

# Indian Society for Assisted Reproduction Consensus Guidelines on Preimplantation Genetic Testing in *In vitro* Fertilization Clinics

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ABSTRACT

**Study Question:** What are the good practice guidelines for Pre implantation genetic testing applicable in INDIA? **What is Already Known:** Pre-Implantation Genetic Testing (PGT) is not new in India. It is used to identify euploid embryos for transfer, thus enabling couples to achieve a healthy pregnancy. There has been a lot of controversy surrounding PGT in the international forums; most of these debates have failed to reach a consensus on whether PGT should be offered or its concerns be validated more. **Study Design, Size, Duration:** This is the report of a 2-day consensus meeting where two moderators were assigned to a group of experts to collate information on Pre implantation genetic testing and embryo biopsy practices in INDIA. This meeting utilised surveys, available scientific evidence and personal laboratory experience into various presentations by experts on pre-decided specific topics. **Participants/Materials, Setting, Methods:** Expert professionals from ISAR representing clinical, embryological and genetic fields. **Main Results and the Role of Chance:** The report is divided into various components defining the terminologies, the various requirements, qualifications, recommendations on PGT -A,M,SR, and quality management: the report and recommendations of the expert panel reflect the discussion on each of the topics and try to lay down good practice points for labs to follow. **Limitations, Reasons for Caution:** The recommendations are solely based on expert opinion. Future availability of data may warrant an update of the same. **Wider Implications of the Findings:** These guidelines can help labs across the country to standardise their PGT services and improve clinical outcomes. **Study Funding/Competing Interest(S):** The consensus meeting and writing of the paper was supported by funds from CooperSurgical India.

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## Introduction

Preimplantation genetic testing (PGT) is a technique used to identify genetic defects in embryos created through *in vitro* fertilization (IVF) before pregnancy. Preimplantation genetic diagnosis (PGD) refers specifically to when one or both genetic parents have a known genetic abnormality and testing is performed on an embryo to determine if it also carries a genetic abnormality. In contrast, preimplantation genetic screening (PGS) refers to techniques where embryos from presumed chromosomally normal genetic parents are screened for aneuploidy. PGT and PGS are presently the only options available for avoiding a high risk of having a child affected with a genetic disease prior to implantation. It is an attractive means of preventing heritable genetic disease, thereby eliminating the dilemma of pregnancy termination following unfavorable prenatal diagnosis.<sup>[1]</sup>

The rapidly changing nature of PGS or PGT, specifically the technologies associated with its use and increasing patient access, has necessitated review and establishment of PGT consensus guidelines in the Indian scenario. This is a logical step considering the comparative difficulty in achieving the highest levels of accuracy and reliability with single cells as part of PGT/PGS compared with more routine genetic testing. PGT/PGS is still relatively unregulated and lacks standardization compared with other forms of diagnostic testing; however, more federal, state, and local governments are beginning to take an interest in PGT and some have begun accrediting laboratories that offer PGT (Harper *et al.*, 2010a).<sup>[2]</sup> One step toward higher quality overall and standardization for PGT/PGS is to build consensus opinion on best practices within the PGT/PGS community, a component of the mission of the committee.

The PGT consensus committee recognizes that owing to variations in local or national regulations and specific laboratory practices, there will remain differences in the ways in which PGT/PGS is practiced (from initial referral through IVF treatment, single-cell analysis to follow-up of pregnancies, births, and children). However, this does not preclude a series of consensus opinions on best practice based on experience and available evidence. The working PGT group members were composed of people with hands-on expertise on the described techniques, aiming at a representation of different settings and nationalities. The group consisted of clinicians, embryologists, biologists, and geneticists. All ten group members, according to their expertise, wrote a section that was later discussed in depth with the entire group until consensus.

Topics covered in this guideline include infrastructural requirements for embryo biopsy, personnel qualification and training and competency, consents and checklists in PGT, polar body biopsy/cleavage-stage biopsy, blastocyst biopsy, use of euploid/aneuploid and mosaic embryos, indications for preimplantation genetic testing for monogenic (PGT-M) preimplantation genetic testing for aneuploidy (PGT-A)/preimplantation genetic testing for structural rearrangement (PGT-SR), genetic counseling, technology, quality control checkpoints, and genetic risk assessment.

The PGT consensus committee hopes that a minimum standard might be achieved across all centers. Achieving this goal ultimately should ensure that all patients receive optimum care regardless of the center in which they are treated. Rather than a drift toward the lowest common denominator, established and fledgling centers alike can learn from practical experiences and be guided by consensus opinion. These opinions are not intended as rules or fixed protocols that must be followed, nor are they legally binding. The unique needs of individual patients may justify deviation from these opinions, and they must be applied according to individual patient's needs using professional judgment. However, guidelines and opinions may be used to frame laws and regulations, and practitioners should ensure that they comply with statutory requirements or clinical practice guidelines in their own clinical settings.

