27 PREIMPLANTATION GENETIC DIAGNOSIS

Dr. Ameet Patki
MD., DNB., FCPS, FICOG; FRCOG
Hon Associate Professor
K.J.Somaiya Medical College, Hospital and Research Centre, Mumbai
Secretary Mumbai OBGYN Society 20011-12
Fellow Representative RCOG-West zone India 2010-12
Governing Council ICOG 2012-14

What is PGD?

Preimplantation genetic diagnosis (PGD)—the technique by which early human embryos are genetically screened and then the genetically normal embryos are transferred into the uterus.

Who Discovered PGD?

In 1967, Robert Edwards and David Gardner reported the successful sexing of rabbit blastocysts, setting the first steps towards PGD (1). Handyside and collaborators' first successful attempts at testing were in October 1989 with the first births in 1990 (2).In these first cases, PCR was used for sex determination for patients carrying X-linked diseases.

Is PGD Safe?

More than a 5000 PGD cycles have been performed until now with a pregnancy rate of around 24%.The procedure is safe and the incidence of abnormalities is equvivalent to that in the general population (3).

What are The Indications for PGD?

PGD is indicated for the following cases

1. Couples with a high risk of transmitting an inherited condition

This can be a monogenic disorder, autosomal recessive, autosomal dominant or X-linked disorders or a chromosomal structural aberration such as a balanced translocation.

Autosomal Recessive Disorders

Cystic fibrosis, Beta-thalassemia, Sickle cell disease, Spinal muscular atrophy type 1

Dominant diseases

Myotonic dystrophy, Huntington's disease, Charcot-Marie-Tooth disease

X-linked diseases

Fragile X syndrome, Hemophilia A, Duchene Muscular dystrophy

2. Pre Genetic Screening (PGS)

- PGS is done in couples that undergo IVF treatment. The embryos are screened for chromosome aneuploidies (PGS) to increase the chances of an ongoing pregnancy.
- The main indications for PGS are
- Advanced maternal age
- History of recurrent miscarriages
- Recurrent implantation failure (defined as three or more failed IVF attempts)
- Patients with obstructive and non-obstructive azoospermia

Advanced maternal age

Aneuploidy screening is probably the most frequent indication for PGD, mainly suggested to couples undergoing IVF with an advanced maternal age and for patients with repetitive IVF failure. The principle behind it is that, since it is known that numerical chromosomal abnormalities explain most of the cases of pregnancy loss, and a large proportion of the human embryos are aneuploid, the selective replacement of euploid embryos should increase the chances of a successful IVF treatment. Different studies provide indications that PGS increases the implantation rate (4,5,6,)and lowers the spontaneous abortion rate, though other studies indicate that there are no significant differences for patients with an advanced maternal age, with a poor implantation rate or with recurrent idiopathic miscarriages. It is thus clear that large randomised-controlled studies are still necessary to measure the real impact of this technique for the different indications.

Repeated spontaneous abortion

PGD is also useful for couples with chromosomal translocations who had repeated spontaneous abortion. Robertsonian translocations, for example, may occur as frequently as 1 in 1000 in many populations.

3. Mitochondrial disorders

Diseases arising from mitochondrial DNA (mtDNA) mutations are usually serious pleiotropic disorders with maternal inheritance. When this mutation is present in a higher percentage of a person's mitochondria—greater than 90 percent to 95 percent—it causes a more severe condition known as maternally inherited Leigh syndrome. Owing to the high recurrence risk in the progeny of carrier females, "at-risk" couples require prenatal diagnosis. However, reliability of such practices remains under debate.

4. Ethically difficult indications

- Human leukocyte antigen (HLA) typing of the embryo, so that the child can be a cord-blood stem cell donor for a sick sibling
- Non-disclosure PGD for Huntington's disease

5. Recent Application of PGD

Late-onset diseases

- PGD for Alzheimer's disease
- Cancer predisposition syndromes

  PGD can be used to avoid the birth of children who are healthy at birth but face a higher than average risk of having cancer or some other serious disease.

  The procedure was performed for patients with predisposition to familial adenomatous polyposis coli (FAP), Von Hippel-Lindau syndrome (VHL), retinoblastoma, Li-Fraumeni syndrome, determined by p53 tumour suppressor gene mutations, neurofibromatosis types I and II and familial posterior fossa brain tumour (hSNF5).

- BRCA 1 and BRCA 2 gene testing by PGD

  In May 2006, the UK Human Fertilization and Embryology Authority (HFEA) approved use of preimplantation genetic diagnosis (PGD) for lower penetrance, late onset cancer susceptibility syndromes such as hereditary breast and ovarian cancer (HBOC). It may be sought for BRCA1 and BRCA2 susceptibility for breast cancer.

