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PREIMPLANTATION GENETIC TESTING (PGT)

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The aim of the Assisted Reproduction Technology is not only 'To give Live birth' but 'To give Healthy Live Birth'. Which can be achieved by advanced technology like Pre Implantation Genetic Test. The field of ART getting advanced day by day since four decades to improve patient care. Embryologists and clinicians can keep their knowledge updated with print media, conferences and electronic media. NEXUS is providing all basic and advance information of Particular subject in the form of a single manual.

In this part of NEXUS, we have tried to cover Basic knowledge of all biopsy procedures step by step and molecular procedure, which is important to know for embryologists.

I am really thankful to IFS and team NEXUS for giving me this opportunity. My sincere thanks to Dr Rashmi Sharma and Pooja Awasthi for their effort to ensure final bulletin of this quality.

With Regards,

Dr Harsha Bhadarka
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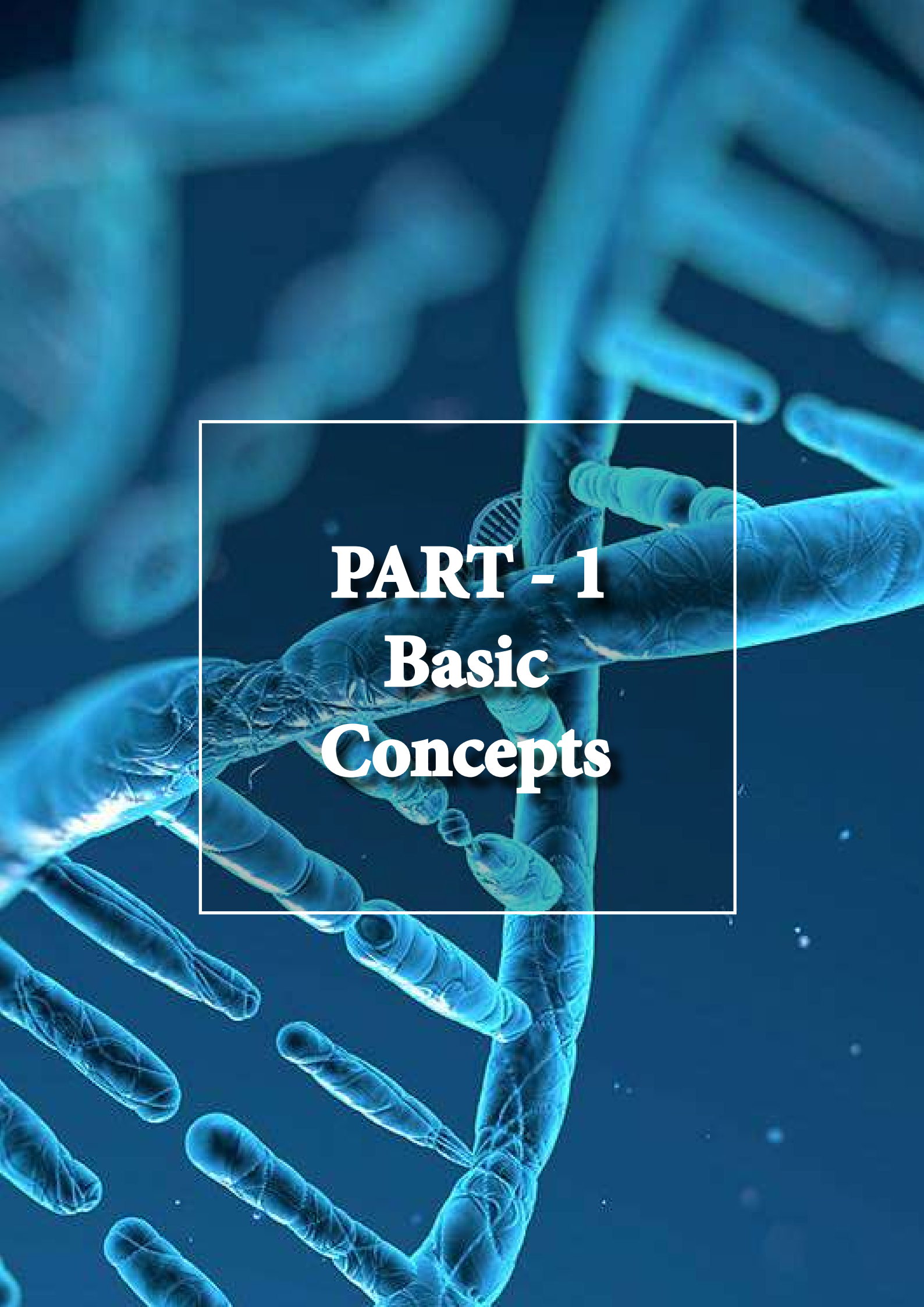

PART - 1
Basic Concepts

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1. INTRODUCTION

The role of molecular Genetics has marked it's significance in Assisted Reproduction Technology. With the upgradation of prenatal diagnostic techniques like amniocentesis and chorionic villi sampling (CVS), it has been possible for couples at risk of genetic diseases to give birth to healthy babies. However, the experience of these diagnosis followed by termination of pregnancy in case of abnormal findings if detected can be a unwelcome memory for a couple.

The advancement in assisted reproduction since last four decades has made favourable changes in the treatment of infertility like improvement in fertilization rate and thus selection of competent embryo becomes the most crucial step for the treatment. The aim of PGS/PGD is to detect whether embryo is chromosomally affected or not, thereby preventing transfer of a chromosomally abnormal embryo. PGD is essentially an alternative to prenatal diagnosis, which helps in negative selection of mutant embryo prior to implantation.

The terms PGS and PGD is now replaced by new terminology in the international glossary of infertility and fertility care.

The new name of the all test is Preimplantation genetic testing (PGT)
PGT-A for anuploidies previously termed as PGS
PGT-M for Monogenic/single gene disorders previously termed as PGD
PGT-SR for chromosomal structural rearrangement previously termed as PGS translocation [1]

2. HISTORY

In 1937 manuscript in the New England Journal of Medicine, Dr John Rock predicted that human IVF, gender selection, and gestational carriers would be utilized in ART treatment. [2]

Dr Robert Edwards and Dr. Richard Gardner has first described use of PGD for sexing of rabbit blastocysts in Nature journal, 1967. [3]