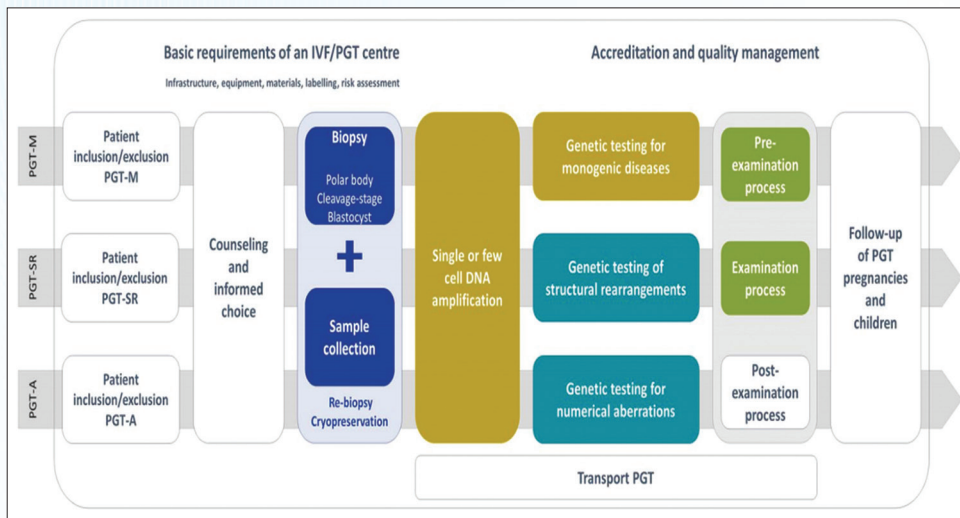
### Definition of Terms in Indian scenario

The terms PGD and PGS, as of 2017, have been replaced by the term PGT according to "The International Glossary on Infertility and Fertility Care, 2017." A consensus was taken by The International Committee for Monitoring Assisted Reproductive Technologies in association with several prominent societies involved in human reproductive medicine.<sup>[3]</sup>



## Preimplantation Genetic Testing in India

In India, PGT is supervised by the Ministry of Family Health and Welfare. According to the Pre-Conception and Pre-Natal Diagnostic Techniques (PCPNDT) Act of 1994, a written consent specified by the guidelines in a language understood by the individual must be obtained before PGT.<sup>[3]</sup>



**Figure 1:** Overview of in vitro fertilization or preimplantation genetic testing process as per the ESHRE recommendations.<sup>[4]</sup> ESGRE=European Society of Human Reproduction and Embryology, PGT=Preimplantation genetic testing, PGT-M=Preimplantation genetic testing for monogenic genes, PGT-SR=Preimplantation genetic testing for structural rearrangements, PGT-A=Preimplantation genetic testing for aneuploidy

### Infrastructural Requirements for Embryo Biopsy

#### Requirements for registration of a genetic counseling center, laboratory, and clinic

PGT centers are under the PCPNDT Act, 1994. IVF laboratory performing embryo biopsy must be registered with PCPNDT under the “Genetic Clinic” category. In order to do so, Form A needs to be submitted. In case the genetics laboratory is in house and is part of the IVF clinic, then the IVF clinic must register with PCPNDT as a Genetics Laboratory. The IVF program should be a successful program before we can escalate to PGT. There should be an excellent communication between IVF laboratory and the genetics laboratory. There is a significant overlap between IVF laboratory and PGT laboratory infrastructure. However, some instruments, consumables, disposables, and media are exclusive to embryo biopsy. A skilled embryologist is an asset and so is a technician with biopsy experience. A genetic counselor should be easily accessible [Figure 1]. As per the PNDT Act and Rules, the components recommended as per schedule 1 are shown in Tables 1-4.<sup>[5]</sup>

**Table 1: Recommendations for a genetic counseling center**

Place	Equipment	Employees
A room with an area of 7 m <sup>2</sup>	Educational charts/models	Any one of the following Medical geneticist Gynecologist with 6-month experience in genetic counseling or having completed 4-week training in genetic counseling Pediatrician with 6-month experience in genetic counseling or having completed 4-week training in genetic counseling

**Table 2: Requirements for registration of a genetic laboratory**

Place	Equipment	Employees
A room with adequate space for carrying out tests	Chromosomal studies	A medical geneticist
	Laminar flow hood with ultraviolet and fluorescent light or other suitable culture hoods Photomicroscope with fluorescent source of light Inverted microscope Incubator and oven Carbon dioxide incubator or closed system with 5%-6% CO <sub>2</sub> atmosphere Autoclave Refrigerator Water bath Centrifuge Vortex mixer Magnetic stirrer pH meter A sensitive balance (preferable electronic) with sensitivity of 0.1 mg Double distillation apparatus (glass)	A laboratory technician having a B.Sc. degree in biological sciences or a degree or a diploma in medical laboratory course with at least 1 year's experience in conducting appropriate prenatal diagnostic tests
	Biochemical studies (requirements according to tests to be carried out)	
	Laminar flow hood with ultraviolet and fluorescent light or other suitable culture hoods Inverted microscope Incubator and oven Carbon dioxide incubator or closed system with 5%-6% CO <sub>2</sub> atmosphere Autoclave Refrigerator Water bath Centrifuge Electrophoresis apparatus and power supply Chromatography chamber Spectrophotometer and ELISA reader or radio-immunoassay system (with $\gamma\beta$ -counter) or fluorometer for various biochemical tests Vortex mixer Magnetic stirrer pH meter A sensitive balance (preferable electronic) with sensitivity of 0.1 mg Double distillation apparatus (glass) Liquid nitrogen tank	

Contd...

