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Preimplantation Genetic Diagnosis (Pgd) in Women with Advanced Maternal Age: A Literature Review

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Abstract

Aim: Pre-implantation genetic diagnosis (PGD) in women at advanced maternal age (AMA) is a test of genetic status performed on the genetic material obtained from a biopsy of the oocyte or embryo of women who are at risk of aneuploidy associated with their AMA.

Method: This process is performed in conjunction with in vitro fertilisation (IVF) whereby, after ovulation induction, the embryo is biopsied.

Results: However, misdiagnosis remains an issue and these women as well as their partners and family need to be counselled. Conclusions: This article reviews PGD in women with AMA, with its positive and negative forecast as well as the ongoing debates concerning associated ethical issues.

Keywords: Aneuploidy; Embryo; Maternal age; Preimplantation genetic diagnosis; Zona pellucida

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Introduction

Pre-implantation genetic diagnosis (PGD) is a technique used to test embryos or oocytes from patients at risk of conceiving a childaffected by a recurrent genetic disorder. PGD is performedso that only embryos not carrying the disorder are pre-selected for transfer [1].

Pre-implantation genetic screening (PGS), on the other hand, is a technique aiming at improving the outcome of assisted reproduction treatment for patientsconsidered sub-fertile, by testing for a number of the most frequent chromosome aneuploidies to improve implantation and reduce the incidence of miscarriage [2].

Pre-implantation genetic testing can therefore be classified as either PGD or PGS. These two techniques require a biopsy of the oocyte or embryo in order to obtain genetic material for diagnosis. Once a biopsy is performed, the type of test applied to the cell depends on the genetic condition being tested. It is offered for three main categories of diseases to identify single gene disorders, which may be recessive or dominant, where the molecular abnormality is tested following amplification by PCR of DNA extracted from single cell; and to detect a variety of chromosomal arrangements such as translocations, inversions, deletions, or insertions on interphase nuclei using fluorescence insituhybridisation (FISH) [3].

Fertile women aged 37 years and older are at risk of transmitting inherited genetic disorders to their child.PGD and PGS allow them to know if their embryos have problemsand to decide whether they wish to interrupt their pregnancy.

Herein,we reviewed the literature onPGD in women with AMA using Pubmed. We limited our search to articles published in

English, Spanish, and French. In this review, we discuss the outcomes of advanced maternal age (AMA) with associated decline in fertility and the increased risk of aneuploidy.We also discuss the type of aneuploidies and the role PGD inin vitrofertilisation(IVF). Embryo biopsy, selection,and transfer aiming at improving pregnancy outcome in female at risk for chromosomal abnormalities are reviewed. We discuss PGDfor aneuploidy screening (PGD-AS),while focusing on AMA and risk of chromosomal abnormalities (monosomyand trisomy).Ethical issues associated with PGDare also discussed **(Figure 1 and 2)**.

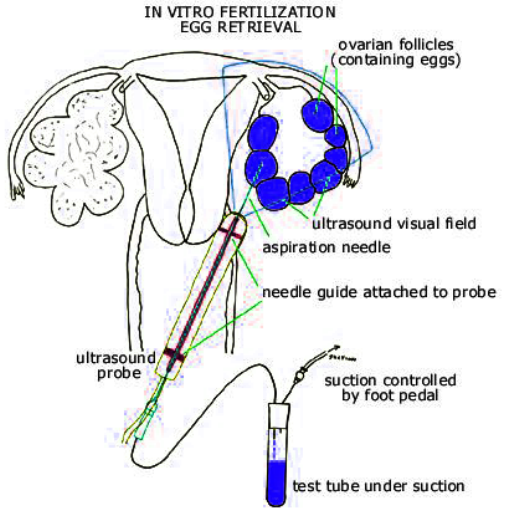

Figure 1: The biopsy technique can be performed at three different stages, on oocytes or zygotes (polar body biopsy), on 6–10-cellembryos (cleavage stage biopsy), or on blastocysts (blastocyst biopsy).

