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Preimplantation genetic diagnosis in Saudi Arabia

Zeinab Abotalib

Department of Obstetrics and Gynecology, King Khalid University Hospital, College of Medicine, King Saud University P.O. Box 60826, Riyadh 11555, Kingdom of Saudi Arabia; Zeinab Abotalib – Email: drzeinab@yahoo.com

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Abstract:
Preimplantation genetic diagnosis (PGD) testing is the practice of obtaining a cellular biopsy sample from a developing human oocyte or embryo, acquired via a cycle of in vitro fertilization (IVF); evaluating the genetic composition of this sample; and using this information to determine which embryos will be optimal for subsequent uterine transfer. PGD has become an increasingly useful adjunct to IVF procedures. The ability to provide couples who are known carriers of genetic abnormalities the opportunity to deliver healthy babies has opened a new frontier in reproductive medicine. The purpose of the PGD is enables us to choose which embryos will be implanted into the mother. In the present study 137 families who had undergone IVF at Habib Medical Centre, were enrolled for the PGD analysis. The couple visited the clinic for the sex selection, recurrent fetal loss and with the recurrent IVF failure. 802 embryos were tested by the biopsy method and 512 are found to be normal and 290 were abnormal embryos. In this study only 24% of the embryos were transferred and the remaining was not transferred because of the abnormalities or undesired sex of the embryos. The structural and numerical abnormalities were found to be 16.8%.

Keywords: Preimplantation genetic diagnosis (PGD), invitro fertilization (IVF), Biopsy, Saudi Arabia.

Background:
Preimplantation genetic diagnosis (PGD) is an established procedure of embryo genetic analysis. It allows couples carrying genetic diseases to have an unaffected child, without facing an invasive prenatal diagnosis and termination of pregnancy. It consists in realizing genetic analyses on embryonic cells and transferring the embryos identified unaffected into the uterus. Advancements in molecular biological and in vitro fertilization (IVF) techniques have enabled the perfecting of PGD **[1]**. PGD is an accepted and realistic alternative to traditional prenatal diagnosis in families with inherited disorders in many countries. In the affected families, the risk of having a child with a severe disease can be as high as 50%, and many carriers of inherited chromosome abnormalities suffer from recurrent miscarriages and subfertility **[2].** Already in 1967, Edwards and Gardner succeeded in sexing rabbit embryos at the blastocyst stage, and predicted the use of similar technology to avoid genetic disease in humans **[3]**. Research with the goal to perform genetic testing of the Preimplantation embryo was initiated in the UK in the mid-1980s. The first successful use of PGD was reported in 1990 when an X-linked disorder was avoided by sex determination and selection of female embryos for transfer to the mother. Handyside et al **[4]** reported the first established pregnancies using this procedure, in two couples known to be at risk of transmitting adrenoleukodystrophy and X linked mental retardation. Two female embryos were transferred after IVF, biopsy of a single cell at the six to eight cells stage, and sexing by DNA amplification of a Y chromosome-specific repeated sequence. Both women were confirmed as carrying normal female twins. In France, the bioethical laws (1994) allowed PGD practice in 1994, and the decrees have been published in 1998. The first birth was then obtained in 2000, for a couple with the woman carrying ornithine transcarbamylase deficiency which is an X-linked dominant metabolic disorder with partial penetration in heterozygous females **[5]**.

Two decades after the first clinical application, PGD is still a challenge due to the small amount of DNA available for analysis as well as time constraints. To allow transfer of unaffected embryos, PGD requires IVF, biopsy of the early human embryo and subsequent genetic analysis of single cells

for the specific disorder. By using PGD the couple can start a pregnancy assured that the disease has not been transmitted to the child, and the risk of having to terminate the pregnancy to be avoid. However, due to technical complexity and high cost (~SR 21,000), PGD is at present available in a rather limited number of centres worldwide **[6].**