**Table 2: Contd...**

Place	Equipment	Employees
	Molecular studies	
	Inverted microscope	
	Incubator	
	Oven	
	Autoclave	
	Refrigerators (4°C and –20°C)	
	Water bath	
	Microcentrifuge	
	Electrophoresis apparatus and power supply	
	Vortex mixer	
	Magnetic stirrer	
	pH meter	
	A sensitive balance (preferable electronic) with sensitivity of 0.1 mg	
	Double distillation apparatus (glass)	
	PCR machine	
	Refrigerated centrifuge	
	UV illuminator with photographic attachment or other documentation systems	
	Precision micropipettes	

PCR=Polymerase chain reaction

**Table 3: Requirements for registration of a genetic clinic**

Place	Equipment	Employees
A room with an area of 20 m <sup>2</sup> with appropriate aseptic arrangements	Equipment and accessories necessary for carrying out clinical examination by an obstetrician/gynecologist	A gynecologist with adequate experience in prenatal diagnostic procedures (should have performed at least 20 procedures under the supervision of a gynecologist experienced in the procedure, which is going to be carried out, for example, chorionic villi biopsy, amniocentesis, cordocentesis, and others indicated at B above)  A radiologist or registered medical practitioner for carrying out ultrasonography. The required experience shall be 100 cases under supervision of a similarly qualified person experienced in these techniques
	Equipment, accessories necessary for other facilities required for operations envisaged in the act	
	An ultrasonography machine*	
	Appropriate catheters and equipment for carrying out chorionic villi aspirations per vagina or per abdomen*	
	Appropriate sterile needle for amniocentesis or cordocentesis*	
	A suitable fetoscope with appropriate accessories for fetoscopy, fetal skin or organ biopsy, or fetal blood sampling shall be optional	
	Equipment for dry and wet sterilization	
	Equipment for carrying out emergency procedures such as evacuation of uterus or resuscitation in case of need	
	Platforms for FISH, NGS, and CGH arrays	

\*These constitute the minimum requirement of equipment for conducting the relevant procedure. FISH=Fluorescence *in situ* hybridization, NGS=Next-generation sequencing, CGH=Comparative genomic hybridization



**Table 4: Biopsy requirements**

Forms required	Before performing an embryo biopsy, it is mandatory to fill form F and G in duplicate
Equipment	Inverted microscope with light field objectives (4×, 10×, 20×) and Hoffman objective (40×) with heated stage Holding micromanipulator and microinjector Biopsy micromanipulator and microinjector Laser shot system (mandatory) Laminar flow hood incorporated with UV light (mandatory)** Laminar hood in different rooms (advisable)** Stereo Zoom microscope for tubing Deep freezer (−20°C) (mandatory) Minicentrifuge (6000 rpm) Micropipettes with adjustable volumes (0.5-10 μL; 20-200 μL; 100-1000 μL)
Consumables/ disposables	Biopsy and holding micropipettes for embryo biopsy Flexipets/denudation pipettes/glass capillaries for embryo and biopsy manipulation Barrier tips for micropipettes: Sterile tips, with white filter; RNase, DNase, pyrogen, and DNA free (should fit your micropipettes 0.5-10 μL and 20-200 μL or 100-1000 μL) Use surface decontaminant like ethanol Sterile disposable surgical gown (mandatory) Sterile latex/nitrile gloves (nonpowdered only)
Medium	The use of standard IVF culture medium during biopsy is “acceptable,” but its effectiveness may be highly dependent upon the developmental stage of the embryo biopsied

IVF=*In vitro* fertilization, UV=Ultraviolet

## Qualification and Training

To ensure that the embryo biopsy is performed by competent staff, it is recommended that embryo biopsy should be performed only by an experienced clinical embryologist with credentials and expertise as follows:

### Clinical embryologist

- The clinical embryologist must be knowledgeable in mammalian embryology, reproductive endocrinology, genetics, molecular biology, biochemistry, microbiology, and *in vitro* culture techniques
- The embryologist must also be familiar with assisted reproductive technology (ART). He/she must be either a medical graduate or have a postgraduate degree or a doctorate in an appropriate area of life sciences. (In the case of a clinic in existence for at least 1 year before the promulgation of these rules, a person with a B.Sc. or B.V. Sc degree but with at least 3 years of first-hand, hands-on experience of the techniques mentioned below and of discharging the responsibilities listed below, would be acceptable for functioning as a clinical embryologist. Such persons would also be eligible to take a test to be designed and conducted by an appropriate designated authority.)
- He/she must be familiar with the following:
  - Principles and practice of semen analysis and cryopreservation of semen
  - Cytology of mammalian and human oocyte to identify stages of oocyte maturation accurately
  - All aspects of embryology including developmental biology
  - Cell biological techniques used in cell and tissue culture
  - Molecular biology and genetics of human reproduction
  - Micromanipulation of sperm and oocytes for carrying out intracytoplasmic sperm injection (ICSI) and single-cell biopsies of embryos for PGD
  - Principles and functioning of all the equipment used in the laboratory

- IVF of oocytes after processing the gametes
- Principles and practice of gamete/embryo freezing.
- It is recommended that the PGT laboratory should be directed by a person or persons who have executive accountability and the competence to assume responsibility for the services provided
- A defined training and quality assurance (QA) program needs to be developed by the PGT center to encompass all areas of work, including the use of all equipment, all relevant standard operating procedures (SOPs), data protection, and training in the IVF center, for example.
  - Training should be supervised by an appropriate person and should be recorded in the logbook of the individual staff member. All staff should keep a log of their continued professional development to ensure continual recording and updating of their certifications/credentials
  - It is acceptable to train staff in both disciplines (fluorescence *in situ* hybridization [FISH]-based and amplification-based PGD/PGS) if each discipline has a training schedule and assessment program to demonstrate proficiency with all the necessary skills and techniques and ability to work independently in either or both sections.
  - Establishing internal continuous training programs and competency assessment of staff is recommended.