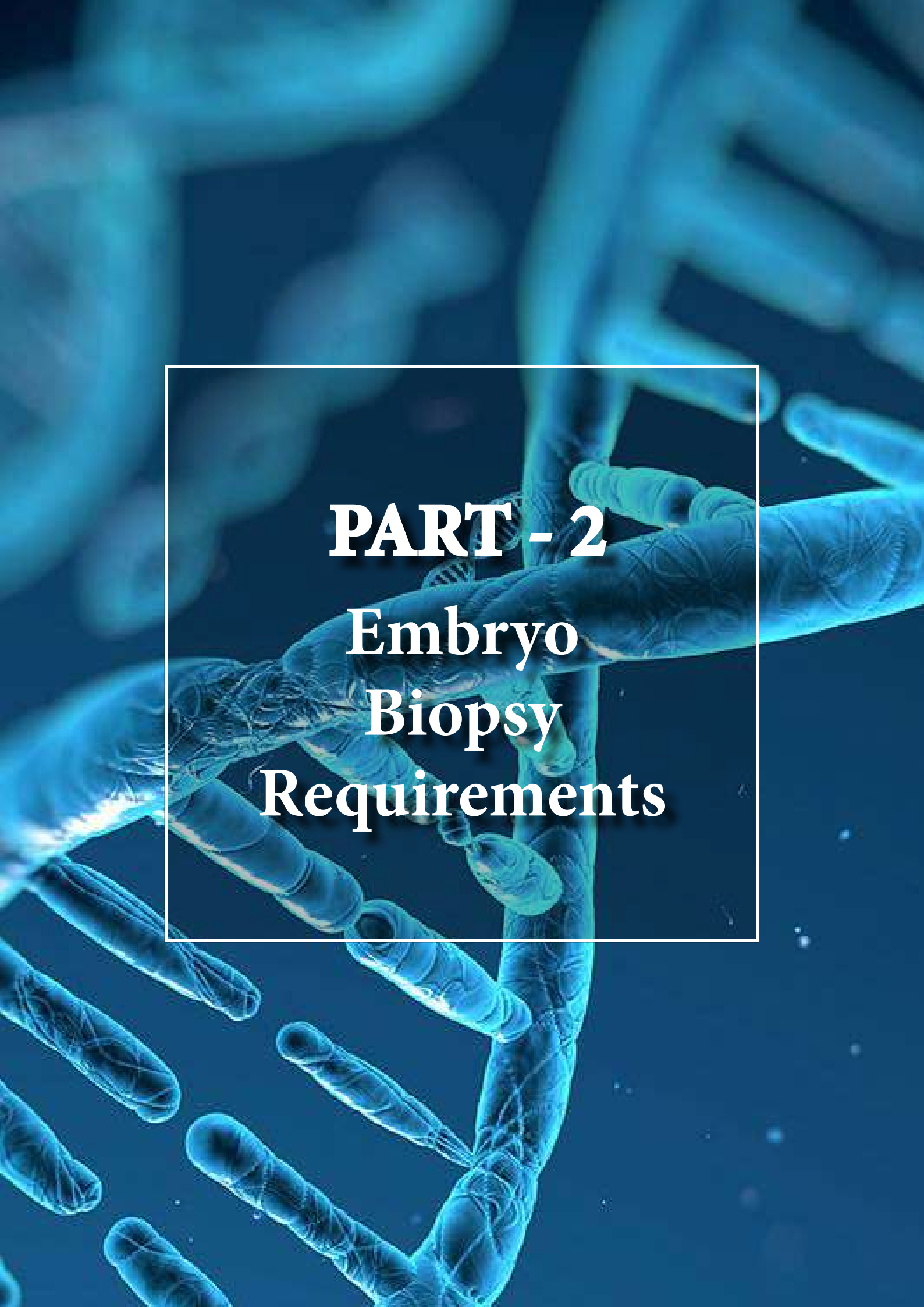
In 1986, Leeanda Wilton initiated the cleavage stage biopsy.
After that in 1987, Marilyn Monk's work had illustrated PGD in a murine model for Lesch-Nyan syndrome. [4]

Later, in 1988, Yuri Verlinsky describes polar body biopsy [5]and Audrey Muggleton-Harris describes trophectoderm biopsy thereby adding two more approaches in obtaining genetic material from embryos. [6]

In 1989, PGD was first used for testing monogenic disorders and sex-linked disorders by Elana Kontogianni which showed PCR for the Y chromosome from a blastomere. Further, working on X-chromosome linked diseases, amplification and detection of Y-chromosome specific repeat sequences allowed selection of embryos that were female which are not at risk of carrying the disease. Due to these approaches, further detection of gene mutations on autosomes and sex chromosomes enables clinicians to select healthy embryos without mutation for embryo transfer.[7]

3. INDICATIONS FOR PREIMPLANTATION GENETIC TESTING

√ Higher age of female patient.
√ History of recurrent pregnancy loss.
√ Repeated IVF failure.
√ Male factor infertility.
√ Patients with family history of X-linked disorders carries 25% risk of having an affected embryo.
√ Mental or physical problems in offspring occurs due to Chromosome translocations.
√ Carriers of autosomal recessive diseases, carries 25% risk of having an affected embryo.
√ Carriers of autosomal dominant diseases, carries 50% risk of having an affected embryo.
√ HLA matching. [8]



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Embryo Biopsy Requirements

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1. SOURCES OF GENETIC MATERIAL FOR PGT

There are potentially three types of cells suitable for PGT analysis including polar bodies (PBs) from the oocyte/zygote stage, blastomeres from cleavage stage embryo, trophectoderm cells from blastocysts or blastocoel fluid and culture medium as non invasive method of PGT.

2. PREPARATION OF BIOPSY PROCEDURE

As per ESHRE PGD Consortium/Embryology Special Interest Group the following recommendations are made in lab for preparations prior to any biopsy procedure on human oocytes or embryos.

- Confirm all equipment is working correctly, calibrated and maintained per requirements of procedures.
- Biopsies must be performed on a warmed stage.
- Laminar air flow should have maintained sterile, cell free environment.
- Ensure the appropriate media, chemicals and tools are available in proper maintained conditions.
- Ensure that biopsy is performed by a suitably qualified person who is trained to a written procedure.
- During procedure use of powder free gloves disposable gown, cap and mask is mendetory to reduce DNA contamination.
- Biopsy dishes should be made up before the procedure, and clearly labelled with the patient name and oocyte or embryo numbers.
- Biopsy dishes should contain a drop of biopsy medium of sufficient size to maintain pH, osmolarity and temperature during the procedure Sufficient rinse drops comprising culture medium should be available to rinse oocytes and embryos after the biopsy procedure.
- It is necessary to make sure that an proper labelling system to identify the cell number and the oocyte/embryo from which it was biopsied and it is critical that all stages have appropriate and recorded witnessing. This must include documented matching of the cell and oocyte/embryo after biopsy, of the cell and slide/tube during preparation and finally of the embryos recommended for transfer on the PGD report prior to embryo transfer.
- Acidified Tyrodes solution (if applicable) should also be readily available to allow pipette priming between biopsies.
- Arrangement of Transportation of cell should be done in advance.
- ICSI is preferable procedure for fertilization and all cumulus cells are removed before biopsy as these cells can contaminate lead to misdiagnosis.

FIG 1 : BIOPSY MEDIA

3. LIST OF EQUIPMENTS AND DISPOSABLES

List of Equipments:

