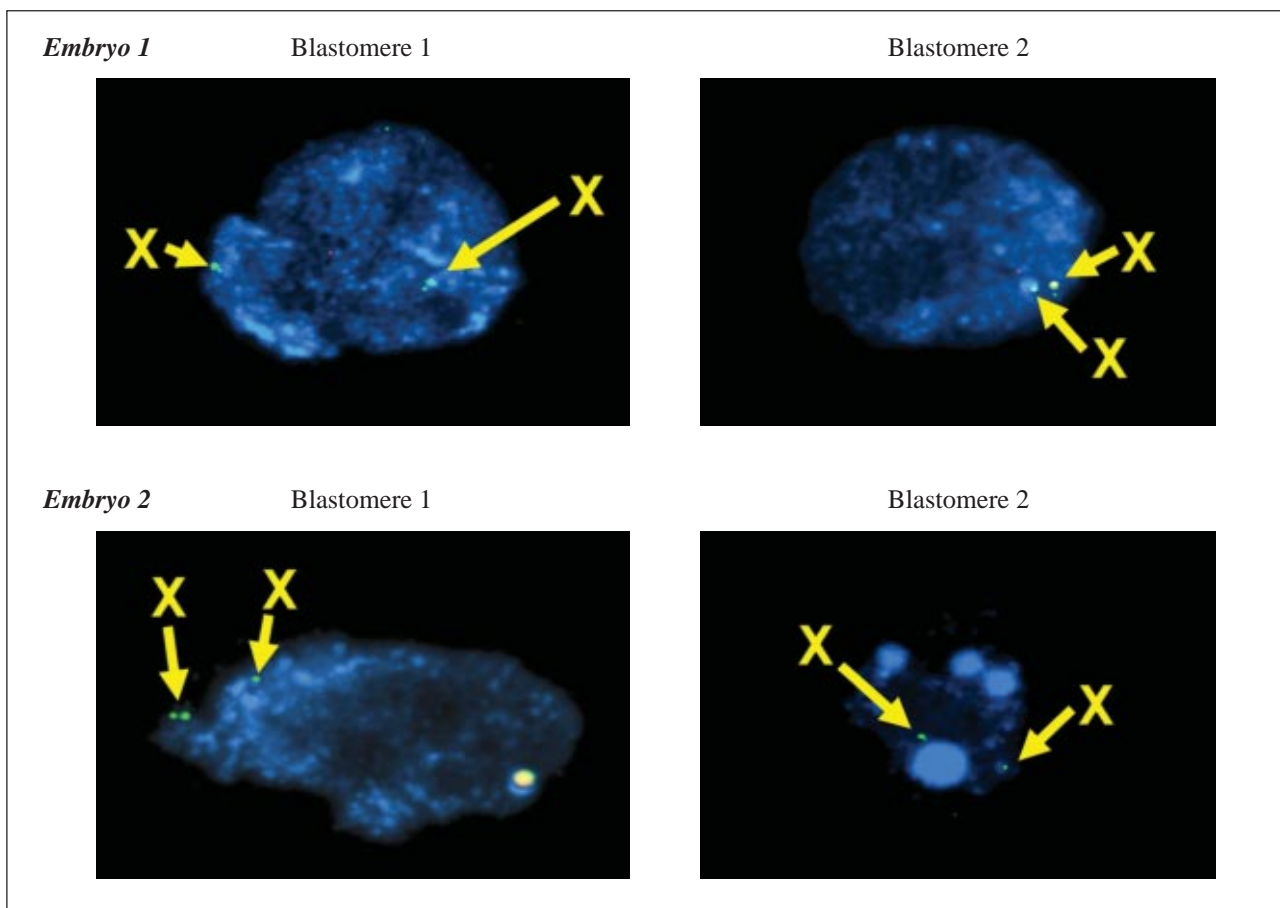**Prenatal Diagnostic and Counselling Department, Tsan Yuk Hospital, 30 Hospital Road, Hong Kong**
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Fig 1. Fluorescence in situ hybridisation result of the first birth

and there were no excess embryos for freezing. In the second cycle, eight oocytes at metaphase II were fertilised and cleaved after ICSI. Two good quality embryos were transferred and there was one frozen embryo from the second cycle. The woman did not conceive in these two treatment cycles, however.

The husband subsequently underwent karyotyping and microdeletion of the Y chromosome assay as part of a research project to investigate the genetic causes of severe male infertility.[4] The results revealed 47,XYY and no Y microdeletion. After detailed and extensive counselling, the couple agreed to undergo a third cycle of IVF/ICSI with PGD in May 2001 to select embryos without any sex chromosomal abnormalities for replacement. Only five of 12 oocytes aspirated were microinjected because only five motile spermatozoa were found after sperm processing. On day 3 after egg collection, PGD was performed by fluorescence in situ hybridisation (FISH) for chromosomes X and Y on two good quality embryos from the fresh cycle and the frozen embryo from the second cycle.