Selected Procedures

Biopsy

There are different PGD approaches according to when the biopsy is performed. The earliest, with regard to embryo development, is sequential or simultaneous biopsy of the first and second polar body. Polar body biopsy can be the first and/or second polar bodies, which are removed after their extrusion from the oocyte and are studied by genetic analysis which is possible to study specific maternal contribution for the embryo and identify chromosomal translocations or genetic mutations from maternal origin. Polar body biopsies can be used as an alternative for preimplantation diagnosis of common aneuploidies in IVF patients with advanced age, to detect and avoid fertilization and transfer of oocytes with common aneuploidies **[1]**. Embryos are obtained by in vitro fertilization with intracytoplasmic sperm injection (ICSI). Indeed, ICSI is recommended for all PGD cases to reduce the risk of paternal contamination by sperm attached to the zona pellucida. Embryo biopsy was performed on 6-10 cell stage embryos, 3 days at the eight cells stage, after insemination, in Ca^{2+}/Mg^{2+}-free medium under oil (SAGE BioPharma) with the use of a double-needle approach, even if some alternatives mentioned later can be proposed. The zona pellucida is opened by one of the three methods reported for this procedure: mechanical, chemical, or laser. The presence of a clearly visible nucleus guides the selection of the blastomere to be biopsied. One or two blastomeres are sampled and the genetic analysis is performed on the same day. Only unaffected embryos are transferred into the uterus. Ovarian reserve has to be optimal because it is particularly important to obtain an adequate number of embryos. Indeed, there will be a selection among them and PGD implantation rate is not as high as the rate observed in classical IVF with ICSI **[6]**.

Embryo Transfer and Amniocentesis

Embryos with normal or balanced FISH signals can be selected and transferred into the uterus of the patient on the 4th or 5^{th} day after oocyte retrieval. Clinical pregnancy will be ascertained by confirming fetal heart beat using ultrasonography at 6 or 7 weeks of gestation. Amniocentesis technique can be used to confirm the results of PGD in clinical pregnancies. Cytogenetic, FISH and molecular analyses can be performed in cases of miscarriage when abortus samples were available **[7].**

Chromosomal Analysis

PGD has been used for the identification of chromosome abnormalities in couples who are at risk for either aneuploidy that is based on maternal age or an unbalanced parental karyotype chromosome rearrangement (such as translocations and inversions). PGD for chromosomal abnormalities or rearrangements provides an alternative to prenatal diagnosis and termination of affected foetuses and theoretic enhancement of implantation and pregnancy rates for the couples **[8].**

FISH

Although fluorescent in situ hybridization (FISH) probes can be designed to identify normal or balanced embryos from polar bodies or blastomeres of cleaving embryos, there are significant limitations to this technique. The process requires controlled ovarian stimulation and IVF and may result in too few oocytes recruited, particularly in women with advanced maternal age, which limits the number of embryos to analyse. In the analysis of translocations, unique FISH probes that flank the breakpoints of each translocation or that require the use of subtelomeric probes (specific to the chromosome ends of the translocated segments) for each affected individual must be designed and validated to detect normal and balanced products in embryonic tissue **[9].** The application of (FISH to a single embryo cell (blastomere) presents special challenges both in practicalities and in interpretation of the signal pattern. The biopsied cell needs to be spread within a pre-defined area on the slide in order to facilitate its localization following FISH; extreme care needs to be taken in ensuring that the cell is lysed, that the cytoplasm has been dispersed, and that the nucleus is visible and intact; and, as the diagnosis depends on the results from this single cell, stringent scoring and interpretation guidelines should be applied. However, in experienced hands, FISH is a robust technique for PGD in clinical practice. The principle of PGD by FISH is that target-specific DNA probes labelled with different fluorochromes or haptens can be used to detect the copy number of specific loci, and thereby to detect chromosome imbalance associated with meiotic segregation of chromosome rearrangements which includes the Robertsonian translocations, reciprocal translocations, inversions, and complex rearrangements. FISH can also be used to select female embryos in families with X-linked disease, for which there is no mutation-specific test. More controversially, FISH has also been used to screen for sporadic chromosome aneuploidy in order to try and improve the efficiency of assisted reproduction; however, the predictive value of this test using FSIH is likely to be unacceptably low in most people's hands and it is not recommended for routine clinical use **[10].**