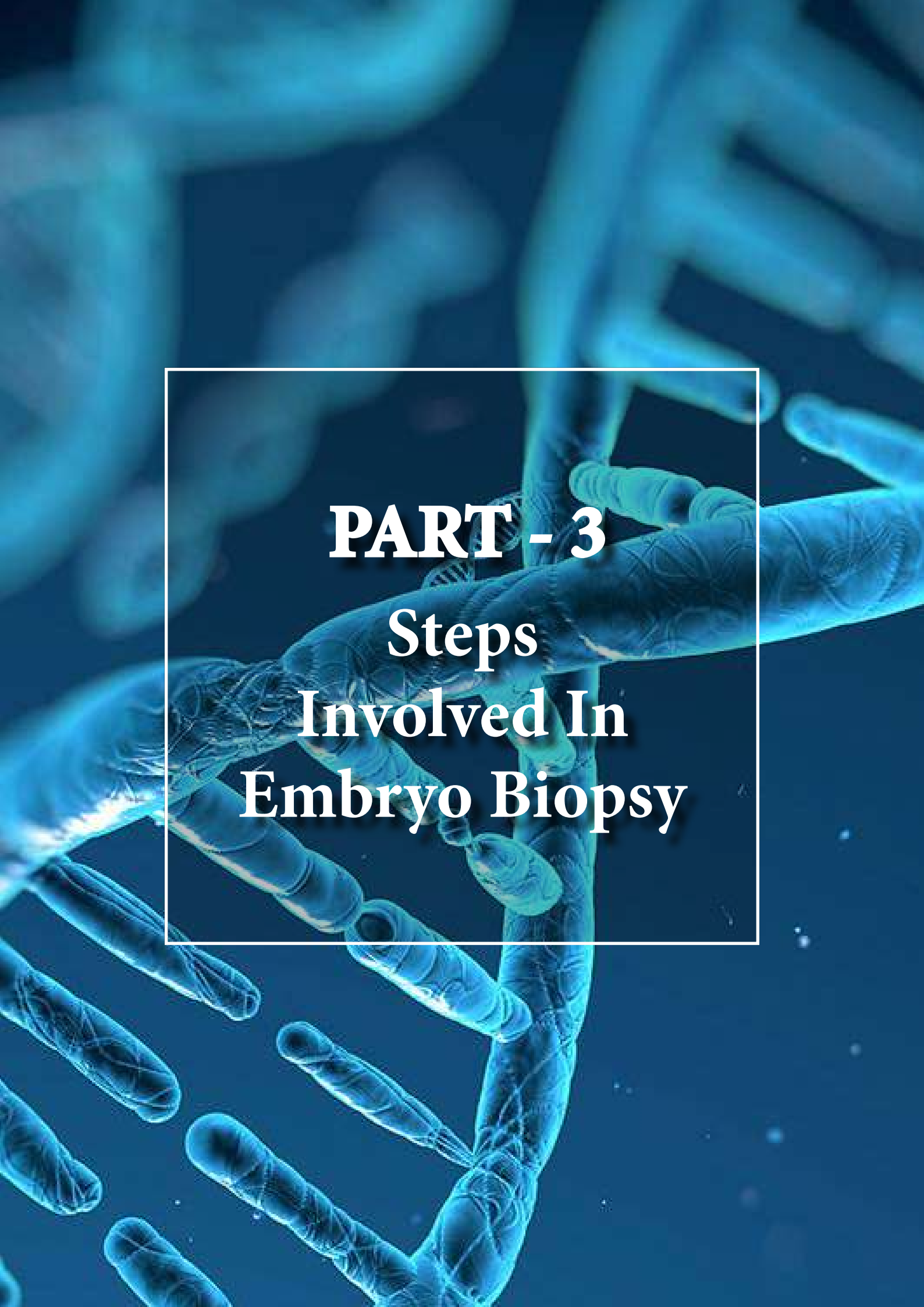
- Laminar air flow.
- Stereozoom microscope for embryo/cell handling.
- Micromanipulator with LAH
- Mini spin-centrifuge for PCR tubes

List of Disposables:

- Petri dishes
- Micro pipette 1 µl- 10 µl.
- Sterile tips, with filter and DNA/RNA free.
- Capillaries of a diameter between 100-130 µm.
- Sterile 0.2 ml PCR tubes stored at -20°C with 2 µl of PBS solution.
- Biopsy needles
- PCR tube cooler rack

Reagents :

- Mineral oil
- Ca^{++} and Mg^{++} free Media (for cleavage stage)



PART - 3

Steps Involved In Embryo Biopsy

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STEPS INVOLVED IN EMBRYO BIOPSY

It involves three main steps:

1) Drilling a hole in Zona Pellucida(ZP)
2) Removal of the Cell/cells
3) Transportation to the Genetic lab

1. DRILLING A HOLE IN ZONA PELLUCIDA(ZP)

There are three methods for zona drilling:

Mechanical drilling:

- It involves partial zona dissection making a cut using sharp and closed micro needle.
- This method is Simple,Safe and no chemicals, laser are used which may affect embryo development.
- It is Time-consuming and technically difficult requires skill to perform.

Chemical drilling :

- It is done by using acidic tyrode's solution which creates larger and rounder hole in zona.
- It may affects embryonic development due to side effect of chemical.

Laser assisted hatching:

- It is most advanced technology in which laser beam is used to drill zona detrimental effects on embryo.
- It is fast, easy and safe method.
- But laser shot may slightly increase surrounding temperature in media droplet and the equipment is costly.

2. REMOVAL OF THE CELL / CELLS

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.

The use of commercially available $Ca^{2}+/Mg^{2}+$ free biopsy medium[9] is recommended for cleavage stage biopsy. Biopsy should be perform as quick as possible to reduce changes in pH, osmolality and temperature.
Three types of cells are for the PGT analysis.

Polar body, Blastomere, Blastocyst cells depending on types of biopsy.

TYPES OF BIOPSY:

1) Polar body biopsy
2) Blastomere biopsy
3) Trophectoderm biopsy

1) POLAR BODY BIOPSY

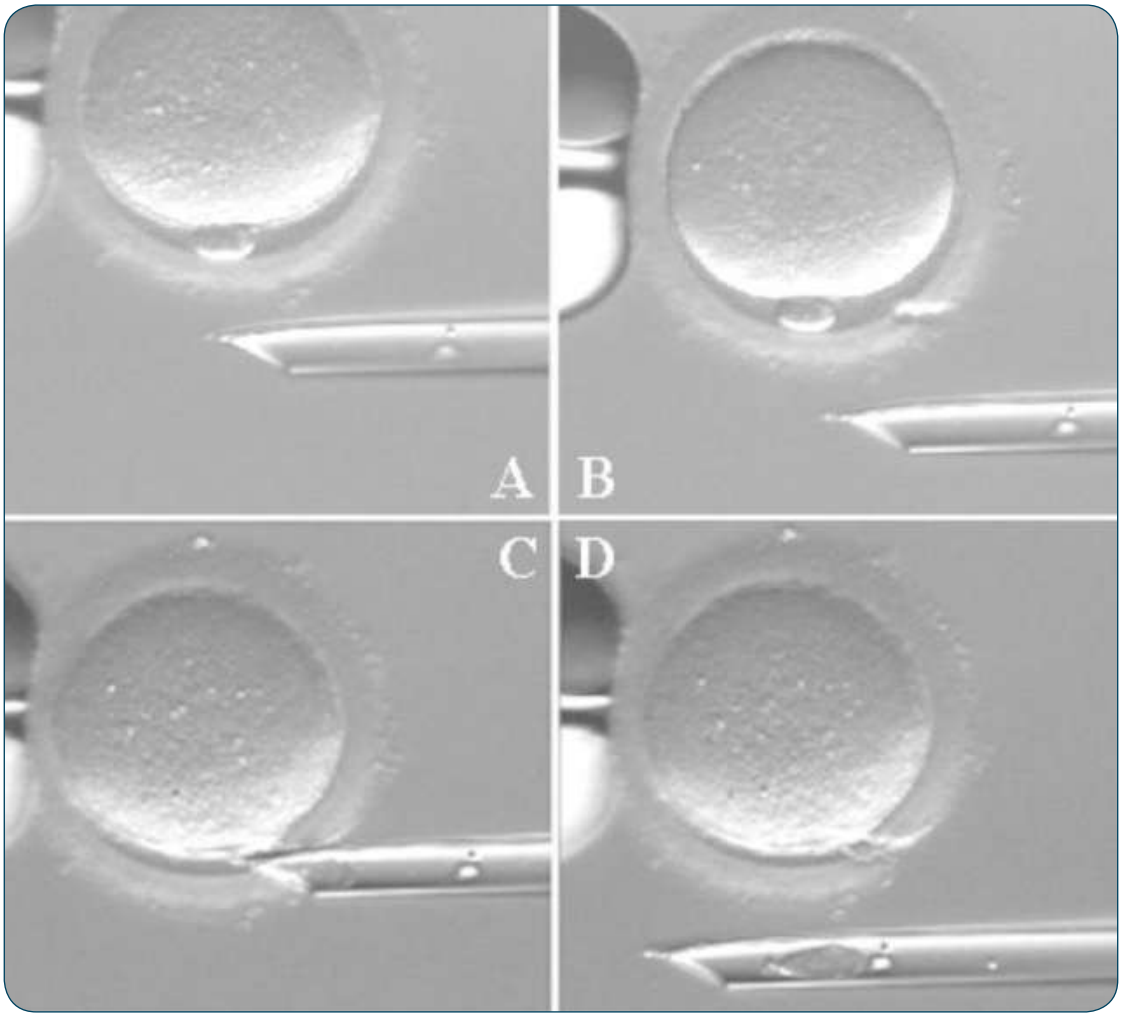
- Its a kind of preconception diagnosis where inherited disease in oocyte are recognized before fertilization.
- It works on the principle that genetic makeup of polar body is complementary to genetic makeup of its oocyte.
- As this technique does not require cleavage of embryo, it is mainly done in a country where there are legel restrictions on embryo research.
- It is also applied for couples having religious, ethical and moral constraints towards discarding surplus embryo.