Two of the biopsied embryos, one from the fresh cycle and one from the frozen cycle, had two X chromosomes and no Y chromosome (Fig 1). Diagnosis could not be made in the third embryo because of the lack of a nucleus in the biopsied cell. The two normal embryos were transferred to the wife on day 4 after egg collection and she subsequently conceived. The wife declined to have amniocentesis because of the risk of miscarriage after the procedure. The antenatal course was uneventful and a baby girl was born vaginally in February 2002. A normal female karyotype was confirmed after delivery.

Case 2

A 26-year-old woman and her 36-year-old husband were referred to the Subfertility Clinic in February 1998 because of primary infertility for 3 years due to severe OAT. Repeated semen analyses showed one to two motile spermatozoa at microscopic examination with low magnification. The husband was in good health and he neither smoked nor drank. There was no previous history of mumps orchitis, genital trauma, or surgical operation. Clinical examination of the husband did not show any abnormalities and his hormonal profile including FSH, luteinising hormone, testosterone, and prolactin level was normal. Karyotyping revealed 45,XY with Robertsonian translocation of chromosomes 13 and 21 and there was no Y microdeletion. The wife had undergone two IVF/ICSI cycles in March 1999 and November 2000 without PGD being performed. She had three frozen embryos from the second cycle.

After detailed and extensive counselling, the couple consented to have PGD done on the three frozen embryos after they were thawed in June 2001 in a hormonal replacement

Embryo 1 (normal for chromosomes 13, 21)
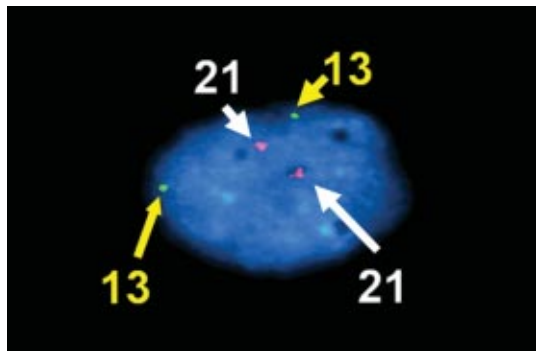
Embryo 2 (normal for chromosomes 13, 21)
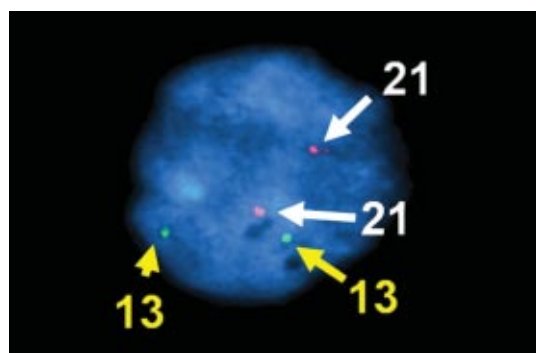
Embryo 3 (monosomy 21)
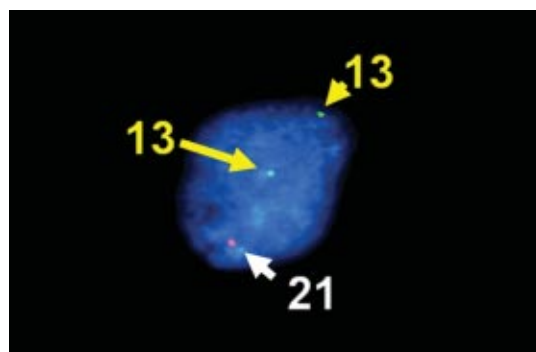
Fig 2. Fluorescence in situ hybridisation result of the second birth

cycle. Fluorescence in situ hybridisation was performed using a five-coloured probe panel (MultiVysion PGT; Vysis, Downers Grove, United States) consisting of multicolour probes for chromosomes 13, 18, 21, X, and Y. Results demonstrated that none of the three embryos had consistent normal signals for the chromosomes tested. Therefore, no embryos were replaced in this cycle. Further analysis of the embryos showed that all embryos were abnormal with either chaotic, diploid mosaic, or abnormal mosaic patterns.[5] The third cycle of IVF/ICSI/PGD was done in December 2001. The patient had good ovarian response and nine of 15 oocytes at metaphase II were normally fertilised after ICSI. Pre-implantation genetic diagnosis

was performed on eight good quality embryos on day 3 after egg collection. Fluorescence in situ hybridisation for chromosomes 13 and 21 demonstrated two normal embryos while other abnormal embryos were found to have mosaic, monosomy 21/mosaic, or chaotic patterns on further analysis (Fig 2). These two normal embryos were replaced and the woman was found to be pregnant with a single intrauterine foetus. Amniocentesis in April 2002 confirmed normal karyotype of the foetus and a healthy baby girl was delivered in October 2002 at 41 weeks of gestation.