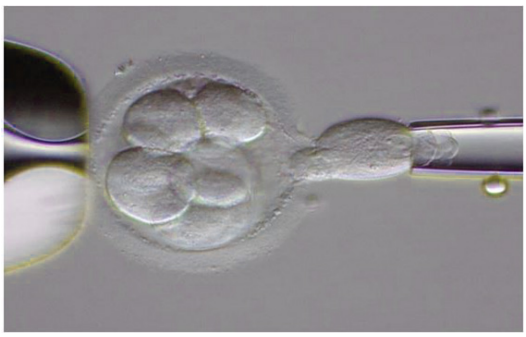

Figure 2: Regardless of the procedure chosen, a biopsy involves two steps: zonapellucida drilling, which can be performed mechanically, chemically, or by laser energy,and cell removal for analysis of the genetic material to transfer only healthy euploid embryos.

Definition of Advanced Maternal Age (AMA)

AMA is defined as a maternal age of 37 or 38 years and over. Theaverage natural fertility declines with aging, resulting in a significant decrease in the pregnancy rateof women over 37 years and inthe success of fertility treatment.This natural decrease in fertility and the increased risk of miscarriage with maternal aging is related to a decrease in both the quantity and quality of available oocytes.Over time, chromosomes in the ovary are less likely to divide properly,resulting in the egg having either an extra or a missing chromosome (aneuploidy),increasing the risk of conceiving an abnormal baby [4-6].

An embryowith aneuploidy is less likely to attach to the uterus or develop to full term. Hence,the difference in the percentage of affected embryos comparedto live born babies is due to the fact thatmost embryoswill not implant or will be miscarried. The lack of implantation and loss rate of aneuploid embryos explain why pregnancy rates decrease with AMA.

Maternal age is the most important predictor of IVF success. Oocyte donationhas been used to resolve, to a certain extent, the aging issues related to decrease reproductive outcome in IVF. However,the complication rates of the resulting pregnanciesare modestly improved. AMAis associated with pregnancy complications, even when the woman own gametes are involved.

Factors associated with poor maternal fertility outcome

Quantity of oocytes and follicles

The number of eggs in theovaries is referredto as“Egg quantity” andfemale germ cells are not replenished during life.The number ofoocytes and follicles is determined in utero and declines from the second trimester of pregnancy to menopause in acharacteristicexponential curve5.During foetal life, germ cells rapidly proliferate by mitosis to produce 6 to 7 millionoogonia, whichtransformed to oocytes after entering the first meiotic division by 16-20 weeks of pregnancy. At birth,the number of germ cells decreases to 1 to 2 million by the onset of pubertyto about 300.000 to 500.000, and only about 400 to 500oocytes will ovulateover the next 35-40 years of reproductive life. By age 37-38,approximately 25,000 oocytes remainandless than 1,000 follicles remainat the time of menopause.

Quality of oocytes and follicles

The quality of oocytes and folliclesrefers to thereadiness and ability of the female eggs to become fertilised. Every woman carries a certain number of eggs in her ovaries ready to be released for fertilisation. These eggs need to present the right shape, be healthy, and contain the right number of chromosomes in order to develop into an embryo and later, a baby. Unfortunately, the egg quality also changes over time. As females age, the eggs become weaker and less able to form a healthy embryo. They begin to decrease in number, leaving fewer and fewer quality eggs available for fertilisation. A 40-year-old woman typically has lower egg quality than a 20-year old woman.

Complications associated withpoor oocyteand follicle quality

Pooregg quality can lead to a variety of complications, includingIVF or intrauterine insemination (IUI) failure,repeated miscarriages,and chromosomal abnormalities. The increased risk for miscarriage in aging women is due to the increase in chromosomal abnormalities in their eggs.

Chromosomal aneuploidy

Spermatozoa or eggs that have extra or missing chromosomes will pass this problem on to the embryo after fertilisation. This situation is known as aneuploidy. There can be extra (trisomy) or missing (monosomy) chromosomes. Both conditions are a problem. If aneuploidy involves larger chromosomes, the embryo may not attach to the wall of the uterus or may stop developing soon after and miscarry. In some cases such as Down syndrome, however, aneuploidy may cause the foetus to be abnormal, but able to develop to birth. The features of the chromosome condition depend upon which chromosome is extra or missing, but can include physical abnormalities and mental retardation.