Method:

- First and/or second polar bodies are biopsied simultaneously or sequentially after LAH.
- First polar body is biopsied immediately after egg retrieval on day 0 when oocyte is at metaphase II stage followed by second polar body on day 1 of fertilization.
- Removal of polar bodies done by aspiration technique. [10]

Limitations:

- Biopsy of only first polar body has limitations as it provides information about only maternal genetic defects.
- Less quantity of material available for testing.
- Hence, biopsy of first and second polar body is must to improve diagnostic effectiveness and so two times manipulation is required for single embryo testing.



2) BLASTOMERE BIOPSY

- It is performed on day 3 post fertilization, when embryo is 6-8 cell stage.
- It works on the principle that at 6-8 cell stage embryo is totipotent and each cell is mirror image of other cells present in embryo so removal of one cell during biopsy will not affect developmental potentiality of embryo.

Method:

- A hole is made in zona pellucida and one/two blastomeres having nucleus are aspirated gently. [11]
- One of the major problem is cells undergoes compaction leading to tight junction. Hence, it requires time, which , increases possibility of blastomere damage.
- Ca^{++} and Mg^{++} free HEPES buffer medium is used to loosen membrane adhesions between blastomere.
- Blastomeres removal done by aspiration. [12]
- Fresh blastocyst transfer followed by clevage stage biopsy is possible.

Limitations:

- Only 1-2 blastomeres are available for genetic diagnosis but higher percentage of cytoplasm removed as compare to blastocyst biopsy.
- Higher chances of mosaicism at this stage.

Increases the cost as all embryo may not reach up to the blastocyst stage. It is preferable not to take biopsy of poor quality embryo.

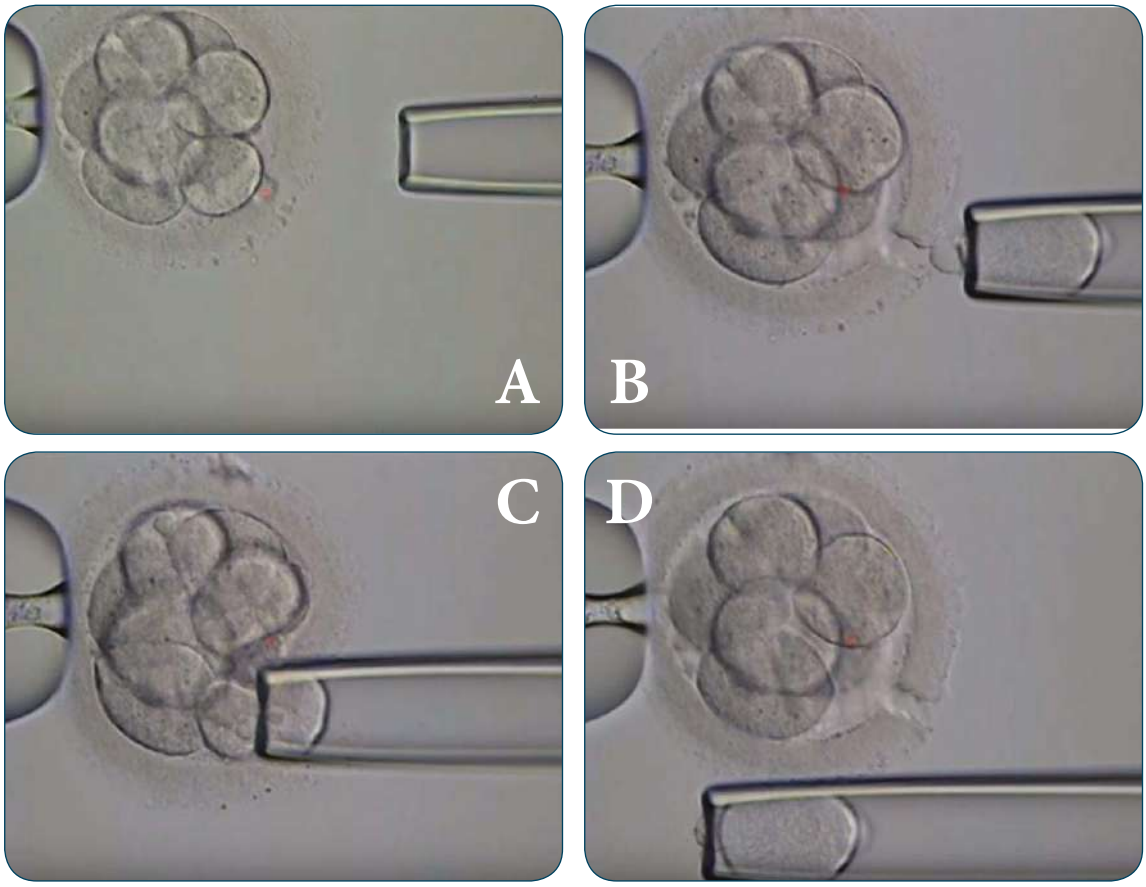
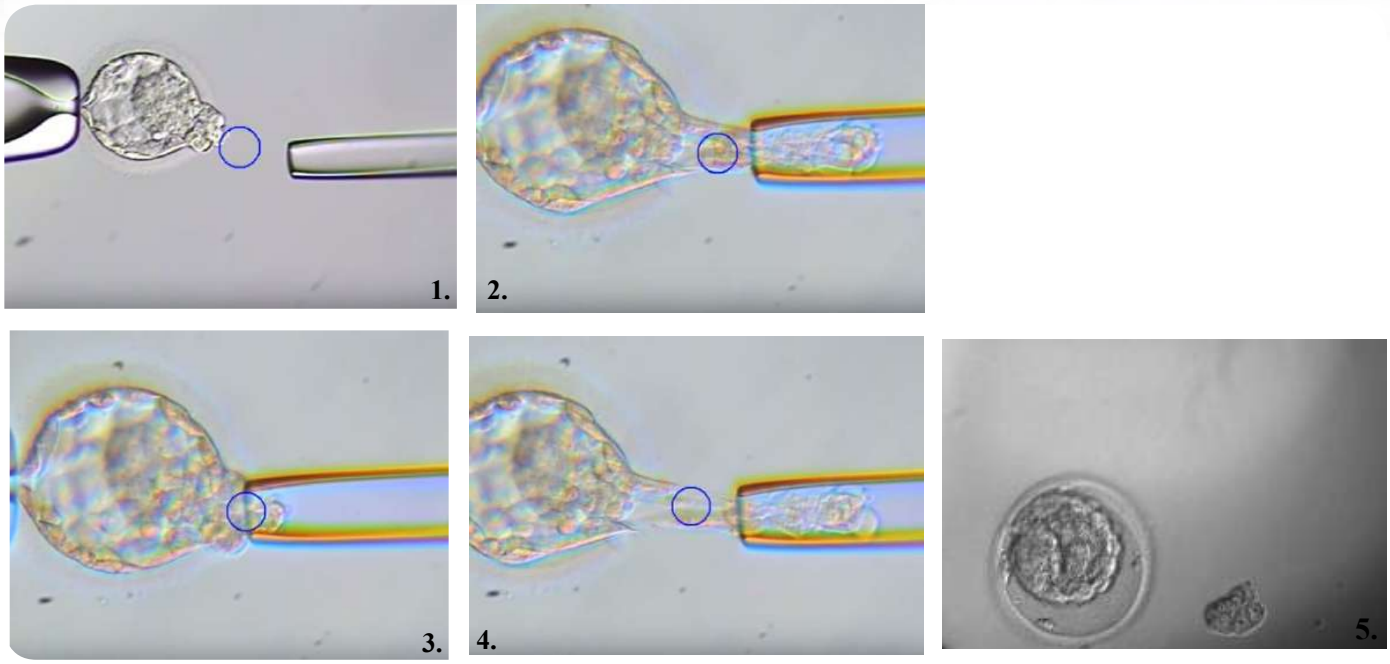