## Counseling Recommendations for Preimplantation Genetic Testing Monogenic Disorders

Before PGT is performed, genetic counseling must be provided to ensure that patients fully understand the risk for having an affected child, the impact of the disease on an affected child, and the limitations of available options that may help to avoid the birth of an affected child.<sup>[6]</sup>

- PGD can reduce the risk for conceiving a child with a genetic abnormality carried by one or both parents if that abnormality can be identified. Prenatal diagnostic testing to confirm the results of PGT-M is encouraged because the methods used for PGT-M have technical limitations that include the possibility for a false-negative result.<sup>[6]</sup>

### Clinical recommendations

- Biopsy practitioner must be proficient in ICSI and micromanipulation procedures such as using laser
- Person who is performing biopsy must be trained and proficient in performing tubing/fixing of biopsied cells depending on type of genetic platform being used
- For training, validation, and QA, the biopsies should be performed on waste embryos under the supervision of an expert and then validated by the genetics laboratory for lack of contamination and DNA quality.

## Counseling Recommendations for Preimplantation Genetic Testing for Aneuploidy

Before PGT A is performed, thorough education and counseling must be provided to ensure that patients fully understand the limitations of the technique, the risk of error, and the available evidence with regard to efficacy of PGT A in improving live birth rates.<sup>[5]</sup>

## Consents and Checklist

### Consent elements for assisted reproductive technology centers

All informed consents must be in writing, signed by all participating parties, and properly witnessed. In addition to information about chance of success and financial obligations, the following issues should be addressed in the process of obtaining consent. It is also important that couples are provided with full information concerning alternative procedures available to manage their specific infertility problems,



including procedures that are not performed by the treating center, as well as nonmedical options such as adoption and nontreatment.<sup>[5]</sup>

The list of elements is as follows:<sup>[5]</sup>

- Consent for IVF/ICSI for embryo formation for PGT
- Consent for adjuncts alongside PGT testing to prioritize embryos
- Risk of embryo biopsy
- Information of genetic diagnostic testing and risk of misdiagnosis
- PGT safety results
- Chance of live birth rate
- Risk of untested anomalies occurring in screened embryos (polyploidy, microdeletions, etc.)
- National/international society guideline information
- Legally binding PCPNDT Information on illegal sex determination
- Recommendation for pre-PGT genetic counseling/psychological support
- Alternatives to PGT
- Information on avoiding natural sex
- Consent for research of affected embryos
- Consent for research on stored DNA
- Consent regarding affected embryo fate
- Possibility of no embryo to transfer
- Confidentiality.

### Consents for genetic laboratories

- Form A certificate copy for patient's records
- Legally binding PCPNDT Information on illegality of sex determination
- PCPNDT formats Form G (invasive techniques)
- Risk of genetic diagnosis beyond scope and information consent
- Benefits/limitations of PGT, testing method, and used equipment/procedures by genetic laboratory
- Embryo biopsy risks
- IVF center-related risks
- Pregnancy-associated risks
- Follow-up recommendation for PND
- Genetic counseling recommendation
- Consent for additional screening tests (e.g., mitochondrial score)
- Confidentiality.

### Checklists for genetic laboratories

- Scheduled kit drop-off to ART center checkpoint
- Scheduled pickup check with biopsy practitioner kit of consent/The International Air Transport Association (IATA) regulation documents for sample pickup in dry ice
- Embryo biopsy labeled form from ART center
- Biopsy consents checklist via phone/scan upload check
- Form G scan upload checkpoint
- Form D – maintenance of records checkpoint
- DNA quality check – NanoDrop
- DNA amplification check – whole-genome amplification
- DNA quality reporting/degraded DNA reporting to biopsy practitioner for second biopsy
- Reporting check for delivery
- Genetic counseling requirement checkpoint.

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## Embryo Biopsy

- The biopsy procedure of preimplantation embryos consists of two main steps: creating an opening in the zona pellucida (ZP) followed by removal or biopsy of embryonic cells such as polar bodies (PBs) or blastomeres or trophoctoderm (TE) cells. ZP opening may be performed either mechanically or chemically or using lasers
- Chemical method for creating an opening in the zona using acid Tyrode's solution has been universally abandoned as it is not well tolerated by the oocyte and may interfere with embryonic development thereafter
- Mechanical method uses simple glass tools but involves multiple steps such as dissection, release, and rotation of the oocyte. It requires skill and time
- The use of LASER is the method of choice as it allows maximum precision and safety
- PB biopsy.

PB biopsy and analysis is an indirect approach for diagnosing the genetic status of the corresponding oocyte. Since both first and second PBs are not involved in fertilization nor embryo development, they make good candidates for performing genetic analysis.

