Preimplantation diagnosis of human genetic disorders

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Recent advances in molecular biology and medically assisted reproductive technologies have made it possible to diagnose genetic disorders prior to the establishment of a pregnancy. Preselecting the sex of the child can reduce the occurrence of X-linked disorders. The genetic material from the polar body of the oocyte can be analysed to determine whether the oocyte is genetically normal. Blastomeres from an embryo can be biopsied, the DNA amplified by the polymerase chain reaction and then analysed for the occurrence of specific genetic defects. Further progress in molecular biology and medically assisted reproductive technologies may permit routine genetic screening of gametes and embryos from high risk individuals and the 21st century may see the elimination of many genetic disorders.

ABOUT 3% of children are born with birth defects[1]. Prenatal diagnosis of genetic disorders can be achieved by karyotyping foetal cells recovered from the amniotic fluid or by sampling the chorionic villus. Both these methods are invaluable but they are not without risk to the developing foetus. Furthermore, if the foetus is genetically abnormal then the parents face a dilemma over the termination of the pregnancy. This state of affairs provided the impetus to develop a method of diagnosing genetic disorders before pregnancy is established.

The advent of assisted reproductive technologies and methods of amplifying DNA, such as the polymerase chain reaction (PCR), have made it possible to analyse the genetic content of single cells including sperms, polar bodies and blastomeres. Handyside *et al.*[2] determined the sex of embryos obtained by *in vitro* fertilization (IVF), transferred only 'female' embryos and thereby prevented the transmission of X-linked disorders. This marked the beginning of the clinical application of preimplantation diagnostic techniques. This article reviews the current status of this area of 'new biology'.

Genetic disorders

Genetic disorders may either be due to chromosomal aberrations or due to the inheritance of abnormal genes.

Chromosomal aberrations

The most common chromosomal aberrations are the occurrence of chromosomes 21 (Down's syndrome) or 18 in triplicate and the presence of a 45X or 47 XXY karyotype which respectively lead to Turner's and Klinefelters' syndrome. In about 95% of children with Down's syndrome, the trisomy of chromosome 21 is due to the presence of two chromosomes 21 in either the sperm or the oocyte. The chances of having a child with chromosomal aberrations increase with an increase in the maternal age[3].

Inheritance of abnormal genes

A child could inherit mutated genes from either parent. The male child is at a very high risk of inheriting 'Fragile X' syndrome, muscular dystrophy or haemophilia as they result from the mutations of the X-chromosome. Cystic fibrosis, sickle cell anaemia, thallasaemia and Tay Sach's disease are a result of mutated recessive genes. Autosomal dominant defects lead to Huntington's disease, polycystic kidney disease and malignant hyperthermia.

The onset and progress of the disorder depends upon the type of genetic abnormality. While Down's syndrome and the other trisomies are recognized at the time of birth, Klinefelters' syndrome is detected at puberty and Huntington's disease manifests itself only in the third decade of life. Many conceptions with chromosomal aberrations result in spontaneous abortions while those which continue to term result in children with a shorter life span. Gene transfer therapy is available for very few human genetic disorders and for most of the genetic disorders, therapy involves supportive treatment like repeated blood transfusions for anaemias, rehabilitative therapy for muscular dystrophy and dietary modifications for inborn errors of metabolism.

The origins of preimplantation diagnosis

The concept of sexing embryos and transferring only female embryos to prevent X-linked recessive disorders was first developed by Gardner and Edwards[4]. Trophe-

ctodermal cells from rabbit blastocysts were excised and analysed for the sex chromosome. Only female embryos were transferred and the sex was confirmed at term[4].

It took two decades before this technique could be extrapolated to human embryos. With improvements in the IVF procedures, it is possible to produce and maintain several embryos in culture for genetic analysis. The development of the PCR, which amplifies genes from a single cell, also aided the progress of preimplantation diagnosis techniques[5].

Sex pre-selection

X-linked disorders can be prevented by inseminating women with enriched preparation of X-chromosome-bearing sperms.