FIG 2 : DAY 3 BIOPSY

MORULA STAGE BIOPSY:

Day 4 embryo, with compaction and without vacuole or fragmentation, were incubated in Ca^{2+}, MG^{+-} free biopsy medium for 15-20 min for decompaction.

After hatching of zona pellucida of decompacted embryo, 3–7 cells were retrieved using a technique similar to blastomere biopsy at the cleavage stage. Here, number of cells are more than clevage stage biopsy and comparable to trophectoderm biopsy.[13]



3) TROPHECTODERM BIOPSY

- Blastocyst embryo have two types of cells:
  1) Trophectoderm cells which forms placenta and
  2) Inner cell mass which forms fetus.
- The genetic makeup of trophectoderm is similar to inner cell mass. So, removal of few trophectoderm cells do not affect development of fetus.

Method:

There are several methods for blastocyst biopsy which includes breaching of zona on day 3 and removal of 5-7 cells of trophectoderm on day 5 or zona breaching and remove 5-7 trophectoderm cells on day 5.
These cells are removed by herniation following drilling with laser.[14]

Limitations:

- Time available for establishing diagnosis is very limited as embryo.
- Require skilled embryologist.
- Mostly it requires all embryo freezing.

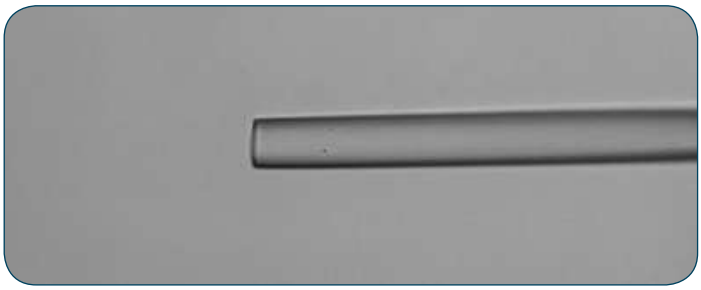


FIG 3: DAY 6 BIOPSY

NON INVASIVE METHODS:

As a newer techniques to reduce biopsy stress to the embryo Blastocoele fluid and spent culture medium, is usedfor chromosomal analysis in PGT nowadays. Presence of DNA material including chromosome, genomic and mitochondrial DNA in the blastocoele fluid and culture medium has been already published. As there are limited published data available for these analysis, more clinical studies are required for improvements in the chromosomal analysis and gene level analysis for both fluid culture medium analysis and blastocoele fluid analysis. Obviously, non-invasiveness gives these two methods a great potential to make PGT even safer and more routinely used in clinics.

3. POINTS TO REMEMBER

FIG 1

- Prepare a Petri dish with 1 row with three droplets of 5 µl of cell washing media for each embryo (include 6 rows max in one plate) and cover with mineral oil. Each blastomere/trophectoderm sample should be rinsed in these droplets. **(Figure 1)**
- Use one new capillary for each individual cell (day-3 biopsy) or trophectoderm biopsy. Always rinse embryo and biopsied material properly in culture media to remove traces of biopsy media.
- Spin the PCR tubes in mini spin with the 2µl of PBS before starting to remove air bubble and confirmation of media at the bottom of tube.
- Lable all PCR tube with initials of patient's name and number.

  For example patient's name Harsha Bhadarka tube and embryo droplet named by HB1, HB2.....For blank tube •HB1, •HB2....Same labeling should be followed at the time of embryo freezing on the device. **(Figure 2)**