Chromosomal problems associated with AMA

The meiotic spindle is involved in organising the chromosome pairs so that proper division of pairs can occur as the egg is developing.When the chromosomes line up in a straight line on the spindle, the division process proceeds normally. However, with a disordered arrangement on an abnormal spindle, the division process may be inappropriate, resulting in an unbalanced chromosomal situation. Eggs in older women are significantly more likely to have abnormal spindles, predisposing to the development of chromosomally abnormal eggs.There are general types of chromosomal abnormalities, numerical and structural abnormalities.Numerical abnormalities are abnormalities in the number of chromosomes, called aneuploidies such as monosomy (Turner syndrome) and trisomy (Down syndrome).Structural abnormality is a problem associated with the structure of a chromosome such as translocations, duplications, and deletions of part of a chromosome.

Couples known to be at risk of transmitting genetic disorders to their children have various options.Theycould decide to conceive naturally and accept the risk of their child inheriting the genetic condition such as recurrent miscarriages as a result of the genetic condition or opt fora gamete donation,a process wherebyone or both parents would not be the biological parent of the child during the process of assisted conception. Adoption or being childlessarethe other options available for these couples.When the couple chose natural conception, they undergo conventional prenatal diagnosis (PND) following conception.

The UK national policy study on aneuploidy screening

According to theUK national policy studyon aneuploidy screening, the odds of the foetusbeingaffectedafter a positive combined test in the first trimester are muchgreaterthanthe oddsbased on AMA alone (1:20 vs 1:75).Thus,the probability that an invasive test wouldconfirm an abnormalfoetalkaryotype is higher.The two commonly invasive procedure tests used post-conception for diagnosis are chorionic villus sampling (CVS) at 11 weeks and amniocentesis at 16 weeks. If the foetuspresents the genetic abnormalities of concern, the parents have to decide whether or not to opt for termination of the pregnancy (TOP). Finding out that an expected baby has a serious anomaly is extremely stressful, not only for the pregnant woman, but also for herfamily. Counseling needs to take into account the parents' culture, religion, and beiefs in order for them to make an informed decision. In South Africa, TOPis performed at any periods of gestation if it is agreed by consensus decision of a multidisciplinary team that continuation of the pregnancy will result in a severely handicapped child. Adequate time after counseling should be provided to the parents to reach an informed decision [7,8].

PGD and PGS

PGS or PGD for aneuploidy screening (PGD–AS) is a form of PGD that has been used to screen genetic abnormalities in embryos of infertile couple undergoing IVF.

In PGS, no specific genetic diagnosis is tested for. PGS just screens embryos for the most common genetic diseases. The parents are presumed to be chromosomally normal and the test is used to look for abnormalities in chromosome numbers. The main indications suggested for PGS are AMA, repeated implantation failure (three or more failed embryo transfer procedures involving high-quality embryos), repeated miscarriage in patients with normal karyotypes (usually at least three previous miscarriages), and severe male factor infertility.

PGD is not to be confused with PGS. The widespread use of PGS without evidence of its ability to improve delivery rates has been reported as a problem in the field of IVF.

Since 2004, there have been randomised controlled trials (RCTs), mainly for AMA, showing no benefit of performing PGS14. In a systematic review and meta-analysis of the RCTs for PGS, no evidence of a beneficial effecton the live birth rate after IVF of PGS as currently applied was reported. On the contrary, for women of AMA, PGS significantly lowers the live birth rate from 26% after IVF without PGS to between 13% and 23% .

PGD started in the 1980s, resulting in the world's first baby born in 1989. The technique involves a genetic analysis of a single cell from an eight-cell embryo performedduring IVF to improve the outcome of a normal pregnancy.It was introduced in 1990 with polar body biopsy and with blastomere biopsy that same year by [1].

Since then, over 60 PGD centres have been opened worldwide, with over 10,000 babies born so far. PGD can be performed at different stages with polar body biopsy, at the cleavage stage embryo, or at the blastocyst stage with the use of FISH to detect chromosome abnormalities or polymerase chain reaction (PCR) techniques to detect genetic conditions.