6. PGD for sex selection

Increasingly, PGD is also used for sex selection for non-medical reasons. A 2006 survey found that 9 per cent of clinics in the US provide this service. Half of these perform it only for "family balancing", which is where a couple with two or more children of one sex desire a child of the other, but half do not restrict sex selection to family balancing. In India, this practice has been used to select only male embryos although this practice is illegal according to PNDT act.

7. PGD for nonmedical traits

Other controversial uses of PGD would arise if genetic tests for nonmedical traits such as hearing, sexual orientation, height, beauty, intelligence, or other factors became available. Untangling the inherited basis of phenotypic traits strongly influenced by learning and environment will not be simple, and such tests, with a few exceptions, are unlikely to be available, if at all, for some time to come.

Although PGD acts negatively by screening out embryos, it accepts the principle that parents might rightfully exercise control over the genomes of offspring. It will then be much easier to take the next steps leading to positive alteration and engineering of offspring traits and the risk of 'designer' children which that will bring (7).

What Is the Procedure for PGD?

PGD is a form of genetic diagnosis performed prior to implantation. This implies that the patient's oocytes should be fertilized in vitro and the embryos kept in culture until the diagnosis is established. It is also necessary to perform a biopsy on these embryos in order to obtain material on which to perform the diagnosis. The diagnosis itself can be carried out using several techniques, depending on the nature of the studied condition. Generally, PCR-based methods are used for monogenic disorders and FISH for chromosomal abnormalities and for sexing those cases in which no PCR protocol is available for an X-linked disease. These techniques need to be adapted to be performed on blastomeres and need to be thoroughly tested on single-cell models prior to clinical use. Finally, after embryo transfer, surplus good quality unaffected embryos can be cryopreserved, to be thawed and transferred back in a next cycle.

Is ICSI Better Than Ivf For PGD?

In the majority of the reported cycles, intracytoplasmic sperm injection (ICSI) is used instead of IVF. The main reasons are to prevent contamination with residual sperm adhered to the zona pellucida and to avoid unexpected fertilization failure.

What are the different Embryo Biopsy procedures used for PGD?

As PGD can be performed on cells from different developmental stages, the biopsy procedures vary accordingly.

Theoretically, the biopsy can be performed at all preimplantation stages, but only three have been suggested:

1. On unfertilised and fertilised oocytes (for polar bodies, PBs)
2. On day three cleavage-stage embryos (for blastomeres)
3. On blastocysts (for trophectoderm cells).

The biopsy procedure always involves two steps: the opening of the zona pellucida and the removal of the cell(s). There are different approaches to both steps, including mechanical, chemical (Tyrode's acidic solution)

and laser technology for the breaching of the zona pellucida, extrusion or aspiration for the removal of PBs and blastomeres, and herniation of the trophectoderm cells.

What is Polar Body Biopsy (PB)? Its Advantages and Disadvantages

The first PB is removed from the unfertilised oocyte, and the second PB from the zygote, shortly after fertilization. They have been used for diagnosing translocations and monogenic disorders of maternal origin, as well as for PGS (8).

First polar body biopsy

In women who are heterozygous for a genetic disease, genetic analysis of the first polar body allows the identification of oocytes that contain the maternal unaffected gene. These oocytes can be fertilized and transferred to the mother without risk of establishing a pregnancy with a genetically abnormal embryo.

Munne et al have used first polar biopsy for patients undergoing in-vitro fertilization (IVF) aged > or = 35 years as it has been estimated that in this age group, 50% of embryos are chromosomally abnormal, with aneuploidy being the major contributing factor. And since the origin of most aneuploidies is maternal meiosis I non-disjunction, unfertilized oocytes were safely screened for aneuploidy by analyzing their first polar bodies. They analyzed polar bodies by fluorescence in-situ hybridization (FISH) using probes simultaneously for chromosomes X, Y, 18, 13 / 21 or X, Y, 18 and 16 (8).

Advantages and disadvantages of polar body biopsy

The main advantage of the use of PBs in PGD is that they are not necessary for successful fertilisation or normal embryonic development, thus ensuring no deleterious effect for the embryo. One of the disadvantages of PB biopsy is that it only provides information about the maternal contribution to the embryo, which is why cases of autosomal dominant and X-linked disorders that are maternally transmitted can be diagnosed, and autosomal recessive disorders can only partially be diagnosed. Another drawback is the increased risk of diagnostic error, for instance due to the degradation of the genetic material or events of recombination that lead to heterozygous first PBs. It is generally agreed that it is best to analyse both PBs in order to minimize the risk of misdiagnosis. This can be achieved by sequential biopsy. ,

What Is Cleavage-stage Biopsy (Blastomere Biopsy)?