For the molecular diagnosis, future improvements include the use of quantitative PCR, DNA fingerprinting and microarray technology. DNA microarrays manufactured to date are not able to analyse limited amount of genetic material in a single cell. Microarrays containing oligonucleotide mutation probes are emerging as useful platforms for the diagnosis of genetic disease. Further automation of part of this technique will enable a greater number of diseases to be accurately diagnosed at the single cell level **[11].**

PCR Analysis

PCR is used to amplify sufficient DNA from cells obtained from an oocyte or embryo to diagnose monogenic diseases **[12]**. Polar body or a blastomere is placed in a solution that lyses the cell and releases the DNA and the PCR reaction mix is then added to begin the PCR. Because of its high sensitivity, contamination of the study sample with extraneous DNA is a danger and has led to the adoption of rigorous laboratory procedures and standards, such as the use of intracytoplasmic sperm injection **[13]**. Moreover, amplification of only one, rather than both, of the genes present in a cell can result in misdiagnosis of disease and the transfer and implantation of affected embryos **[14]**. To overcome this potential difficulty, dubbed allele-drop out,

various techniques have evolved for the analysis of PCR fragments; fluorescent PCR and fragment analysis on automated sequencers were introduced first and, later, multiplex PCR was developed. Since then, the introduction of automated sequencing, minisequencing, and real-time PCR has further refined the diagnostic capabilities **[15]**.

PGD Procedure

Before PGD was initiated, it was important that the couple received genetic counselling to ensure that they were informed regarding the nature of the genetic disorder, the pattern of inheritance and the risk for their offspring. The genetic defect has to be identified and DNA must be available from both parents, and sometimes other members of the family, in order to establish a reliable test. The counseling included information about alternative reproductive options such as traditional prenatal diagnosis. If PGD was a realistic alternative from genetic point of view, the possible success of an IVF treatment was evaluated. Briefly, this includes estimation of the ovarian reserve and the probability for conception based on factors such as female age, physical examination, ovarian transsonographic examination, hormonal profile and semen quality **[8].**

Methodology:

This is a retrospective review of couples who had undergone IVF at Habib Medical Centre, Riyadh between March 2011 through March 2012, and specifically for the purpose of using PGD to identify chromosomally normal/balanced embryos for transfer and to test the sex chromosomes for sex selection. The indication for PGD was a history of recurrent pregnancy loss, history of translocation in the family, recurrent failure of IVF cycles and advance maternal age. All patients in the PGD program were received advice appropriate to their circumstances. Indications for PGD and prenatal diagnosis are often rather similar, but the efficiency of the methods as well as the consequences of the tests and treatment may be different. We have provided the necessary information to the patients to understand the risks, discomfort, costs, benefits and various alternatives to the PGD. All couples completed the informed consent process as approved by the Institutional Review Board. All the patients underwent ICSI and PGD procedure was according to the biopsy method mentioned above **[8]**. The characteristics of the PGD patients are summarized in **Table 1 (see supplementary material)**.

Statistical analysis

Statistical analysis was performed using SAS software (SAS Institute, Cary, NC). A chi-square test was used to compare frequency distributions among collapsed categorical groups of score ranges. Nonparametric analysis was conducted to make comparisons in the overall distributions. Bonferroni correction was used to calculate a significant P value based on the number of comparisons that were made. A P value of <0.05 was considered statistically significant.

Results:

In the present study 137 patients were enrolled for the PGD analysis. PGD procedure was followed in the invitro laboratory and on day 3 a total of 802 embryos were biopsied and among them 193 (24%) were transferred and 609 (76%) were not transferred because of the chromosomal abnormalities (n=290) and undesired sex of the embryos (n=309). The average age of women in the study was 34.83$\pm$4.99. Respondents represented a cross-section of Saudi families, with a wide range of age, education and background. The normal embryos (n=512) were in the age range of 34.30$\pm$4.43, whereas abnormal embryos (n=290) were in the age group of 34.65$\pm$4.48. When we perform t-test for both age group normal and abnormal embryos we did not find any significance value (p=0.82). In this study the couple had visited the clinic for the sex selection, recurrent fetal loss, recurrent IVF failure and advanced maternal age. 57.7% of patients came for sex selection, 24% of the women were having the history of recurrent fetal loss and 18.3% of the women were having recurrent IVF failure. The frequencies of abnormalities of sex selection, recurrent fetal loss and recurrent IVF failure groups are 68.3%, 57.5% and 12% respectively. The abnormal embryos constituted 36.2% (290), the abnormal sex chromosomes were 12.2% trisomies and triploidies were 3.2% (n=25) and 3.5% (n=25).There is only one Tetraploidy were present i.e. 0.2% (n=01). Chromosomal analysis was tabulated in the table 2. The abnormal chromosomes presented in this study are categorized as abnormal sex chromosomes (structural abnormalities, numerical abnormalities), trisomies and tetrasomies. The demographic details of the overall chromosomes are displayed in the **Table 2 (see supplementary material)**.