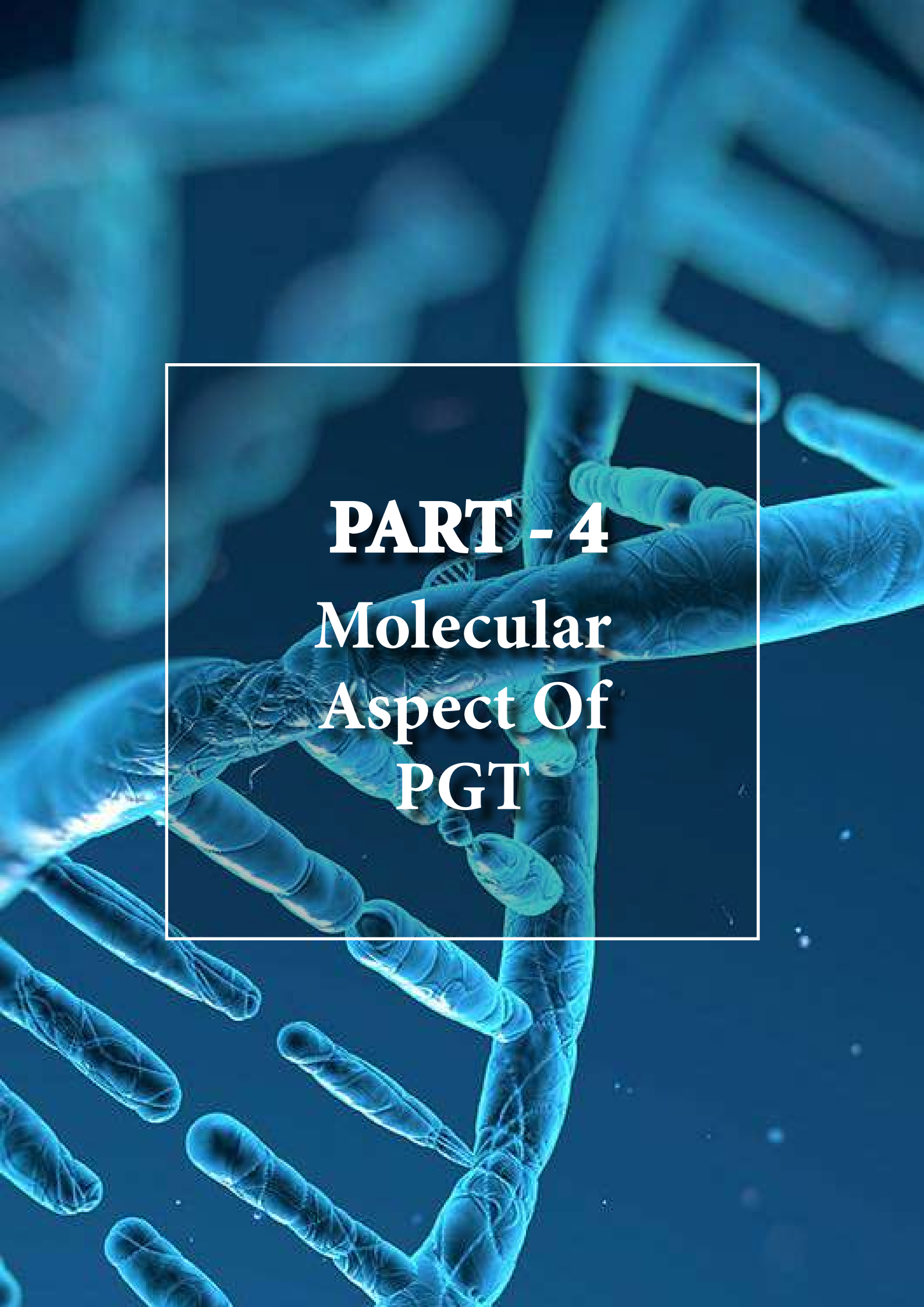
FIG 2

3. TRANSPORTATION TO THE GENETIC LAB

- Place the biopsied cells sample into the PCR tube with minimum volume of washing media under the stereozoom microscope.
- There should be one blank for each embryo sample, in which remaining fluid is placed after releasing each cell into the collection tube.
- Place PCR tubes in the cooler rack and cover the cool rack with parafilm. Keep it inside the shipping box with ice packs with taking care that cooler does not move inside the box during transportation.
- Fill all the forms and details of patients properly.

4. REBIOPSY OF EMBRYOS

- This practice is only acceptable in the case of lost or anucleated cell and non informative results.
- Prior counselling of patients about risk of embryo damage should be done.
- The embryo cell number and day(day3 or 5) of rebiopsy should be considered.
- Rebiopsy should be done from same site of zona hatching. [15]



PART - 4
Molecular Aspect Of PGT

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MOLECULAR ASPECTS OF PGT

For genetic analysis there are three main techniques available for testing.

FISH, ACGH and NGS.
NGS is the latest and most promising technique, which is followed by most of the genetic labs for PGT now a days. Fluorescent in situ hybridization (FISH) was initial method used as molecular analysis because of has it's technical limitations like the number of probes required to get reliable reports and the requirement of specific parent karyotyping prior to testing. [16][17][18] It analyzes a limited number of chromosomes at a time (13, 16, 18, 21, 22, X and Y). These constraints have led to the development of new and improved 24 chromosomal copy number analysis technologies, including comparative genomic hybridization (CGH), array comparative genomic hybridization (aCGH), digital-PCR, real-time quantitative PCR, SNP microarray and next generation sequencing (NGS), which is the latest method to date.

With recent technologies, new methods for comprehensive chromosome screening (CCS) like a CGH, qPCR and SNP array can detect both euploid and aneuploidy embryos but are unable to detect mosaicism. However high-resolution NGS has the ability to detect euploid and aneuploidy embryos as well as mosaicism.[19]

All of these different technologies have their own advantages and disadvantages according to the time of analysis, the cost, the resolution, the labor needed, the procedures and the abnormalities which can be detected.
At present, Illumina and Ion torrent are widely used as NGS platforms in human genetics. Illumina uses sequencing

1. DIFFERENT PLATFORMS USED IN NGS

by synthesis with reversible terminator. The newer platforms of Illumina can yield about 24 million short fragments of DNA sequence per run or "read" which gives it an ultrahigh- throughput and makes it cost effective. **The main limitation of Illumina is in the increased base substitution error rate.**

Ion torrent use emulsion PCR as their amplification method and Illumina uses bridge PCR. Ion torrent uses ion semiconductor sequencing and can read up to 200bp. It has high error rates in calling insertions and deletions. Although it takes days to have one genome sequence with Illumina, it is the most widely used because of its cost effectiveness, good sequencing throughput and accuracy [20]

Workflow overview of Ion-Torrent Sequencing

Sampling

Samples (single cell) were collected from human embryo (Standard collection techniques were used as per the protocol)

⇩

The samples were diluted in Phosphate Buffer Saline (PBS)

Library preparation

⇩

Extract gDNA(Genomic DNA)

⇩

Prepare Ion SingleSeq™ libraries

⇩

Pool, purify and quantify the libraries

Template preparation

⇩

Prepare template-positive Ion PGM™ Template IA Ion Sphere™ Particles

⇩

Enrich the template-positive Ion PGM™ Template IA Ion Sphere™ Particles

Sequencing

⇩

Create a Planned Run

⇩

Clean and initialize the Ion PGM™ Sequencer

⇩

Load the chip and start the sequencing run

Analysis

⇩

Analyze the run

2. TERMINOLOGY IN PGT

ANEUPLOIDY: When the number of chromosome in a cell changes from the normal chromosomal composition, it is called aneuploidy.

TRISOMY: Addition of a single chromosome.
TETRASOMY: The addition of a pair of chromosomes.
MONOSOMY: The loss of a single chromosome.
NULLISOMY: the loss of a pair of chromosomes

3. SINGLE GENE DISORDER

Single gene disorder, also known as monogenic disease, is when a single mutation in a specific gene leads to a hereditary disease which can occur early during childhood or have a late onset.

With the use of PGD, we can now select embryo which are free from these mutations. In fact, in 1992, the first baby free from cystic fibrosis was born after PGD and since then PGD has been increasingly used to detect monogenic disorders [21]

Usually these disorders lowers quality of patient's life and sometimes may even be associated with a shorter life span.

4. MOSAICISM

Mocaisim is defined as the presence of two or more different cells lines with different chromosomal number or structure in one embryo resulted by errors in chromosomal segregation during mitosis.

There are chances that mosaicism could be only in the ICM and not in the TE. Hence it diagnosed as a euploid embryo and could result in an unfavorable outcome. It is advisable to perform amniocentesis / CVs for confirmation of test results.

Detecting Mosaicism

Trophectoderm biopsies includes approximate 5-10 cells.

In a mosaic biopsy of only 5 cells, the lowest possibility aneuploidy is 20% (if atleast one cell abnormal), and the highest is 80% (max. 4 cell abnormal). PGDIS have recommended that PGT-A samples with <20% aneuploidy be classified as euploid, 20-80% as mosaic, and >80% as aneuploid.

5. MitoScore

Mitochondria plays an important role in energy production and their own DNA that is known as mitochondrial DNA or mtDNA,which is responsible for predicting implantation potential of embryo. The mitochondrial score "MitoScore" represents the total mtDNA content in euploid embryos. [22]

The amount of mitochondria present in cell, relates the function and viability of that cell. ATP production capacity is important for maturation of the cytoplasm and nucleus, completion of meiosis II and fertilization. [23] [24][25]

Normal MII oocytes containing ideal mitochondrial content and ample levels of ATP (approximate 2 pM),[26] which results in best quality embryo. [27]

As cell division begins, the total amount of mitochondria within each blastomere decreases due to dilution with no new mitochondrial biosynthesis. [28]

At the time of cellular division, the cells do not express the replication factors required to increase copy numbers of mitochondria.Hence, there is no biosynthesis of new mitochondria.So, the total amount of mitochondria decreases in each cell due to dilution during cell division [29] and ultimately results into very low mtDNA in the trophectoderm cells.[30] The amount of mtDNA is inversely proportional to the implantation rate of embryo In conclusion, euploid embryos have higher implantation rate and lower Mitoscore values.

6. DIAGNOSIS

Misdiagnosis

- There remains an empirically determined 1-2% chance of a misdiagnosis, either by a false negative or a false positive result.