Cleavage-stage biopsy is generally performed on day three post-fertilization, when embryos reach the eight-cell stage. The biopsy is usually performed on embryos with less than 50% of anucleated fragments and at an 8-cell or later stage of development. A hole is made in the zona pellucida and one or two blastomeres containing a nucleus are gently aspirated or extruded through the opening.

What are the Advantages and Disadvantages of Blastomere Biopsy?

The main advantage of cleavage-stage biopsy over PB analysis is that the genetic input of both parents can be studied. On the other hand, cleavage-stage embryos are found to have a high rate of chromosomal mosaicism, putting into question whether the results obtained on one or two blastomeres will be representative for the rest of the embryo. It is for this reason that some programs utilize a combination of PB biopsy and blastomere biopsy. Furthermore, cleavage-stage biopsy, as in the case of PB biopsy, yields a very limited amount of tissue for diagnosis, necessitating the development of single-cell PCR and FISH techniques. Although theoretically PB biopsy and blastocyst biopsy are less harmful than cleavage-stage biopsy, this is still the prevalent method. It is used in approximately 94% of the PGD cycles reported to the ESHRE PGD Consortium. The main reasons are that it allows for a safer and more complete diagnosis than PB biopsy and still leaves enough time to finish the diagnosis before the embryo transfer, unlike blastocyst biopsy. Of all cleavage-stages, it is generally agreed that the optimal moment for biopsy is at the eight-cell stage. It is diagnostically safer than the PB biopsy and, unlike blastocyst biopsy, it allows for the diagnosis of the embryos before day 5. In this stage, the cells are still

totipotent and the embryos are not yet compacting. Although it has been shown that up to a quarter of a human embryo can be removed without disrupting its development, it still remains to be studied whether the biopsy of one or two cells correlates with the ability of the embryo to further develop, implant and grow into a full term pregnancy.

What is Blastocyst Biopsy / Trophoectoderm (TE) Biopsy?

Blastocyst biopsy has been suggested, in an attempt to overcome the difficulties related to single-cell techniques as it provides a larger amount of starting material for diagnosis. As in the case of cleavage-stage biopsy, the chromosomal differences between the inner cell mass and the trophoectoderm (TE) can reduce the accuracy of diagnosis, although this mosaicism has been reported to be lower than in cleavage-stage embryos (9).

How is Trophoectoderm Biopsy performed?

Human blastocyst-stage biopsy for PGD is performed by making a hole in the Zona Pelucida on day three of in vitro culture. This allows the developing TE to protrude after blastulation, facilitating the biopsy. On day five post-fertilization, approximately five cells are excised from the TE using a glass needle or laser energy, leaving the embryo largely intact and without loss of inner cell mass. After diagnosis, the embryos can be replaced during the same cycle, or cryopreserved and transferred in a subsequent cycle.

What are the drawbacks of Blastocyst Biopsy?

There are two drawbacks to this approach, due to the stage at which it is performed. First, only approximately half of the preimplantation embryos reach the blastocyst stage. This can restrict the number of blastocysts available for biopsy, limiting in some cases the success of the PGD. On the other hand, delaying the biopsy to this late stage of development limits the time to perform the genetic diagnosis, making it difficult to redo a second round of PCR or to rehybridize FISH probes before the embryos should be transferred back to the patient.

Which techniques are used for Genetic analysis in PGD?

Fluorescent in situ hybridization (FISH) and Polymerase chain reaction (PCR) are the two most commonly used technologies in PGD, although other approaches have been proposed or are currently in development (such as whole genome amplification and comparative genomic hybridization) . PCR is generally used to diagnose monogenic disorders and FISH is used for the detection of chromosomal abnormalities (for instance, aneuploidy screening or chromosomal translocations).

What is FISH?

FISH is the most commonly applied method to determine the chromosomal constitution of an embryo. In contrast to karyotyping, it can be used on interphase chromosomes, so that it can be used on PBs, blastomeres and TE samples. The cells are fixated on glass microscope slides and hybridised with DNA probes. Each of these probes are specific for part of a chromosome, and are labelled with a fluorochrome. Currently, a large panel of probes are available for different segments of all chromosomes, but the limited number of different fluorochromes confines the number of signals that can be analysed simultaneously.

The use of probes for chromosomes X, Y, 13, 14, 15, 16, 18, 21 and 22 has the potential of detecting 70% of the aneuploidies found in spontaneous abortions.

The FISH technique is considered to have an error rate between 5 and 10% (3).

What are the drawbacks of PGD using FISH?

The main problem of the use of FISH to study the chromosomal constitution of embryos is the elevated

mosaicism rate observed at the human preimplantation stage. Up to 70% of the embryos studied by FISH have been found to be mosaic for some kind of chromosomal abnormality. In another study Li et al found that 40% of the embryos diagnosed as aneuploid on day 3 turned out to have a euploid inner cell mass at day 6. As a consequence, it has been questioned whether the one or two cells studied from an embryo are actually representative of the complete embryo, and whether viable embryos are not being discarded due to the limitations of the technique (3).