Enrichment of X-chromosome-bearing sperms

The X-chromosome-bearing sperms have a larger head than the Y-chromosome-bearing sperms[6] and therefore they also differ in their density. Percoll, Ficoll and albumin density gradients are used to separate the two types of sperms. An enriched preparation of 'X' sperms with 90% purity has been achieved on a Percoll gradient[7]. When semen is filtered through a Sephadex column, 95% of the sperms that elute are 'X' sperms and subsequent washing of the column leads to an enriched fraction of 'Y' sperms. Forty-eight pregnancies which resulted from insemination with the 'X' sperm enriched fraction led to the birth of female babies[6].

Enrichment of Y-chromosome-bearing sperms

The most popular method of obtaining an enriched fraction of 'Y' sperms is to separate them on an albumin gradient[8]. Ericsson *et al.* have achieved an enriched fraction of 'Y' sperms with 70% purity. However, several groups have been unable to validate Ericsson's claims[9, 10].

Flow cytometry for checking the efficacy of the enrichment techniques

Flow cytometric techniques permit the accurate typing of X and Y sperms on the basis of their DNA content. The DNA probes are toxic to sperms and therefore clinical application of flow cytometry is not possible. Flow cytometry is currently being used to evaluate the efficacy of the enrichment techniques[11].

Genetic analysis of the oocyte

Genetic analysis of oocytes from women who are carriers of genetic disorders and selection of oocytes with a normal genotype for IVF can reduce the risk of X-linked and autosomal recessive diseases. Direct analysis of the DNA content of oocytes is impractical as it destroys the oocyte. Therefore the genotype of the oocyte is inferred on the basis of the genetic content of the first polar body[12].

Polar body analysis

The genetic content of the polar body is analysed with the help of probes for the normal allele. If the polar body is homozygous for the defective gene, then the oocyte is considered to be normal. If the polar body is homozygous for the normal allele, then the oocyte definitely inherits the defective gene on completion of the second meiotic division. If the polar body is heterozygous, then the oocyte may inherit the abnormal allele at completion of the second meiotic division. The state of the oocyte genes is then determined by the analysis of the second polar body.

Techniques of polar body analysis

Polar body analysis has been successfully performed by Verlinsky and Strom in infertile women treated by IVF-ET[13, 14]. The oocytes are incubated in culture medium for three hours after which the polar body is aspirated with a fine bevelled Pasteur pipette. The polar body is lysed while the oocyte is fertilized *in vitro*.

Genetic analysis

The DNA of the polar body is amplified by the PCR and subsequently analysed with specific probes for the abnormal genes. For the PCR, the target DNA is mixed with a primer (specific for the region of the target DNA to be amplified) and Taq polymerase. The primer acts as template and the newly synthesized strands are separated by repeated heating and cooling. Each of these act as a template for the synthesis of more strands. There is therefore an exponential increase in the number of specific DNA fragments. The amplified fragments are separated by electrophoresis and stained with fluorescent or radiolabelled specific probes for abnormal genes[15].

Application of polar body analysis

Oocytes free of polar body can fertilize and develop into morphologically normal embryos. About 50% of the polar bodies from patients at risk of cystic fibrosis, haemophilia and α antitrypsin deficiency show homozygous alleles[13]. This technique shows a lot of promise for the detection of X-linked and autosomal recessive abnormalities in oocytes from female carriers.

Preimplantation diagnosis of early embryos

The blastomeres of early embryos are totipotent. If one of the blastomeres is removed, the rest of the embryo as well as the excised blastomere is capable of developing normally. Bovine embryos have been bisected and after transferring them to the uterus, they led to the birth of identical twins[16].

Embryo biopsy

After IVF, the embryos are cultured for three days so that they develop to the 6–10 cell stage. A hole is drilled into the zona pellucida with a stream of acid Tyrodes medium and one of the blastomeres is aspirated out with the help of a fine Pasteur pipette[17].

Genetic analysis

Embryos can be sexed with the use of radiolabelled, biotinylated or fluorescent Y chromosome-specific probes[18]. The limited amount of genetic material in single cells makes it difficult to detect the probes following *in situ* hybridization[19]. These probes can be detected by amplifying the DNA by the PCR[15].