### When and how?

- Removal of the first and second PBs can be done at separate times (day 0 and day 1: sequential approach) or at the same time (day 1: simultaneous approach)
- Sequential biopsy may be preferred if a fragmented PB1 is already present at the time of injection on day 0
- Simultaneous biopsy of the two PBs is "acceptable" for FISH analysis since they can provide distinguishable results
- Sequential biopsy of PBs is "recommended" for PCR analysis to determine recombination events between the first and second PBs.

### Cleavage-stage biopsy

Traditionally embryo biopsy can be performed at any one of the three developmental stages of an embryo: PB, cleavage, or blastocyst stage. Cleavage-stage embryo biopsy was the most widely practiced form of embryo biopsy globally but now has been replaced by TE biopsy. This process of embryo biopsy involves two stages: the first stage involves creating an opening in the ZP followed by the second stage where a single cell is removed without compromising the embryo.

- The main characteristics of the three biopsy approaches are summarized in Table 5.

**Table 5: The main oocyte and embryo biopsy approaches to conduct preimplantation genetic testing**

	PB biopsy	Blastomere/ cleavage-stage biopsy	Blastocyst/TE biopsy
Fragment origin	Waste products of maternal meiosis	Totipotent cells	TE gives origin to the placenta and the extraembryonic membranes
Number of cells retrieved	2 (both required)	Two might be "one" retrieved, but it is discouraged	5-10 trophoctoderm cells

Contd...

**Table 5: Contd...**

	<b>PB biopsy</b>	<b>Blastomere/ cleavage-stage biopsy</b>	<b>Blastocyst/TE biopsy</b>
Complexity in the acquisition of the skill	Day 0+ day 1 approach Moderate Day 1 only approach Moderate to high (PB1 and PB2 should be reliably recognized)	Moderate	Day 3 hatching-based strategy: Low-to-moderate Morula hatching-based strategy: Low to moderate Same day hatching-based strategy: Low to moderate Simultaneous ZP opening and TE cells retrieval strategy: Moderate to high
Complexity in the performance of tubing	Moderate to high	Moderate	Moderate to high
Embryo developmental competence	Unpredictable at this stage	Unpredictable at this stage	Only embryos developing to the blastocyst stage are biopsied
Laboratory workload	High (all oocytes/zygotes should be biopsied regardless of their developmental competence)	Moderate (all oocytes/zygotes/cleavage-stage embryos should be biopsied regardless of their developmental competence)	Multiple time slots required (day 5-7) and cryopreservation mostly mandatory Day 3 hatching-based strategy: Moderate to high (all embryos should undergo ZP opening at the cleavage stage regardless of their developmental competence) Morula hatching-based strategy: Moderate to high (all morulae should undergo ZP opening regardless of their developmental competence) Same day hatching-based strategy: Moderate to high (all blastocysts should undergo ZP opening and monitoring of TE cells hatching) Simultaneous ZP opening and TE cells retrieval strategy: Moderate
Extended embryo culture	Suggested but not mandatory	Suggested but not mandatory	Not mandatory
Cryopreservation following biopsy	According to laboratory policy	According to laboratory policy	Mostly mandatory
Meiotic errors assessed	Only maternal	Yes	Yes
Mitotic errors assessed	Not	Not	Possible within given technical, methodological, and biological limitations (e.g., molecular platform and bioinformatic parameters - dependent, inevitable sampling bias)
Inconclusive diagnosis (%)	10	10	<10
Impact on reproductive competence	Not reported, but more data are still required	Reduced implantation potential post biopsy	Not reported, but more data are still required

The parameters "low," "moderate," and "high" were agreed unanimously after a thorough discussion among all the components of the working group. TE=Trophectoderm, PB=Polar body, ZP=Zona pellucida

## Blastocyst biopsy

To overcome the challenges of cleavage-stage single-cell analysis and mosaicism, blastocyst stage biopsy was proposed, which is now being used extensively to detect both aneuploidies for PGS as well as single-gene disorders (SGDs) for PGD. TE biopsy at the blastocyst stage enables the removal of several cells for genetic testing while being noninvasive to the inner cell mass (ICM) which is destined for fetal development. Blastocyst biopsy is usually performed on the morning of day 5 or 6 postinsemination and needs considerable expertise as compared to a blastomere biopsy. Despite this, application of TE biopsy coupled with comprehensive chromosomal screening represents the most effective clinical approach for PGS/PGD till date.

The ideal stage for performing a TE biopsy is when the blastocyst is either expanding or hatching on day 5 or 6, since such embryos tend to have higher cell numbers as compared to early blastocysts. TE biopsy can be achieved quite easily if the blastocyst undergoes spontaneous hatching and herniation. If herniation does not occur spontaneously, then it is possible to initiate herniation either late on day 4, or on 5 or 6 of culture, by introducing a small hole of  $\sim 10 \mu\text{m}$  in the ZP, at a site farthest away from the ICM with the help of laser, followed by a period in culture during which the expansion of the blastocoel cavity forces the TE to herniate out of the hole.

If blastocysts are fully hatched, biopsy is still feasible and excision of TE cells is advisable using a combination of laser pulses and a flicking movement.

- It is recommended to biopsy 5–10 TE cells for genetic testing (according to the stage of development and number of cells constituting the blastocyst). The impact of removal of more than 10 TE cells on embryo development remains an area of further investigation
- $\text{Ca}^{2+}/\text{Mg}^{2+}$ -free medium should not be used for blastocyst biopsy
- There is a theoretical risk of cross-contamination when the same biopsy pipette is used to biopsy more than one blastocyst. However, it is acceptable to thoroughly rinse the biopsy pipette before each biopsy, thus allowing the same pipette to be used for multiple biopsies, but it should be verified in the laboratory that this suffices to avoid cross-contamination
- It is also recommended that following biopsy, blastocyst is immediately transferred in culture medium or cryopreserved once tubing of the biopsy has been confirmed.