PGDcan be offered when one or both genetic parents have, or are carriers of, a known genetic abnormality. Testing is performed on the embryos created through IVF to determine whether they are at risk of genetic disease. Tests are carried out for the specific disorder that the embryos are known to be at significant risk of inheriting. Unaffected embryos are selected for transfer to the

uterus,hoping that a normal birth will ensue.

IVF with PGDbecause of AMA or in couples with unexplained recurrent pregnancy loss can increase implantation rates and decrease miscarriage risk.However,the modest improvements in live birth rates achieved thus far cannot justify the associated costs for couples without other specific indications for IVF5.

PGD has become an alternative to commonly used PND; PGD helps to transfer only unaffected embryos, thereby avoiding the risk of pregnancy termination. In addition, PGDis currently applied for other indications than PND, including common diseases with genetic predisposition and pre-implantation human leukocyte antigen typing, with the purpose of establishing potential donor progeny for stem cell treatment of siblings. Many healthy, unaffected children have been born after PGD, supportingits reliability and safety. PGD appears to be of special value for avoiding age-related aneuploidies in patients of advanced reproductive age, improving reproductive outcome [9].

PGD is used for carriers of balanced translocations to have unaffected children of their own.PGD allows the selection ofeuploid embryos for transfer in assisted reproduction.

Risks to the child conceived via IVF and PGD

PGD allows couples at significant risk of having a child with a genetic disorder, to have a child who is genetically related to them and at very low risk of being affected. Information about the effectiveness of PGD in reducing the risk of passing on a genetic disease compared with a couple getting pregnant spontaneously is based on observational studies, with the largest case series being from the European Society of Human Reproduction and Embryology (ESHRE) PGD Consortium with over10,000 PGD cycles that took place between January 1997 and December 2008.

The indications for PGD include patients with AMA, aneuploidy screening, recurrent miscarriage, recurrent IVF failure, genetic or chromosomal conditions, sex linked diseases, and family balancing. Aneuploidy rates in embryos destined for transfer increases with age. At TMC Fertility Centre, patients below 35 years old have an aneuploidy rate of 30.6%, which increases to 70% for patients above 40 years of age. In patients with recurrent miscarriage, pregnancy rates improved following PGD. Chromosomes, which are tested with PGD, often include 13, 16, 18, 21, 22, Xand Y.

PGD also improve implantation rates17.For balance translocation, PGD increases delivery rate from 11.5% to 81%.

Clinical pregnancy rates following PGD embryo transfer range from 23% to 51% worldwide with a delivery rate ranging from 18% to 43%. PGD presents the following advantages:very early diagnosis and thetransfer of unaffected (or carrier) embryos.Thedisadvantagesof PGD include its costand low implantation rates. Additionally, affected or unused embryosare discarded,which creates ethical concerns.

PGD process

The PGD process involves the following :counselling, induction of ovulation and oocyte collection, fertilization byintracytoplasmicsperm injection (ICSI), pronuclear formation (+2nd polar body), pronuclear fusion, and zygote.

Once a genetic PND (ESHRE guidelines) is reached, 1 to 2 suitable embryos are implanted and pregnancy is confirmed or not.

PGD and counselling

Before PGD is performed, genetic counselling must be provided by a multidisciplinary team to ensure that patients fully understand the risk of having an affected child, the risk of misdiagnosis,and the impact of the disease. Patientsshould be informed about the available options and the multiple technical limitations, including the possibility of an erroneous result.

Induction of ovulation

In order to obtain a large number of oocytes, the patients undergo controlled ovarian stimulation with the use of Follicle Stimulating Hormone (FSH), followed byultrasound-guided trans-vaginal oocyte retrieval [10].

Biopsy

Biopsy can be performed at three different stages: on oocytes or zygotes (polar body biopsy), on 6-10 cells embryos (cleavage stage biopsy), or on blastocysts (blastocyst biopsy). Independently from the procedure chosen, a biopsy involves two steps: 1) zonapellucidadrilling, which can be performed mechanically, chemically, or by laser energy, and cell removal.