PCR can be used to amplify defective DNA fragments for preimplantation diagnosis of disorders whose molecular basis has been identified. Embryos can be sexed by amplifying the Y chromosome specific regions for the X-linked disorders whose molecular basis is not known. Failure of amplification results in the misinterpretation of a male embryo as a female embryo[20]. The use of Y specific repeated sequence can prevent such misinterpretations[21].

Effects of biopsy/genetic analysis on embryonic development

The developmental potential of biopsied human embryos has been studied with reference to their ability to form blastocysts leading to pregnancies following transfer. More than 70% of the biopsied embryos develop into blastocysts in contrast to the development of only 59% of unmanipulated embryos[22]. Fifty-six per cent of the biopsied embryos even hatched from the zona pellucida. The reduction of the inner cell mass of the blastocyst causes a proportional decrease in the uptake of energy substrates[23]. The transfer of biopsied embryo has resulted in the birth of normal female babies[1].

Preimplantation diagnosis of blastocysts

Blastocysts are obtained either by culture of IVF oocytes or by uterine lavage. Very few human embryos develop to the blastocyst stage *in vitro*[24] and therefore it is advisable to obtain blastocysts by uterine lavage for fertile couples needing preimplantation diagnosis.

Biopsy of blastocysts

Blastocysts can be biopsied by any of the following methods with each of the method having its own limitations: 1) Blastomeres can be aspirated from intact blastocysts but this can increase the susceptibility of the embryos to environmental toxins and mechanical damage[25]. 2) The cells floating freely in the blastocoel have been aspirated[26]. Sometimes cells may not be present in the blastocoel and if present may not be viable or may not represent the embryonic cells[27]. 3) Bovine blastocysts have been bisected and one part used for genetic analysis and the other transferred[16]. The small size of human blastocysts restricts their bisection[28]. 4) The cells remaining attached to the zona after hatching can be collected for analysis but like aspiration of cells from the blastocoel, one may not always be able to retrieve 'representative' cells from within the zona[29]. 5) Trophectoderm is biopsied by slitting the zona. A few cells which herniate from the zona are used for analysis[30]. Trophectoderm biopsied embryos do hatch *in vitro* but such embryos have not yet been replaced in women.

Advantages of the different preconception and preimplantation diagnostic techniques

Enrichment of X- or Y-chromosome-bearing spermatozoa

The techniques of gender preselection, if efficient, are definitely superior to the other invasive methods of preimplantation diagnosis especially for preventing X-linked disorders. However, none of the methods available for enriching X- or Y-chromosome-bearing spermatozoa give consistent results.

Polar body analysis

Removal of the polar body is not hazardous to the developing embryo as the polar body serves no function. The results of the analysis can be obtained by the time of embryo transfer. However, genetic defects of paternal origin will not be detected.

Early embryo biopsy

There is no risk of abnormal embryonic development following biopsy as blastomeres from early embryos are totipotent. As only one or two cells are available for

Table 1. Comparison of preimplantation diagnosis methods

Polar body analysis	Blastomere analysis
First polar body from an oocyte analysed	Blastomeres from early embryos or blastocyst analysed
Detects abnormalities of maternal genome only	Detects chromosomal aberrations and genetic defects of paternal and maternal origin
Indirect evaluation of maternal genome	Direct evaluation of embryonic genome
Can detect genetic disorders only if the specific probes are available	Even in the absence of specific probes, X-linked defects can be prevented by sexing and transferring only female embryos
Only one cell available for analysis	More than one blastomere from blastocysts can be analysed

analysis, a failure of DNA amplification can lead to misinterpretations[20]. For example, the inability of Y-specific regions to amplify can lead to the interpretation of a male embryo as a female one. Secondly, there is a high incidence of anucleate cells in early human embryos and therefore more than one blastomere has to be analysed[31].

Biopsy of blastocysts

Although it is possible to obtain more cells for analysis from blastocysts than from early embryos it is not always possible to culture embryos up to the blastocyst stage[24]. A comparison of polar body and blastomere analysis is presented in Table 1.

The future of preimplantation diagnosis

The successful application of preimplantation diagnosis as a routine clinical feature necessitates advances in both assisted reproduction and molecular biology.