Discussion:

In the present study the most frequent indication for PGD was the sex-selection (57.7%), 24.3% was recurrent fetal loss & 13% were recurrent IVF failure. In all IVF cycles, the microscopic evaluation of the embryo is carried out at varied stages to identify those with the best overall appearance and developmental characteristics to be chosen for embryo transfer. In some IVF cycles PGD is performed to identify the embryos with abnormalities in the number and structure of chromosome present. In our study 36.2% of the human embryos were found to have abnormal chromosomes. 12.2 % of them were abnormal sex chromosomes and 3.2% were trisomies. This is compatible to the study done by Plachot et al **[16]** who find 4.8% of trisomies in abortions in spontaneous pregnancies and 6% in IVF pregnancies **[17]**. Chromosomal abnormalities such as aneuploidies are thought to be responsible for implantation failure, miscarriage and birth defects. There was only one Tetraploidy in our study and this was also comparable to Plachot et al study in 1988. Mosaicism XO is the most frequent abnormality in the sex chromosome and this was also comparable to the study done by Boue **[17]** and Shields **[18].** The most common indication for PGD in general is advanced maternal age which increases the rates of chromosomally abnormal eggs which after fertilization would become chromosomally abnormal embryos. However, in our study the most common reason for PGD was sex selection (79%). The controversy then arises as to whether PGD testing of the early embryos before transfer will increase the chances of a successful outcome or not **[8]**. PGD can clearly benefit the couple if both are carriers of a recessive disease (such as cystic fibrosis), if child (conceived naturally) would have a 25% chance of having this terrible disease. By having IVF /PGD, they can have "normal" embryos transferred so that (if the IVF is successful) their child should not have cystic fibrosis. However, if the embryos are damaged significantly from the testing procedure, then the risk of losing more than gaining from the test results. For example, couple (the husband or the wife) who has a

balanced chromosomal translocation they are normal until they try to have a child **[9]**. When their chromosomes join with those of their partner in the fertilized egg they make a high percentage of chromosomally abnormal embryos. These embryos are at very high risk for miscarriage or could result in the birth of a child with birth defects. This is another situation where PGD can help by having IVF/PGD, they can have chromosomally normal embryos transferred, greatly reducing their risk for miscarriage and birth defects **[19].** In our study the frequency of chromosomal abnormality in this group was 57.5 %. PGD is a technique used mainly in two broad indication groups. The first group consists of individuals at high-risk of having a child with a genetic disease, with the carriers of a monogenic disease or of chromosomal structural abnormalities such as translocations, infertility, recurrent miscarriages. The second group is those being treated with IVF, who might have a low genetic risk but whose embryos are screened for chromosome aneuploidies to enhance their chance of an ongoing pregnancy. PGD can increase the choices available to families at risk of having children with genetic abnormalities **[15].**

PGD may improve the chance of a viable pregnancy for couples suffering from recurrent miscarriages as a result of chromosomal abnormalities. One of the advantages of PGD in IVF programs is to screen for aneuploidies and transfer only embryos with a genetically normal complement and also avoid the possible adverse consequences of embryo manipulation in Prenatal Diagnosis (Amniocentesis and Chronic Villi Sampling) **[20]**. However, major problems with the use of PGD in IVF programs are that the number of embryos available for cryopreservation after embryo transfer is likely to be less, and biopsied embryos may have a lower potential for survival in cryopreservation and thawing processes. The current evidence was that routine use of PGD to improve IVF outcome is inconclusive. PGD can be used for the selection of embryos with similar HLA types to that of an existing child suffering from a certain hematopoietic disorders such as Fanconi anemia, leukemia and thalassemia requiring donation of cells from a "savior sibling". Cells from the umbilical cord of the donor child may then be grafted to the affected child. This technique has also been successfully applied to a group of immunodeficiency syndromes **[20].**