What are problems faced during PGD using PCR?

- Small amout of genomic DNA

The minute amounts of genomic DNA available for PCR during PGD poses a unique problem not seen in routine genetic analysis. As PGD is performed on single cells, PCR has to be adapted and pushed to its physical limits, and use the minimum amount of template possible: one strand. This implies a long process of fine-tuning of the PCR conditions and a susceptibility to all the problems of conventional PCR, but several degrees intensified. The high number of needed PCR cycles and the limited amount of template makes single-cell PCR very sensitive to contamination.

- Allele drop out (ADO) Phenomenon

It is specific to single-cell PCR. It consists of the random non-amplification of one of the alleles present in a heterozygous sample. ADO seriously compromises the reliability of PGD as a heterozygous embryo could be diagnosed as affected or unaffected depending on which allele would fail to amplify. This is particularly concerning in PGD for autosomal dominant disorders, where ADO of the affected allele could lead to the transfer of an affected embryo (3).

What are the criteria's for Embryo selection after PGD?

The establishment of a diagnosis in PGD is not always straightforward. The criteria used for choosing the embryos to be replaced after FISH or PCR results are not equal in all centres. In the case of FISH, in some centres only embryos are replaced that are found to be chromosomally normal (that is, showing two signals for the gonosomes and the analysed autosomes) after the analysis of one or two blastomeres, and when two blastomeres are analysed, the results should be concordant. Other centres argue that embryos diagnosed as monosomic could be transferred, because the false monosomy (i.e. loss of one FISH signal in a normal dipoloid cell) is the most frequently occurring misdiagnosis. In these cases, there is no risk for an aneuploid pregnancy, and normal diploid embryos are not lost for transfer because of a FISH error. Moreover, it has been shown that embryos diagnosed as monosomic on day 3 (except for chromosomes X and 21), never develop to blastocyst when they are transferred, which correlates with the fact that these monosomies are never observed in ongoing pregnancies.

What Is preimplantation Genetic Haplotyping?

Preimplantation Genetic Haplotyping (PGH) is a new clinical method of Preimplantation genetic diagnosis (PGD).

PGH is a clinical method of preimplantation genetic diagnosis (PGD). PGH was first developed in 2006 at London's Guy's Hospital and greatly advances PGD by using DNA fingerprinting rather than identifying the actual genetic signature (such as point mutations) (10).

Compared to previuos PGD techniques PGH involves

The ability to screen male embryos

- Hence male embryos can be screened for X-Linked disorders like Duchennes muscular dystrophy and beckers muscular dystrophy
- Hence having both male and female embryos screened so that an increased number of embryos can be implanted.
- A much greater number of individual tests
- Increased reliability
- Higher success rates

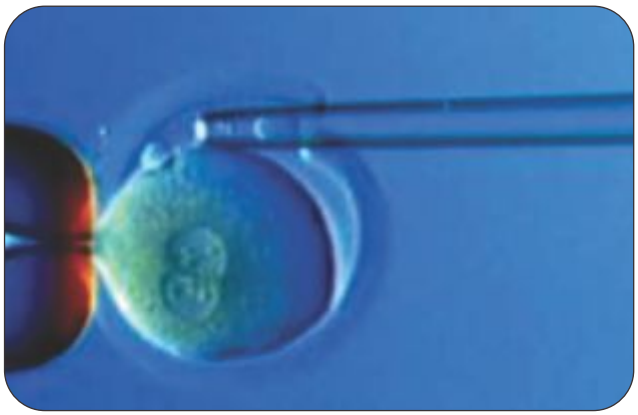
Who is the regulatory authority?

In the UK, the Human fertilisation and embryology authority - HFEA has legal authority over whether a clinic is licensed to do PGD at all and for what indications (7).

In the United States no agency exists at the state or federal level that plays a role comparable with that of the HFEA. Congress exercises some control by refusing to fund research or use of PGD, but this often means that the activity escapes meaningful external review in the private sector. How PGD is used and for what indications is thus left largely to the discretion of providers offering those services and the patients who seek it.