Many improvements are needed in the *in vitro* culture techniques so that more embryos can be cultured up to the blastocyst stage. Some of the genetic analyses are time-consuming and it therefore becomes necessary to freeze the embryos till the results of the genetic analysis are available. Although several frozen-thawed embryos have led to live births, not all human embryos survive freezing and thawing. Therefore there could be a situation where the embryo which is diagnosed to be genetically normal fails to survive freezing and thawing procedures. The success rate of IVF-ET in terms of take home baby rates remains between 15 and 20%. The couples seeking preimplantation diagnosis may no. be infertile and therefore the success rates of IVF in these couples may be better than in infertile couples. However, a large number of infertile couples seeking IVF-ET are in the older age group and have greater susceptibility to chromosomal aberrations and therefore preimplantation diagnosis with a successful embryo transfer should also be available to them. Thus, these problems of IVF-ET need to be tackled to improve the efficacy of preimplantation diagnosis.

The genomic location of several of the 300 recessive X-chromosome-linked disorders has not been identified and therefore no specific probes are available. X-linked disorders in high risk couples can be prevented by selective transfer of female embryos. In the absence of specific probes even genetically normal male embryos get discarded. Causes for the failure of DNA amplification should be identified to prevent misdiagnosing male embryos as female when Y specific probes are being used. Once the entire human genome has been mapped, genomic abnormalities associated with different inherited disorders need to be identified and specific probes for their detection developed to improve the efficacy of preimplantation diagnosis techniques.

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Reproductive health, population dynamics and contraception

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THE most widely known factors affecting human health are those associated with our feeding habits and the environment in which we live. Reproductive health, particularly those of the mother and her infant, is affected by our procreative patterns and habits. Frequent child bearing is a health hazard to both the mother as well as to the new born infant. Avert reactions to unwanted pregnancies can lead to abortions which can cause maternal death especially if they are performed clandestinely by unqualified persons as happens in countries where abortion is illegal.

Our reproductive habits can no longer be considered a matter of mere personal concern; frequent child bearing contributes adversely to population growth rates – a problem which affects all of us inhabiting this planet whose finite natural resources will not be able to meet the demands of an exponentially growing population.

This article examines some of the issues related to reproductive health, population growth and contraception.

Maternal and infant mortality rates in different parts of the world

Maternal mortality *ratios* and maternal mortality *rates* are the two indices commonly used to determine the health status of women. Maternal mortality ratios are calculated as the number of maternal deaths divided by the number of live births during a defined time span – usually a year. Maternal mortality ratios are a direct indicator of the dangers associated with pregnancy. A recent report by the World Health Organization[1] indicates that at least half a million women die each year due to causes related to pregnancy or childbirth. In developed countries this ratio is between 10 and 30 per 100,000 live births. In the USA it is as low as 14 per 100,000 live births[2]. In developing countries the ratio ranges from 100 to 1000 per 100,000 live births and the mean is about 450/100,000. The highest is in Africa where it is about 640/100,000.

Maternal mortality rate, on the other hand, is the number of deaths in a year per 100,000 women in the reproductive age group of 15–44 years. Maternal mortality rates are not only related to the hazards of pregnancy in women in this age group but also to their fertility. The number of pregnancies and deliveries determines a woman's lifetime risk of 1 in 21, 1 in 54 and 1 in 73 in Africa, Asia and Latin America, respectively, and lowest risk of 1 in 5 to 10,000 in North America and Northern Europe[3].

Contraceptive usage improves maternal health

A case study of Matlab, Bangladesh[4], a developing country, showed that in 1976, when Family Planning programme was first introduced, maternal mortality rates were about 90/100,000 women and maternal mortality ratios were about 500/100,000 live births. Ten years later, at the end of 1985, maternal mortality rates dropped to about 60/100,000 women. In contrast, maternal mortality rates increased in a comparison area. The observed reduction in maternal deaths in Matlab resulted from a 25% reduction in fertility.

Thus, there is compelling evidence from these and other studies[5] that maternal mortality ratios are influenced by family planning only when it reduces pregnancies in women at high obstetric risk and when there is a shift in pregnancies occurring in women at high obstetric risk (older women with high parity) to younger, low-risk women. Family planning reduces maternal mortality rates through the reduction of unwanted pregnancies.