Indications

Pre-implantation genetic diagnosis is indicated for couples at risk of having babies with serious genetic disorders, which can be due to chromosomal abnormalities or gene defects. Approximately 20% of male infertility can now be explained by abnormalities in mitotic and/or meiotic chromosomes. An abnormal karyotype has been shown in 13.7% of men with azoospermia and 4.6% of men with oligozoospermia.[6] Sex chromosome abnormalities (mainly 47,XXY) are predominantly found in the azoospermic group, whereas autosome anomalies (Robertsonian and reciprocal translocations) are mainly present in oligozoospermic men. The men in the two couples described here were found to have abnormal karyotypes—it is usual for subfertile men with a sperm count of <5 million per mL to check the karyotype and Y microdeletion prior to IVF treatment. In this programme, nearly 20% of the patients screened had abnormal karyotypes.[7] Chromosomal abnormalities are also increased for couples with a history of having chromosomally abnormal babies, history of recurrent miscarriages, or in subfertile couples undergoing IVF treatment.[8]

In the first case, the husband had a karyotype of 47,XYY. The frequency of spermatozoa with abnormal sex chromosomes (24,XY and 24,YY) in fertile controls are approximately 0.5%, whereas this frequency increases in men with 47,XYY karyotype and ranges from 0.6% to 15.0%.[9-11] During the ICSI procedure, it is impossible to distinguish a spermatozoon carrying abnormal chromosomal makeup from another one with normal chromosomal content as both of them may be phenotypically normal. Therefore, karyotypes of the resulting embryos/babies may have abnormal sex chromosome numbers such as 47,XXY or 47,XYY when PGD is not performed.

Balanced carriers of Robertsonian translocations, which has a prevalence of 1 in 1000, are phenotypically normal but may present with infertility, recurrent miscarriage, or pregnancy with an abnormal phenotype after abnormal segregation of the translocated allele at meiosis. Unbalanced spermatozoa can be found in 8% to 26% of spermatozoa from translocation carriers.[12-14] Approximately half of the embryos from these translocation carriers are chromosomally normal whereas the remaining are aneuploidy or chaotic.[12] Therefore, there is a high chance for a woman

to have an affected pregnancy if PGD is not performed. After PGD selection, there is a significant reduction in spontaneous miscarriage from 95% to 13% in patients with translocations.[15] In the second case, the karyotype of the husband was 45,XY with Robertsonian translocation of chromosomes 13 and 21 and the resulting embryos can be monosomy 13, monosomy 21, trisomy 13, trisomy 21, 45 rob (13, 21) or normal.

The thalassaemia syndromes (α- or β-thalassaemia) are the most common monogenetic diseases and are most prevalent in South-East Asia. Couples carrying the same thalassaemia trait, that is either α- or β-thalassaemia trait, have a 25% chance of having a baby affected by the homozygous state of the disease. Homozygous α-thalassaemia causes hydrops foetalis and homozygous β-thalassaemia causes severe postnatal anaemia that necessitates life-long blood transfusion. Thus prenatal diagnosis or PGD is indicated for these individuals.

The observations of a significant incidence of aneuploidy in early human embryos lead to the application of PGD techniques for patients who had a poor prognosis after IVF treatment such as advanced maternal age and repeated implantation failure. The chromosomes selected for aneuploidy screening are those commonly seen as trisomic in spontaneous abortions and include chromosomes X, Y, 13, 18, and 21. The clinical pregnancy rate per embryo transfer was 36% for advanced maternal age and 11% for repeated implantation failure.[2] Based on these results, it seems that aneuploidy screening offers little advantage to women who have repeated implantation failure during IVF treatment.

Pre-implantation genetic diagnosis has been performed for sex selection for family balancing[16] and detection of genes for adult-onset diseases such as early-onset Alzheimer disease.[17] Pre-implantation genetic diagnosis has also been used to select a human leukocyte/lymphocyte antigen (HLA)–compatible donor from embryos without the genetic mutation so as to provide stem cells for affected siblings in a family with Fanconi or β-thalassaemia anaemia.[18] These indications are controversial, however, and have aroused much debate about the associated ethical issues.[19-20]

Advantage of pre-implantation genetic diagnosis over prenatal diagnosis

The above genetic disorders can also be detected by conventional prenatal testing, including ultrasound, amniocentesis or chorionic villus sampling. The majority of women with affected foetuses undergo termination of pregnancy in the second trimester of pregnancy, although some do not agree to termination of pregnancy because of psychological or religious reasons. Pre-implantation genetic diagnosis is an alternative to prenatal diagnosis and has the advantage that the couples do not need to have the physical and psychological trauma of undergoing termination of pregnancy.