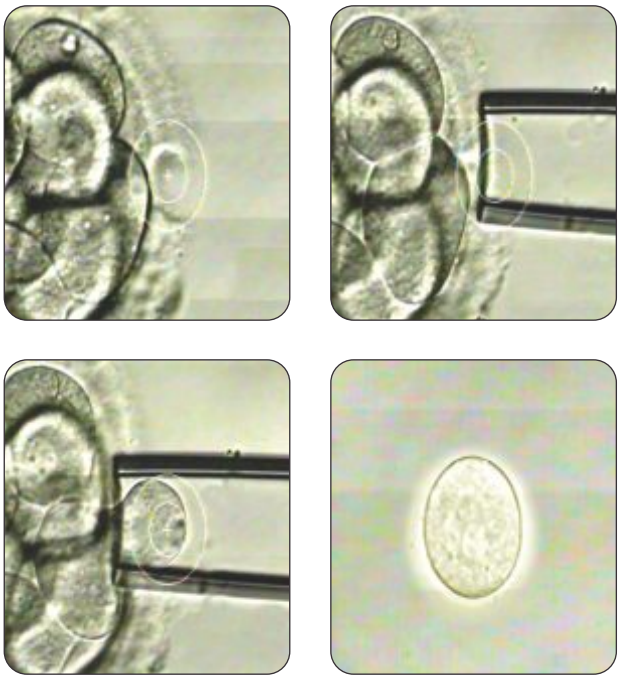
In India the ICMR has issued guideline for the use of PGD in 2005, which are available on www.icmr.nic.in for downloading from the internet. But these need to be updated as the scope for PGD is increasing and more and more centres in India have started offering PGD. As until now there are only guidelines which need to be followed. However there is no law pertaining to PGD except the PNDT act, as sex determination is banned in India (11).