Biopsy of polar bodies

Polar body biopsy consists in the removal of the first polar body from an oocyte in order to diagnose the genetic status before insemination. This procedure is referred as to pre-conception genetic diagnosis.Polar body biopsy allows the evaluation of the genetic maternal contribution by theidentification of chromosomal abnormalities orgene mutations.Additionally, it has recently been applied for HLA typing and for the diagnosis of X-linked disorders.The first polar body is extruded during Meiosis-I and does not require correct fertilisation and/or embryo development. Polar bodies can be biopsied without affecting an egg's rate of fertilisation or eventual cleavage of the embryo, and can be used to deduce the genotype of the oocyte. To obtain polar bodies, a slit is made in the zonapellucida by mechanical means, e.g., sharp needles or with laser technology. The polar body is then gently drawn out of the egg with a biopsy pipette. In a large series of first and second polar-body analyses for single-gene disorders, Verlinsky and co-workers (1990) correctly identified a genetic disorder in 98% (157 of 160) of oocytes tested.

Laboratory techniquesfor analysis of cells obtained after biopsy

Polymerase chain reaction (PCR)

PCR is used to amplify sufficient DNAfrom cells obtained from an oocyte orembryo to diagnose monogenic diseases.In brief, a polar body or a blastomereisplaced in a solution that lyses the cell andreleases DNA. A PCR reaction mix isthen added and PCR begins. Because ofits high sensitivity, contamination of thestudy sample with extraneous DNA is adanger and has led to the adoption ofrigorous laboratory procedures andstandards such as the use of ICSI. Moreover, amplification of only one ratherthan both of the alleles present in a cell can result inmisdiagnosis and the transfer and implantationof affected embryos. To overcome this potentialdifficulty, dubbed allele-drop out, and various techniques for the analysis of PCR fragments, including fluorescence PCR, and fragment analysis on automated sequencers,wereintroduced first and, later, multiplex PCR was developed.Since then, the introduction of automated sequencing,minisequencing, and real-time PCR has further refined ourdiagnostic capabilities.

PCR-based tests only detect disorders at target loci.Other mutations may exist elsewhere.Thus, prenatal amniocentesis or CVSis usually recommended. PCRis indicated formonogenic disorder.

Comparative genomic hybridisation (aCGH)

Array Comparative GenomicHybridisation (aCGH) or detection Chromosomal Microarray Analysis (CMA) allows the detection of smaller pathogenic chromosomal variants that are undetectable using standard cytogenetic analyses (G-band karyotyping). aCGH does not allow the detection of balanced chromosomal rearrangements,triploidy, and some instances of mosaicism. The biggest challenge presented by aCGH is the detection of chromosomal variants of unknown clinical significance.

CGH is a new techniqueintroduced by two groups, where pre-amplified DNAfrom a single test cell islabelled with one fluorochrome (red)and then mixed with pre-amplified DNA from acontrol sample labelled with a different fluorochrome (green) to which it is compared. The mixture isapplied to a normal metaphase spread and the colourratio is measured. Areas with more red indicate that the testsample contained more of this genetic material, whereasareas showing more green indicate that the test samplecontained too little of this genetic material. The advantage of comparative genomic hybridisation over FISH is that the whole chromosome complement isanalysed, though polyploidy and balanced translocationscannot be detected. The disadvantage is that the wholeprocedure takes about 72 h, which is why the method isapplied either to polar bodies, allowing 5 days for analysis,or to cells obtained at the cleavage stage of development,after which embryos are cryopreserved and transferred ina later cycle if identified as healthy. The time required tocomplete the procedure could be shortened by replacingthe metaphase spread with microarrays of carefully chosensequences from all 24 chromosomes, so-called microarraycomparative genomic hybridisation [11].