A survey of attitudes towards PGD in women at risk of giving birth to a child with α- or β-thalassaemia has been performed in Hong Kong.[21] Of 205 questionnaires sent out, 141 completed questionnaires were analysed. The results indicated that 82.3% of the women considered PGD either the same as or better than conventional prenatal diagnosis and women with an affected child or subfertility problems were more willing to accept PGD and to undergo this procedure in their future pregnancies. Avoidance of termination of an affected pregnancy was regarded as the most important advantage of PGD. This study highlights the need for implementation of PGD as an alternative for these women.

Procedure

Couples requiring PGD have to undergo the standard IVF/ET treatment with ICSI, which involves injection of a single spermatozoon into the cytoplasm of an oocyte. This method of fertilisation eliminates the possibility of contamination by the husband's spermatozoa and reduces the chance of misdiagnosis in PGD. The zygotes obtained are cultured for 48 hours, when the good quality embryos should be at the six- to eight-cell stage. One or two blastomeres from each good quality embryo are then taken out using laser micromanipulation. A hole is made on the zona pellucida of the embryos to be biopsied with an infrared (1480 nm) laser. One to two blastomeres are removed from the embryos through the hole using a fine needle with internal diameter of 35 μm (Fig 3). The nucleus of the each blastomere is obtained by successively lysing the cell with hypotonic potassium chloride solution and hydrochloric acid-Tween-20 solution.

The genetic make-up of the biopsied cells is analysed by genetic tests such as FISH or polymerase chain reaction (PCR). The first successful report on PGD described the use
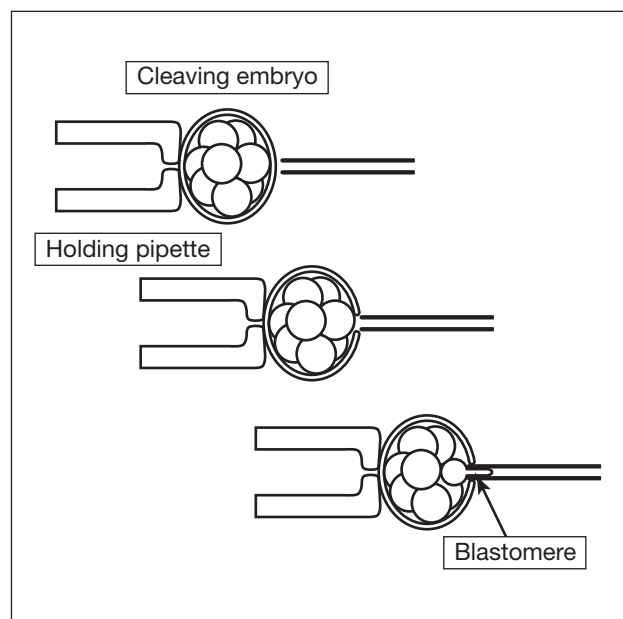

Fig 3. Embryo biopsy

of PCR to sex embryos from couples at risk of sex-linked diseases.[1] With advances in molecular biology, FISH was introduced and replaced PCR for sexing of embryos because of improved accuracy. Fluorescence in situ hybridisation is indicated for numerical and structural chromosomal abnormalities while PCR is mainly reserved for monogenic diseases such as cystic fibrosis and thalassaemia. The genetic tests usually take 8 to 26 hours, depending on the type of tests performed. Genetically normal embryos that continue to grow after biopsy will be selected for replacement to the woman, while the genetically abnormal embryos will be discarded.

Fluorescence in situ hybridisation is often used for aneuploidy screening in PGD. One of the limitations of using this technique is the difficulty in the simultaneous detection of all the chromosomes in a single cell. It is believed that the benefit of aneuploidy screening may be increased if more chromosomes are analysed. Therefore, comparative genomic hybridisation has been applied to a single blastomere biopsied from human embryos for complete karyotyping and has resulted in the first birth from an embryo that has been fully karyotyped prior to transfer.[22] The analysis of this technique, however, takes at least 5 days to complete because of the long hybridisation time required and the laborious analysis of template chromosomes.

Couselling

Patients should be extensively counselled about the indication, procedure, and accuracy of PGD prior to the treatment cycle. The rate of misdiagnosis is approximately 1.8% (0.9% for FISH and 3.4% for PCR).[2] Patients should also be informed of the complications and risks of IVF/ ICSI and accept that there may not be any normal embryos for replacement after PGD. In most programmes, prenatal testing such as amniocentesis is advised to confirm the genetic diagnosis made during PGD. However, some pregnant women may decline the amniocentesis because of the associated risk of miscarriage after the procedure. Although the neonatal outcome of PGD seems to be reassuring,[23] long-term paediatric follow-up would be desirable for monitoring the effects on growth and mental development.

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