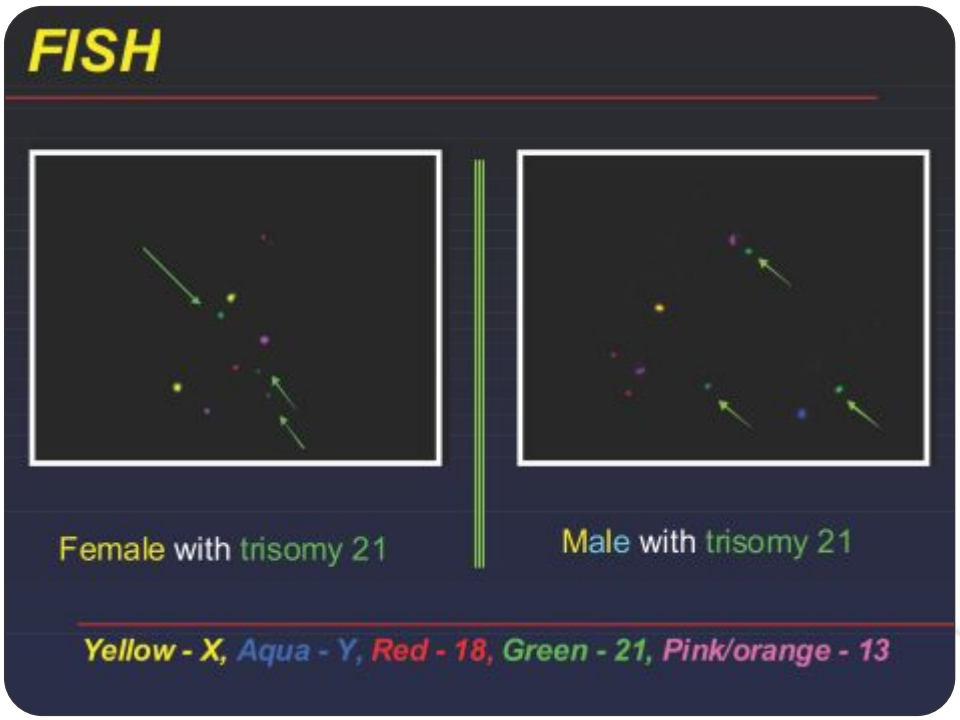
POLAR BODY BIOPSY

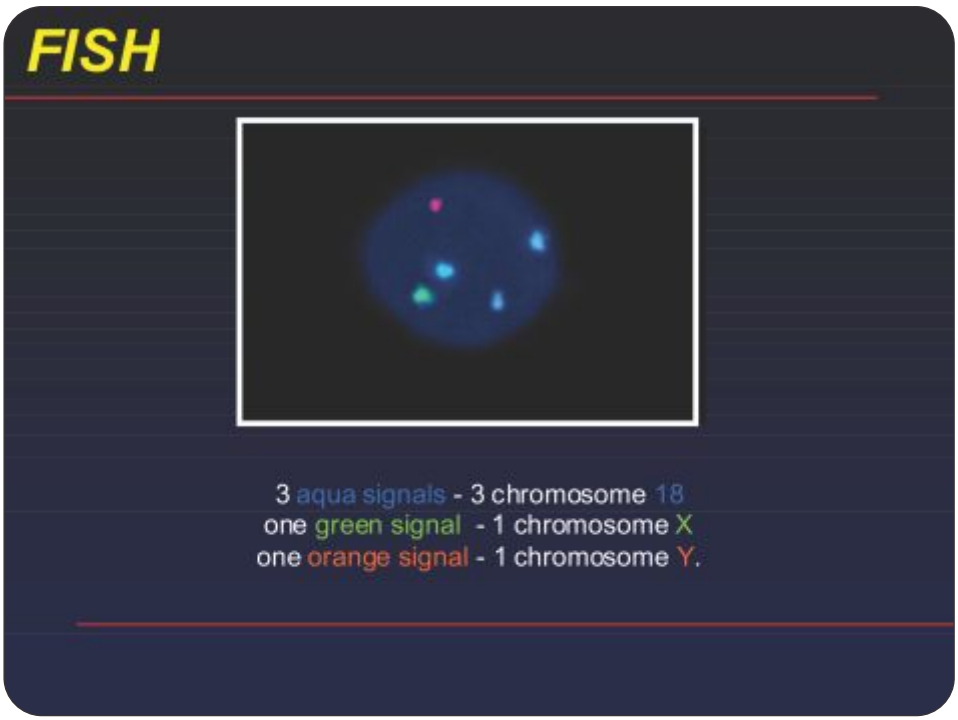


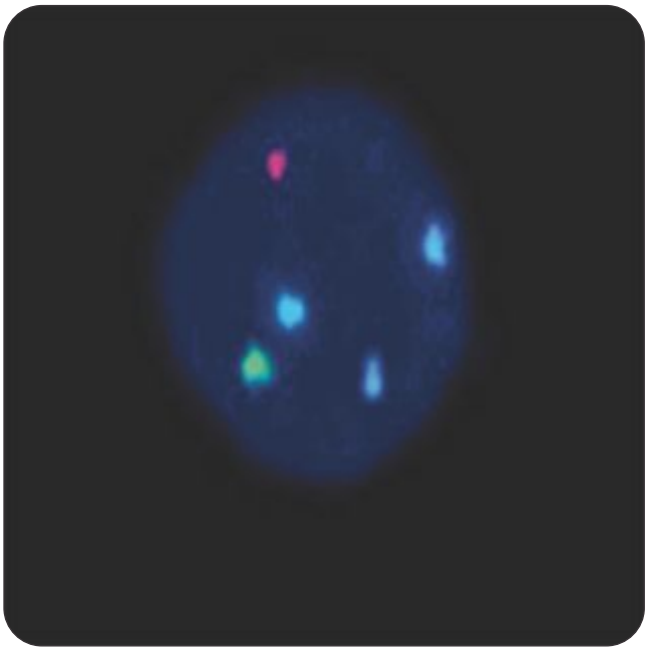
Preimplantation Genetic Diagnosis *contd.*

CLEVAGE STAGE BIOPSY






FISH
3 aqua signals - 3 chromosome 18
one green signal - 1 chromosome X
one orange signal - 1 chromosome Y.



TRISOMY 18

References

1. Edwards RG, Gardner RL: Choosing Sex Before Birth. New Scientist 1968; 38: 218-20
2. Handyside AH et al, Pregnancies from biopsied human preimplantation embryos sexed by Y-specific DNA amplification, Nature. 1990 Apr 19;344(6268):768-70.
3. International Working Group on Preimplantation Genetics, International Congress of Human Genetics: Preimplantation Genetic Diagnosis: Experience of Three Thousand Cycles. Report of the 11th Annual Meeting of International Working Group on Preimplantation Genetics, in association with 10th International Congress of Human Genetics. Vienna, Austria; May, 2001
4. Gianaroli L , Magli MC, Munne S et al Preimplantation diagnosis for aneuploidies in patients undergoing in vitro fertilization with a poor prognosis: identification of the categories for which it should be proposed. Fertil Steril 1999 Nov;72(5):837-44.
5. Munne et al, Preimplantation genetic diagnosis reduces pregnancy loss in women aged 35 years and older with a history of recurrent miscarriages. Fertil Steril 2005 Aug;84(2):331-5
6. Pehlivan T, Rubio C et al , Impact of preimplantation genetic diagnosis on IVF outcome in implantation failure patients. . Reprod Biomed Online2003 Mar ;6 (2) 232-7.
7. John A. Robertson, Ethical issues in new uses of preimplantation genetic diagnosis, Human Reproduction, Vol. 18, No. 3, 465-471, March 2003 © 2003 European Society of Human Reproduction and Embryology
8. Munne S, Dailey T, The use of first polar bodies for preimplantation diagnosis of aneuploidy. Hum Reprod 1995 Apr;10(4):1014-20.
9. Mcarthur SJ, Leigh D et al, Pregnancies and live births after trophectoderm biopsy and preimplantation genetic testing of human blastocysts. Fertile Steril 2005 Dec;84(6):1628-36.
10. Renwick, Pamela J, Trussler, Jane et al , Proof of principle and first cases using preimplantation genetic haplotyping - a paradigm shift for embryo diagnosis. Reproductive BioMedicine Online, Volume 13, Number 1, July 2006 , pp. 110-119(10).
11. www.icmr.nic.in (2007)