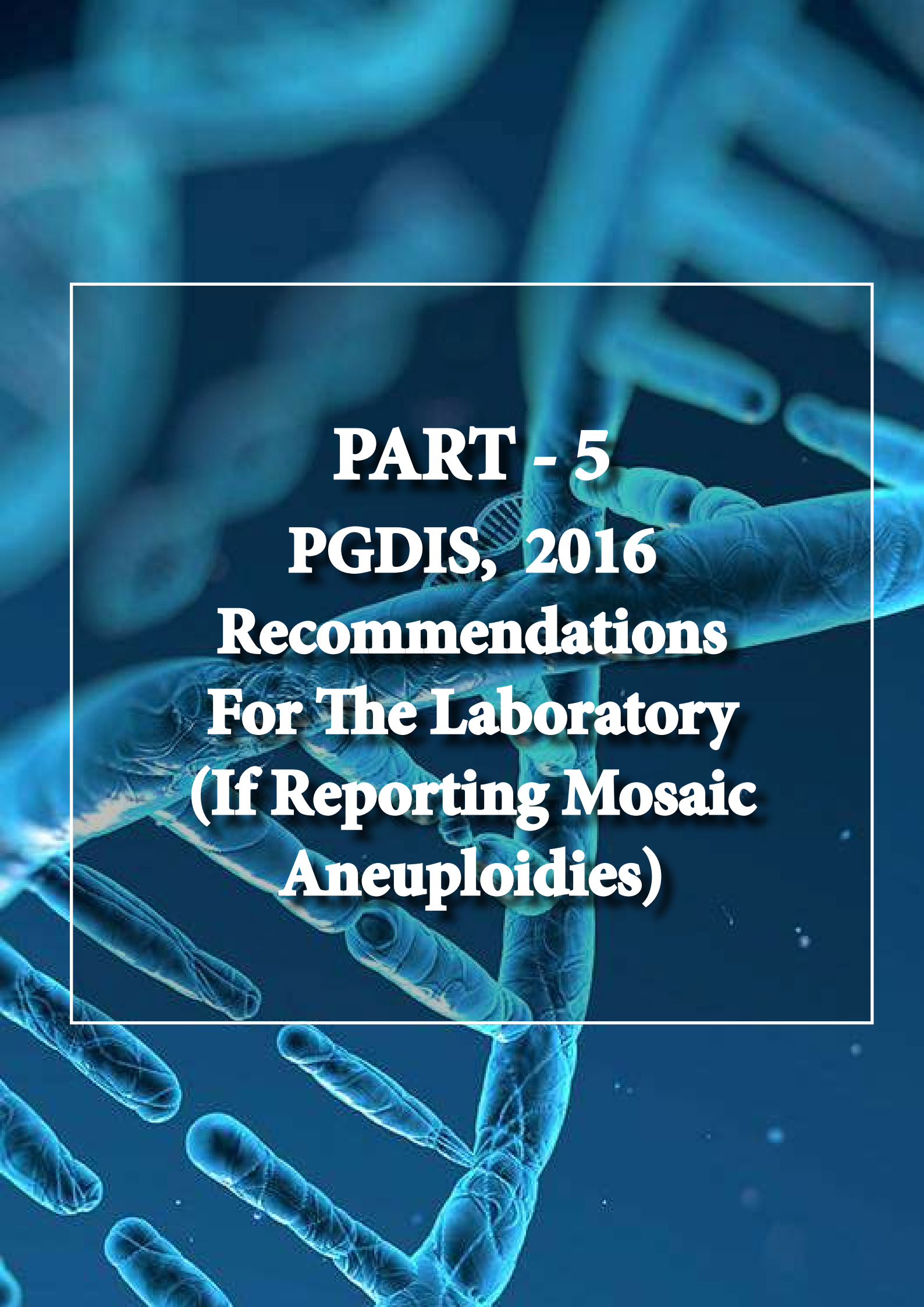
No diagnosis

- Mosaicism can cause a misdiagnosis if the cells that are analysed are not representative of the embryo.
- PGT cannot detect mosaicism when only one or few cells are analyzed.

  Here genetic testing cannot be performed due to improper biopsy techniques, loss of biopsied cells, or poor DNA quality.

7. NON INFORMATIVE REPORTS

The risk of inconclusive chromosomal-assessment after trophectoderm biopsy was approximate 2.5% because of cell DNA degradation, DNA amplification failure or other technical issues the data can not conclude the final result. These results will be reported as Non informative.



PART - 5
PGDIS, 2016 Recommendations For The Laboratory (If Reporting Mosaic Aneuploidies)

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PGDIS, 2016 RECOMMENDATIONS FOR THE LABORATORY (IF REPORTING MOSAIC ANEUPLOIDIES)

1. For reliable detection of mosaicism, ideally 5 cells should be biopsied,
2. Only a validated NGS platform that can quantitatively measure copy number should be used for measurement of mosaicism in the biopsy sample. NGS can accurately measure 20% mosaicism in a sample.
3. For reporting results, the suggested cut-off point for definition of mosaicism is >20%,
   - < 20 should be treated as normal (euploid).
   - > 80% abnormal (aneuploid), and the remaining ones between 20-80% mosaic (euploid-aneuploid mosaics).

1. RECOMMENDATIONS FOR THE CLINICIAN

1. Patients should be councelled that any genetic test based on one or small number of cells taken from embryos cannot be 100% accurate for a combination of technical and biological factors, including chromosomal mosaicism.
2. The patient information and consent forms for aneuploidy testing should mention the possibility of mosaic aneuploid results and any potential risks of it's transfer.
3. Priority should be given to normal euploid embryo as compare to mosaic aneuploid embryo for transfer.
4. In the case of the transfer of a blastocyst with only mosaic aneuploidies counsel the patient for:
   - A new cycle of IVF with PGT to increase the possibility of identifying a normal euploid blastocyst for transfer
   - Transfer of a blastocyst with mosaic aneuploidies for low risk chromosomes only, after appropriate genetic counseling.
   - Proper follow up and antenatal diagnosis of pregnancy should be done, preferably by early amniocentesis (> 14 weeks gestation).

2. SUGGESTED GUIDELINES TO PRIORITIZE MOSAIC EMBRYOS FOR TRANSFER

Based on the reproductive outcomes of fetal and placental mosaicism from prenatal diagnosis, the following can be used as a guide by the clinician when a mosaic embryo is being for transfer:

1. Embryos showing mosaic euploid/monosomy are preferable to euploid/trisomy, given that monosomic embryos (excepting 45, X) are not viable.
2. 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.
   - 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.
   - Embryos mosaic for trisomies that are associated with potential for uniparental disomy (14, 15) are of lesser priority.
   - Embryos mosaic for trisomies that are associated with intrauterine growth retardation (chromosomes 2, 7, 16) are of lesser priority.
   - Embryos mosaic for trisomies capable of liveborn viability (chromosomes 13, 18, 21) are of lowest priority, for obvious reasons.

PART - 6

FAQs

1. FAQS

1) CAN PGT HARM EMBRYO?

The cells from Day 3 embryo are totipotant in nature so removal of one or two cell will not harm.
The 5-10 cells are removed from the trophectoderm that will form the placenta so the embryo is less likely to be harmed if the procedure is done by skilled embryologist.

2) HOW TO PREVENT CONTAMINATION?

By Using powder free latex gloves.
Wear disposable medical gown and cap.
Turn on the laminar flow for at least 30 minutes before procedure .
The work station, all the platform and all disposable material (tip boxes, automatic pipettes, capillaries boxes, etc) should be wiped with 70% Alcohol.
If possible a designated separate room for biopsy from the IVF lab to reduce cell contamination.