## Cryopreservation of biopsied embryos

- Results published regarding the survival rate of biopsied blastocysts are conflicting. In a recent study, a lower survival rate was found for cryopreserved biopsied blastocysts compared with intact blastocysts, while vitrification showed a higher survival rate than slow freezing<sup>[7]</sup>
- For the time being, it is recommended that each center decides its own policy regarding the cryopreservation of biopsied embryos based on its experience and performance on embryo cryopreservation.

## Rebiopsy of embryos

- Rebiopsy incidence is rare
- This practice is “acceptable” in the case of lost or anucleate blastomeres and failed diagnosis.

## Clinical recommendations on biopsy procedures

- Embryo biopsy may be performed at any of the three embryo stages, namely polar body, cleavage-stage, or blastocyst-stage embryo
- It is most beneficial to perform embryo biopsy at the blastocyst stage since embryo viability and implantation potential postbiopsy remains unaffected
- Blastocyst biopsy ensures that only the most competent embryos are selected to undergo genetic screening, which therefore helps in avoiding unnecessary biopsies and the associated costs



- Chromosome mosaicism may be an important limiting factor in the effectiveness of chromosome aneuploidy screening since it may be a source of diagnostic error. Consequently, many groups have been moving away from cleavage-stage biopsy since the incidence of mosaicism is higher at the cleavage stage as compared to the blastocyst stage
- Polar body biopsy may be chosen as an alternative to embryo biopsy only if maternal mutations or aneuploidies are investigated
- Meiotic errors from both parents can be detected, but mitotic errors leading to chromosomal mosaicism cannot be estimated from a single blastomere.

## How to Use Euploid Embryos

### Fresh or frozen transfer?

- Frozen euploid ET is indicated in repeated IVF failure patients, discordant embryos and transport PGT with more than 24-h turnaround time
- Fresh euploid embryo transfer if possible is acceptable in good prognosis patients.

### How many euploid embryos to transfer?

- Based on the recommendations given by the Practice Committee of the American Society for Reproductive Medicine (ASRM) and the Practice Committee of the Society for Assisted Reproductive Technology (SART) published in April 2017 and PGT consensus group, single euploid cleavage-stage or blastocyst embryo should be transferred irrespective of the age group.<sup>[8,9]</sup>

## Clinical Consensus on What to do with Aneuploid Embryos

- Aneuploid embryos can be discarded or used for research only after consent from a couple
- No role of repeat biopsy in case of aneuploid embryos
- Aneuploid embryos must not be transferred into the patient
- The clinicians should have a right to deny patient requests in such a case, but this needs to be stated in the consent form beforehand.

## What to do with Mosaics?

When embryos exhibit the presence of a mixture of chromosomally different cell lines in an embryo due to a variety of genetic changes such as chromosomal aberrations, single-nucleotide variations, small insertions/deletions, they are referred to as mosaics.

### Nomenclature

- If two or more different abnormal cell lines are present in an embryo, they are termed as aneuploid/aneuploid mosaics
- If normal and abnormal cell lines are present in an embryo, they are termed as euploid/aneuploid mosaics.

### Prevalence of mosaicism

- Overall occurrence is low
- Mosaicism in products of conception of early miscarriages has been reported to be low<sup>[10]</sup>
- Embryos with aneuploid ICM are lost during the first trimester owing to spontaneous miscarriages
- Most successful established pregnancies when mosaic embryo transfer (MET) done/in cases of extensive genetic testing done post-PGS have shown placental mosaicism only.<sup>[11]</sup>

Mosaic embryo: To transfer or not to transfer!

### Detection of mosaics: Impact of technique

- All current methods, single-nucleotide polymorphism (SNP) arrays, comparative genomic hybridization (CGH), next-generation sequencing (NGS), etc., use preimplantation embryos and software which is designed to infer the embryo as euploid/aneuploid. Thus, there are a million points of analysis leading to gray areas for the software, and most likely, we are interpreting the gray area as mosaics
- Thus, for conformity, we might have to run PGS on different platforms to decisively comment on mosaicism prevalent in analyzed cells.

### Clinical consensus on mosaic embryo transfer

- Mosaic embryos when transferred may not implant
- Miscarry spontaneously if implantation occurs
- Result in intrauterine growth retardation
- Result in birth of child with minor – severe birth defects and/or mental retardation
- Result in birth of a normal healthy child.

### Recommendations on the Order of Prioritization of Mosaic Embryos by The Preimplantation Genetic Diagnosis International Society, 2016

The recommendations by the Preimplantation Genetic Diagnosis International Society (PGDIS) were based on fetal and placental mosaicism from prenatal diagnosis (PGDIS, 2016).