Dr. Nandita Palshetkar
M.D, F.C.P.S, F.I.C.O.G.,
Infertility Specialist,
Lilavati Hospital IVF Centre Mumbai
1st Vice President FOGSI. (2011)
Treasurer MOGS & IAGE

Dr. Shivanand Sakhare
M.S.,DNB,FNB
Reproductive Medicine

Dr. Rohan Palshetkar
MBBS

Semen analysis: WHO 2010 :

The updated version of the WHO manual was completed at the end of 2009 and published in 2010(1).According to the new manual the sperm motility is classified only as progressive and non-progressive, slow and rapid motility, as it is difficult for a operator to differentiate between slow and rapid motility and hence the arising bias.

Semen Parameters	5th percentile	95% confidence interval
Volume	1.5 ml	1.4-1.7 ml
Total sperm count	39 million/ejaculate	33-46 million/ejaculate
Sperm concentration	15 million/ml	12-16 million/ml
Total motility(PR+NP)	40%	38-42%
Progressive motility	32%	31-34%
Normal sperm morphology	4%	
Sperm vitality	58%	55-63%

Sperm DNA Fragmentation :

The chromatin of the sperm must decondense correctly after fertilization in order to fertilise the egg and produce healthy and viable embryo. The percentage of sperms with high SDF is less likely to fertilize the egg invivo or invitro. Approximately 25% of infertile males have elevated SDF values. This is due to advanced age, increased testicular temperature (varicocele),reactive oxygen species in the semen, obesity, smoking, etc.

- Sperm DNA fragmentation (SDF) threshold identifies the samples(of couple) compatable with invivo pregnancies (2).
- Sperm DNA fragmentation (SDF) is correlated with pregnancy outcome in intra-uterine insemination (IUI). When SDF levels are above 30%, the probability of delivery is significantly reduced from 19.0% to 1.5%(3).
- Couples presenting values of SDF above the 30% threshold should undergo ICSI. In a recent study by Bungum et al demonstrate that by selecting ICSI over IVF for couples presenting SDF values over 30%, there is a 23% (significant) increase in deliveries(3).

Newer Advances in Female Infertility :

PCOS and infertility:

ESHRE Consensus on infertility treatment related to polycystic ovary syndrome(4) :

The currently available evidence, the consensus reached by a group of experts regarding the therapeutic challenges raised in these women. Before any intervention is initiated, preconceptional counseling is required. The recommended first-line treatment for ovulation induction remains the anti-estrogen clomiphene citrate (CC). Recommended second-line intervention, should CC fail to result in pregnancy, is either exogenous gonadotrophins or laparoscopic ovarian surgery (LOS). The use of exogenous gonadotrophins is associated with increased chances for multiple pregnancy and, therefore, intense monitoring of ovarian response is required. LOS alone is usually effective in <50% of women and additional ovulation induction medication is required under those circumstances. Recommended third-line treatment is *in vitro* fertilization. Metformin use should be restricted to women with glucose intolerance.

Fibroids and infertility :

Consensus statement from ACCEPT Australasian CREI Consensus Expert Panel on Trial evidence 2011 (5).

Fibroid management is surrounded by considerable controversy and uncertainty. The location of a fibroid within the uterus influences its effect on fertility.

a. Subserosal fibroids do not appear to impact on fertility outcomes.

b. Intramural (IM) fibroids may be associated with reduced fertility and an increased miscarriage rate (MR); however, there is insufficient evidence to inform whether myomectomy for IM fibroids improves fertility outcomes.

c. Submucosal fibroids are associated with reduced fertility and an increased MR, and myomectomy for submucosal fibroids appears likely to improve fertility outcomes.

d. The relative effect of multiple or different sized fibroids on fertility outcomes is uncertain, as is the relative usefulness of myomectomy in these situations.

Endometriosis and infertility :

Management of endometriosis before IVF remains controversial. Monthly depot gonadotrophin-releasing hormone analogues are the preferred choice of medical treatment of endometriosis before IVF, with an average duration of treatment of 3 months. When surgery is compared with expectant management, there appear to be no statistically significant differences in pregnancy rate and ovarian response to exogenous stimulation. According to ESHRE guidelines the endometrioma (>3 cm) prior to IVF should be surgically treated by drainage and excision of the cyst wall.

SINGLE EMBRYO TRANSFER :

The rise in multiple pregnancies has led to a resurgence of this approach.It lowers the risk of multiple pregnancies, compared with e.g. Double Embryo Transfer (DET) , with a twinning rate of approximately 3.5% in sET compared with approximately 38% in DET (6). In a RCT by D J McLernon, Elective single embryo transfer results in a higher chance of delivering a term singleton live birth compared with double embryo transfer. Although this strategy yields a lower pregnancy rate, but the additional frozen single embryo transfer leads to higher cumulative pregnancy rates (7).