In this study normal Chromosomes are 63.8% and among them 60.9% of the embryos were XX and 39.1% XY. The abnormal sex chromosomes present in the study were 12.2%. Only 3.2% trisomies and 3.5% triploidies and 0.2% tetrasploidies were observed. The reason for testing sex chromosomes can be the important role of X chromosome in transferring certain diseases For example: Duchenne muscular dystrophy which affects only the boys and mother passes the hemophilia disease to their sons through X chromosome **[21]**. The purpose of PGD for sex selection is the potential for inherent gender discrimination, inappropriate control over nonessential characteristics of children, unnecessary medical burdens and costs for parents, inappropriate and potentially unfair use of limited medical resources. Sex selection can be used based on the prevention of transmittable genetic diseases is strong enough to clearly avoid or override concerns regarding gender equality. For the above reasons, in Saudi Arabia sex selection just for the sake of choosing certain gender is provided only in private hospitals. PGD for certain sex linked diseases is provided in some governmental hospitals (for example- King Faisal specialist hospitals) **[22]**. PGD is permissible in Islam provided the sperms and oocytes are from the husband and wife. Muslim jurists have agreed that PGD is a technique that is permissible in Islam because IVF does not conflict with God's desire and might **[23]**. Furthermore, this technique is not considered a modification of God's creation, because it is a kind of treatment. It has been argued that Muslims might reject PND and termination of pregnancy because of religious convictions **[24, 25]**. PGD may be preferable to PD for Muslim parents, because it is done when embryos are only at the eight-cell stage and 'breathing the soul' has not occurred at this stage **[26].** Alsulaiman A **[22]** was the first group to study the attitude of patients towards PGD in the Saudi Arabia. This study reported attitudes towards PD and PGD of Saudi couples offered genetic counseling following the birth of a child with a single gene or chromosomal condition. Eight of the 30 couples (27%) selected for the PGD, four (13%) of them selected for the PD and three (10%) were either technology. The remaining couples were not interested in either test or were unsure. The main concerns of those who would accept neither technology were related to personal religious views. Specific concerns about PGD related to the IVF procedure, the risk of multiple pregnancies, the chance of mistakes and the chance of not getting pregnant. A high proportion of couples (six out of seven ~86%) who had a child with thalassaemia expressed interest in PGD and all would be prepared to use technology to avoid having an affected child **[19]**.

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Supplementary material:

Table 1: Characteristics of the PGD Patients.

S.No	Description	PGD (n=137)
1	Total no of Patients	137
2	Age of mothers	34.83±4.99
3	Total no of embryos tested	802
4	Total no of embryos transferred	193
5	Frequencies of abnormalities for all patients	290 (36.2%)
6	Average age of mothers with abnormal embryos	34.9
7	No. of mothers who come for Sex selection	79 (57.7%)
8	No. of mothers who come for Recurrent fetal loss	33 (24%)
9	No. of mothers who come for Recurrent IVF failure	25 (18.3%)
10	No. of abnormalities who come for Sex selection	54 (68.3%)
11	No. of abnormalities who comes for Recurrent fetal loss	19 (57.5%)
12	No. of abnormalities who comes for Recurrent IVF failure	03 (12%)

Table 2: Types of abnormalities present in this study

S.No	Normal Chromosomes (n=512) (63.8%)	No Signal (n=139) (17.3%)	Abnormal Sex Chromosomes (n=97) (12.2%)	Trisomies (n=25) (3.2%)	Triploidy (n=28) (3.5%)	Tetraploidy (n=01)(0.2%)
1	XX=312 (60.9)		XO-87 (89.6)	T13=03 (12)	XXX=11 (39.3)	XXXX=01(100)
2	XY=200 (39.1)		YO-09 (9.3)	T18=18 (72)	XXY=10 (35.7)	
3			YY-01 (1.1)	T21=04 (16)	XYY=07 (25)	