Cryopreservation of biopsied embryos

After PGD, few embryos usually remain forcryopreservation. Although most centres do cryopreservesurplus embryos, the survival rate of the embryos isextremely low, and only one pregnancy has beendescribed after standard procedures were followed. However, Jericho and colleagues(2003) adapted thesestandard procedures to make the freezing and thawingprocesses more gentle and successfully applied theirnew technique to embryos analysed with CGH. The group noted a survival rateof 75% compared with 43% when using the standardprotocol and an implantation rate of 12%. Fiftyembryoswere transferred in 36 transfer procedures, leading tosix ongoing pregnancies.

useof sexing for social reasons and provoked mixedreactions. Opponents argue that sex selection fornon-medical purposes is sexist and tantamount todiscrimination against women. Those in favor argue thatsex selection at the embryonic stage of development ispreferable to sex selection after PND, or evenafter birth. Moreover, they claim that overall sexdistribution will not be skewed because only few peoplewill use PGD to choose their child's sex. A distinctionshould be made, however, between sex selection forconvenience and for so-called family balancing. In thelatter instance, the family should have at least one or twochildren of one sex before they can ask for a child of theopposite sex. In family balancing, the issues of sexdiscrimination and skewed sex ratios are avoided, givingthe family increased autonomy without conflicts withother ethical principles.

Using PGD inherently makes assumptions about the quality of life, challenging basic tenets of society such as equality, weighing the goal of pregnancy and live birth against the medical and moral risks of multiple gestationsrequires society to make a decision on when life begins.

Discussion

With the advent of the microarray techniques, transcripts of thousands of genes can be tested at once. First trimestercombined screening reduces the numberof invasive prenataldiagnosticprocedures.After a positive combined test, an invasive test wouldconfirm an abnormal foetalkaryotype. Thus, first trimestercombined test reverses the traditionalpyramid of prenatal care.

A CGH is not a substitute for conventional karyotyping and should be used for specific diagnostic purposes in selected pregnancies, not for general screening.

PGD has become an alternative to commonly used PND in women with AMA at risk of aneuploidy as a screening test to transfer only unaffected embryos. PGD is currently applied for indications other than PND, including diseases with genetic predisposition. Many healthy, unaffected children have been born after PGD,

supporting its reliability and safety despite ethical issues that remain unresolved and still the subject of debate.

References

1. Handyside AH, Lesko JG, Tarin JJ, Winston RM, Hughes MR (1992) Birth of a normal girl after in vitro fertilization and preimplantation diagnostic testing for cystic fibrosis. N Engl J Med 327: 905-909.
2. Kuliev A, Verlinsky Y (2004) Thirteen years' experience of preimplantation diagnosis: report of the Fifth International Symposium on Preimplantation Genetics. Reprod Biomed Online 8: 229-235.
3. Grace J, Toukhy T, Braude P (2004) Pre-implantation genetic testing. BJOG 111: 1165-1173.
4. Allen VM, Wilson RD, Cheung A (2006) Pregnancy outcomes after assisted reproductive technology. J ObstetGynaecol Can 28: 220-250.
5. Wells D, Brynn L (2003) Cytogenetics in reproductive medicine: the contribution of comparative genomic hybridization (CGH). BioEssays 25: 289-300.
6. Shenfield F, Pennings G, Devroey P, Sureau C, Tarlatzis B, et al. (2003) Preimplantation genetic diagnosis. Hum Reprod 18: 649-651.
7. Sermon K, De Rijcke M, Lissens W (2002) Preimplantation genetic diagnosis for Huntington's disease with exclusion testing. Eur J Hum Genet 10: 591-98.
8. Pickering S, Polidoropoulos N, Caller J, Scriven P, Ogilvie CM, et al. (2003) Strategies and outcomes of the first 100 cycles of preimplantation genetic diagnosis at the Guy's and St. Thomas' Center. FertilSteril 79: 81-90
9. The NHS Fetal Anomaly Screening Programme (FASP) (2013)
10. Morgan S, Delbarre A, Ward P (2013) Impact of introducing a national policy for prenatal down syndrome screening on the diagnostic invasive procedure rate in England. Ultrasound Obstet Gynecol 41: 526-529.
11. Gagnon A, Wilson RD, Allen VM, Audibert F, Blight C, et al. (2009) Evaluation of prenatally diagnosed structural congenital anomalies. J Obstet Gynaecol 31: 875-81.