Metabolomics (Metabolic biometrics):

It is a rapid, non-invasive procedure used to enhance *in vitro* fertilization (IVF) outcomes by identifying best embryos with higher metabolic rate and thus having the greatest reproductive potential. As embryos develop

they undergo specific metabolic changes and produce biological signals or "biomarkers" that are absorbed into the culture media that nourishes these cells. These biomarkers are identified by mass spectroscopy/Raman effect in the spent culture media, creating a 'fingerprint' or biomarker profile to help determine embryo viability. Data presented by Emre Seli, at the 26 th annual meeting of ESHRE concluded that metabolic assessment of an embryo could yield up to 15% better predictability than morphology alone.

Motile sperm organelle morphology examination (MSOME) :

It is the morphological evaluation of motile spermatozoa in real time. MSOME is performed with an inverted light microscope equipped with high-power Nomarski optics enhanced by digital imaging to achieve a magnification of 6300.

According to Bartoov et al (2003) The criteria for a morphologically normal sperm are :

- The average lengths and widths of sperms 4.75 +/- 0.28 cm and 3.28 +/- 0.20 cm respectively.
- The nucleus should be smooth, symmetric, and oval configurations.
- The nuclear chromatin should not have one or more vacuoles that occupies more than 4% of the normal nuclear area .

In a study by bartoov et al the incidence of morphologically normal spermatozoa was found to have a positive and significant correlation with the fertilization rate following ICSI (8).In a most recent study by oliveira et al there were lower miscarriage rates within the IMSI group compared to ICSI group(9).

Oocyte Vitrification and Ovarian tissue freezing in young cancer patients:

It is a reliable method for preservation of the oocytes in women diagnosed with cancer, prior to chemotherapy or radiotherapy and those who would like to preserve their future fertility because they do not have a partner or wants to extend the childbearing. Oocyte vitrification is extremely beneficial and a viable alternative to ovarian grafting in cancer patients (10).

Ovarian tissue freezing is a relatively new approach to preserving a woman's fertility for cancer patients prior to chemoradiation is ovarian tissue freezing. The vast majority of eggs are located in the cortex within primordial follicles, 1 mm from the surface of the ovary, the cortical tissue is biopsied via laparoscopy and vitrified.

EmbryoScope :

The EmbryoScope instrument is tri-gas incubator, with built in camera for automated image acquisition. Continuous and instant time-lapse observation of embryos is possible without the need to remove embryos from a controlled environment. It has accurate regulation of CO2 and temperature control.

References :

1. World Health Organization. WHO Laboratory Manual for the Examination and Processing of Human Semen, 5th edn. Geneva: World Health Organization; 2010.
2. Bungum M.Sperm DNA integrity assesment in prediction of ART outcome.Human Reprod 2007;22:174-9.
3. Evenson DP,Wixon R.Meta analysis of sperm DNA fragmentation using the sperm chromatin structure assay.Reprod Biomed Online 2006;12:466-72.

4. Azziz R (March 2006). "Diagnosis of Polycystic Ovarian Syndrome: The Rotterdam Criteria Are Premature". *Journal of Clinical Endocrinology & Metabolism* 91 (3): 781–5.
5. Kroon B, Johnson N, Chapman M, Yazdani A, Hart R; on behalf of the Australasian CREI Consensus Expert Panel on Trial evidence (ACCEPT) group.Fibroids and infertility.Aust N Z J Obstet Gynaecol 2011,Aug 51 (4),289-295.
6. Mullin CM, Fino ME, Talebian S, Krey LC, Licciardi F, Grifo JA (April 2010). "Comparison of pregnancy outcomes in elective single blastocyst transfer versus double blastocyst transfer stratified by age". *Fertil. Steril.* 93 (6): 1837–43.
7. McLernon DJ, Harrild K, Bergh C, Davies MJ.Clinical effectiveness of elective single versus double embryo transfer: meta-analysis of individual patient data from randomised trials.BMJ 2010 Dec 21;341.
8. Bartoov et al.Real-Time Fine Morphology of Motile Human Sperm Cells is Associated With IVF-ICSI Outcome.J of Andrology 2002 jan;vol 23 no 1.
9. Berkovitz A, Eltes F, Soffer Y, Zabludovsky N, Beyth Y, Farhi J, LevranD, Bartoov B. ART success and in vivo sperm cell selection depends on the ultramorphological status of spermatozoa. Andrologia. 1999;31:1–8.
10. Sanchez-partida LG.The generation of live offspring from vitrified oocytes.PLos one 2011;6(6).