### Suggested guidelines to prioritize mosaic embryos for transfer

Based on our current knowledge of the reproductive outcomes of fetal and placental mosaicism from prenatal diagnosis, the following can be used as a guide by the clinician (or a genetic counselor if available) when a mosaic embryo is being considered for transfer<sup>[12]</sup>:

- a. Embryos showing mosaic euploid/monosomy are preferable to euploid/trisomy, given that monosomic embryos (excepting 45,X) are not viable
- b. If a decision is made to transfer mosaic embryos trisomic for a single chromosome, one can prioritize selection based on the level of mosaicism and the specific chromosome involved
- c. The preferable transfer category consists of mosaic embryos trisomic for chromosomes 1, 3, 4, 5, 6, 8, 9, 10, 11, 12, 17, 19, 20, 22, X, Y. None of these chromosomes involve the adverse characteristics enumerated below
- d. Embryos mosaic for trisomies that are associated with potential for uniparental disomy (14, 15) are of lesser priority
- e. Embryos mosaic for trisomies that are associated with intrauterine growth retardation (chromosomes 2, 7, 16) are of lesser priority
- f. Embryos mosaic for trisomies capable of live-born viability (chromosomes 13, 18, 21) are of the lowest priority for obvious reasons.

### Clinical consensus on what to do with mosaic embryos?

- Incidence is very small
- There is a question mark on clinical relevance of mosaicism
- Technology is at par to detect as low as 20% mosaicism
- PGDIS guidelines to be followed in case transfer of mosaic embryo must be performed.



## Consensus on Preimplantation Genetic Testing for Aneuploidy Indications

Since last year, PGS and PGD have been re-termed as PGT, in which 90% of cases performed are for PGT for aneuploidies (PGT-A), for indications such as advanced maternal age, recurrent miscarriage, repeated implantation failures ( $\geq 2$  prior failed embryo transfer with good quality embryos) and in general, to improve the selection of embryos with the most potential to implant and produce a viable pregnancy.<sup>[13]</sup> Franasiak *et al.* investigated the relationship between the age of the female partner and the prevalence and nature of human embryonic aneuploidy. A review of 15,169 consecutive TE biopsies evaluated with comprehensive chromosomal screening. Aneuploidy increased predictably after 26 years of age. A slightly increased prevalence was noted at younger ages, with  $>40\%$  aneuploidy in women 23 years and under. The aneuploidy embryo rate was lowest (2%–6%) in women aged 26–37, was 33% at age 42, and was 53% at age 44. The lowest risk for embryonic aneuploidy was between ages 26 and 30. The overall risk did not measurably change after age 43.<sup>[14]</sup>

### Is preimplantation genetic testing for aneuploidy indicated for every in vitro fertilization cycle?

- As per the recommendations by the experts, the findings from the STAR trial<sup>[15]</sup> revealed that there was a statistically significant ( $P < 0.035$ ) improvement in an ongoing pregnancy rate of 14% for women aged 35–40 years in the PGS arm. These findings are consistent with 2014 SART data and other published studies
- The SART data show that implantation rates hover around 50% for PGT irrespective of maternal age, whereas, without PGT, rates decrease after the age of 35 years
- Similarly, recently Ozgur *et al.* demonstrated that, in young patients aged  $<35$  years with at least two or more 2BB blastocysts, PGT-A blastocyst selection did not result in an increased live birth rate
- The recommendations of the practice committees of ASRM and SART published in April 2017 and PGT consensus group in March 2018 are as follows
- The use of PGT-A does not improve outcomes in good prognosis patients  $<37$  years of age
- No difference in outcomes for patients with recurrent pregnancy losses when compared to expectant management
- In women aged  $>37$  years, PGT-A resulted in higher live birth rates with single euploid embryo transfer
- An advanced maternal age is a clear indication for PGT-A.

### Clinical recommendations on preimplantation genetic testing for aneuploidy indications

- PGT-A is recommended for
  - Advanced maternal age (36–40 years)
  - Repeated pregnancy loss – known etiologies.
- PGT-A is not recommended for
  - Young, good prognosis patients ( $<35$  years)
  - Unexplained RPL
  - Low AMH – limited eggs; multiple IVF cycles may be necessary in order to obtain one euploid blastocyst.

As PGT-A may help in diagnosis of only a small subset of repeated implantation failure (RIF) where recurrent aneuploidy may be the cause, there is no evidence for efficacy of PGT-A in patients with RIF. Patients' willingness for single embryo transfer.

### Consensus on preimplantation genetic testing for monogenic indications

Carrier screening for selected populations based on ethnicity and genetic diversity should be advised. Common genetic disorders' carrier status should be tested for few genetic diseases which would help a couple to make reproductive decisions prior to conception.



The recommendations for PGT-M conditions are to use linked markers to allow a more confident determination of genetic status in preimplantation embryos. Historically, this was recommended to prevent misdiagnosis due to allele dropout and contamination. However, the requirement to create complex patient-specific tests incorporating multiple linked markers has increased the time and cost associated with the development of PGT-M testing.<sup>[16]</sup>

In Indian scenario, the silent carriers remain undetected-increases the gene pool, there is no proper awareness about genetic testing, couples seek treatment after having one abnormal baby. In this scenario, panelist experts recommend providing carrier screening for common SGD and couples seeking IVF treatment must undergo carrier screening.

### Preimplantation genetic testing for monogenic indications: Case scenarios

- Previous child with a defect
- Couple screened positive for a variant (carriers)
- An alternative to prenatal genetic diagnosis for couples with a significant risk for transmitting the defect
- Avoiding abortions
- Family history of any X-linked, autosomal dominant, and recessive conditions.

### Preimplantation genetic testing for monogenic clinical point of view

- Screening result or mutation report positive is a must before counseling patient for PGT-M
- Number of embryos required for a biopsy depending on the inheritance pattern
- Need for multiple cycles for oocyte/embryo pooling
- PGT-M haplotyping/regular to be decided by the geneticist.

### Clinical recommendations on preimplantation genetic testing for monogenic indications

- Can be offered to all patients with single or multiple gene disorders with a positive mutation report
- Cannot be offered for diseases which are multifactorial and nongenetic based diseases
- In case PGT-A is desired, it should be performed on PGT-M screened embryos.

## Consensus on Preimplantation Genetic Diagnosis for (Chromosomal) Structural Rearrangement

- One or both parents has a balanced translocation (structural rearrangement in the chromosome)
- Chance of having a pregnancy with an unbalanced structural abnormality (extra or missing genetic material). Typically results in implantation failure/pregnancy loss
- PGT-SR for chromosomal structural rearrangements (previously PGS translocation)
- A genetic test designed to detect inherited rearrangements and increase the chance of a successful pregnancy
- Reduces the risk of having a pregnancy with an unbalanced structural abnormality.

### Chromosomal rearrangements

- Chromosomal rearrangements are associated with chromosomal changes in terms of normal size or arrangement
- Many carriers of balanced chromosomal rearrangements are healthy and are unaware of their carrier status until they try to have children
- People with chromosomal rearrangements are at an increased risk of producing embryos with the incorrect amount of genetic material, which typically do not lead to a successful pregnancy.

## Commonly seen chromosomal rearrangements: Clinical perspective

- Certain rearrangements are seen with greater frequency in the general population
- Hot spots can be found at 11q23, 17q11, and 22q11
- Leading to the recurrent translocations t(11;22) and t(17;22)
- The site on chromosome 22 that is often implicated in translocations is the same locus associated with DiGeorge/velocardiofacial syndrome (22q11 deletion disorder)
- Robertsonian translocations are among the most common balanced structural rearrangements seen in the general population, with a frequency in new-born surveys of about 1 in 1000
- Rob (13q14q) accounts for around 75% of all Robertsonian translocations.

## Guidelines suggested for preimplantation genetic testing for structural rearrangements

PGT for chromosome structural rearrangements (PGT-SR) is an accepted and routine procedure in most IVF/PGT centers. It has been developed for patients unable to achieve a pregnancy or at high risk of pregnancy loss and of abnormal live-born births, resulting from inheritance of unbalanced products of the rearrangement.

- When one parent has known chromosomal aberration<sup>[17]</sup>
- Parental karyotyping to be done to get accurate chromosome breakpoints
- Minimum 850 banding pattern (high-resolution)
- Assay to be carried out on a parental sample to test the probes
- Same probes to be used on biopsy specimens
- Total procedure = 2–10 days
- Same cycle transfers possible in some cases depending on the in-house availability of technology.

Different inclusion/exclusion criteria may apply based on the technology used (FISH, quantitative real-time polymerase chain reaction, comprehensive testing methods [array-based CGH, SNP array, or NGS]).

## Clinical recommendations on preimplantation genetic testing for structural rearrangement indications

- In general, PGT-SR is only recommended if the technique applied can detect all expected unbalanced forms of the chromosomal rearrangement
- When comprehensive testing strategies are applied, it is acceptable to use information on copy number of nonindication chromosomes to refine embryo transfer strategies
- Offered to balanced translocation carrier couple either with repeated implantation failure or repeated miscarriages
- Karyotyping is the gold standard to detect chromosomal abnormalities
- Some couples may be advised to undergo PGT-SR straight away after diagnosis
- FISH-based PGT-SR can be offered for patients where chances of getting blastocysts can be low
- Ample number of embryos required for testing based on female/male translocation carrier
- NGS-based consensus circular sequence only on validated pipeline by the geneticist
- NGS and FISH both are robust and sensitive.

## Accreditation and Quality Management

Accreditation, along with proficiency testing through internal quality assessment and external quality assessment, provides a means to achieve and maintain the highest quality standards. Accreditation is the formal recognition that an authoritative body gives to a laboratory/department/center when it demonstrates competence to carry out defined tasks and involves all aspects of management, along with technical requirements. Where possible, IVF/PGT centers should be accredited/certified, even when it is not legally required.

Because PGT is of a multidisciplinary nature, the various units involved should each be accredited/certified for their defined tasks and according to the most appropriate quality standards. For each unit, responsibilities should be clearly outlined/described and transition of responsibility from one unit to the other during the PGT process should be well defined and guaranteed.



## Quality Management Systems

- It is recommended that a *quality management system* is integrated to the IVF/PGT center. Quality management ensures that an IVF/PGT center and the PGT service it provides, is of consistent quality
- It has four main components: quality planning, QA, quality control, and quality improvement
- Aspects of quality management to be implemented include quality policy, quality manual, document control, compliance to SOPs, risk management, continual improvement, audits, and management review
- Technical requirements include personnel, laboratory conditions and environment, laboratory equipment, all stages of examination procedures, results reporting, and QA.
  - Validation of all methods used is recommended
  - Written SOPs should be available for all steps of the PGT procedure
  - Laboratory staff should have profound knowledge of the SOPs as these are the fundamental backbone of the service
  - Deviations from protocols should be recorded.

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