3) IS IT MANDATORY TO PERFORM ICSI FOR PGT ?

ICSI is recommended for all PGT cases to reduce the chances of paternal contamination from extraneous sperm attached to the zona pellucida [31]

4) IS IT MANDATORY TO REMOVE CUMULUS CELL PRIOR BIOPSY FOR PGT?

It is strongly recommended that all cumulus cells are removed before biopsy as these cells can contaminate and lead to misdiagnosis [32]

5) IS IT MANDATORY TO DO SINGLE CULTURE OF EMBRYO ?

Biopsied oocytes and embryos must be cultured singly in individual drops,well or dishes with a clear identification system to ensure tracking of polar bodies or blastomeres removed and easy identification of oocytes and embryos post-diagnosis.

2. TROUBLESHOOTING DURING PROCEDURE

1) WHAT IF LASER IS NOT WORKING ON THE DAY OF BIOPSY?

- Restart laser software,
- Check and tighten all cable connections of computer and laser equipment.
- Check for any updates with the laser software.
- Call the engineer from the company.
- Postpone biopsy to the next day.
- Freeze blastocyst and plan thaw-biopsy procedure,

Preventative measures:

- Daily laser checks, regular laser software maintenance
- Purchase a 2nd laser setup (depending on work flow)
- Learn the chemical and mechanical method for hatching.

2) WHAT IF THERE IS NO BIOPSY NEEDLES, KITS OR BUFFERS IN STOCK?

- Ask near by IVF center or genetic lab. for help.
- Postpone biopsy embryos to the next day.
- Freeze blastocyst and plan thaw- biopsy procedure,

Preventative measures:

- Check availability of all materials on the day of oocyte retrieval or weekly.

3) WHAT IN CASE OF STICKY BIOPSY SAMPLES?

- If Biopsy sample is stuck to the outside of biopsy needle tap the needle holder carefully to cause the needle jerk in the media it will freed the cells.

Preventative measures:

- Fire only between cells, the less cellular damage so it reduces stickiness of tissue. Fire 2-3 times and use manual force by rubbing tissue with holding needle to tear biopsied tissue from blastocyst.

4) WHAT IF ICM HATCHES OUT FROM EMBRYO?

- Hold the blastocyst away from the hatching ICM and make another hatching in the zona and continue the biopsy.

Preventative measures:

- No Assisted Hatching of Day 3 embryos, Wait until blastoceol cavity forms and do hatching on the opposite side of the ICM.

5) WHAT ARE THE POSSIBILITIES OF ERRORS DURING TUBING SAMPLES?

- loss of cells.
- Cell stucks on the side/cap of the tube.
- More buffer in tube
- Labeling errors.

6) WHAT ARE THE POSSIBILITIES OF ERRORS DURING TRANSPORTATION?

- Forgot to inform courier company about biopsy planning.
- Courier person can not reach because of natural calamities like rain or earthquake.
- Cold chain is not maintained during transportation.

PART - 7
Regulations In India

REGULATIONS IN INDIA

In India, Ministry of Family Health and Welfare, regulates the concept under – **"The Pre-Conception and Prenatal Diagnostic Techniques (Prohibition of Sex Selection) Act, 1994".**

An act to provide for the prohibition of sex selection, before or after conception, and for regulation of pre-natal diagnostic techniques for the purposes of detecting abnormalities or metabolic disorders or chromosomal abnormalities or certain congenital malformations or sex-linked disorders and for the prevention of their misuse for sex determination leading to female feticide.

RECORDS TO BE MAINTAINED

Detailed record of patients undergone councelling and tests in register.

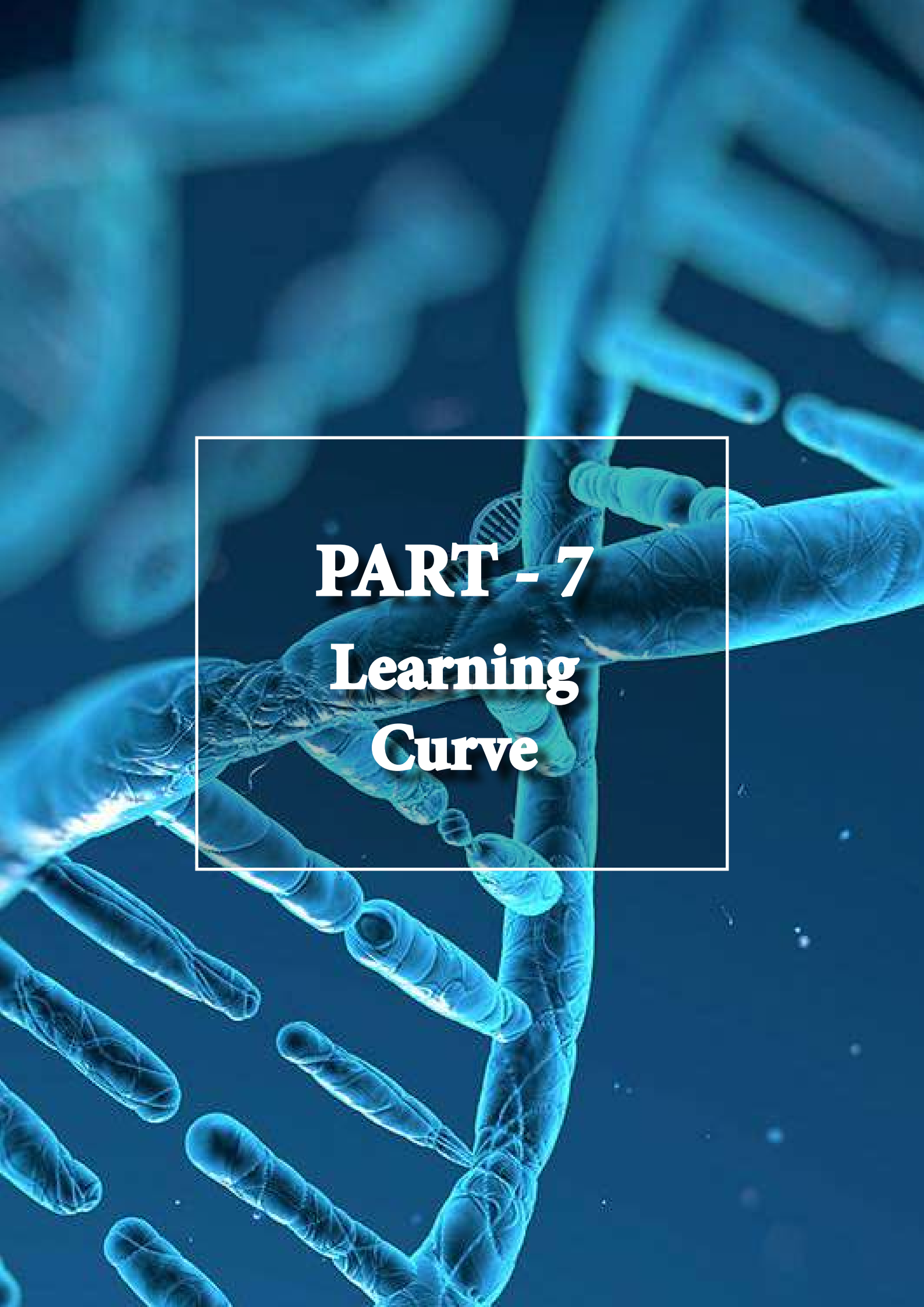
FORM D : Form for maintenance of records by the genetic counselling centre.

FORM E: Maintenance of records by genetic laboratory

FORM F : Form for maintenance of record in case of prenatal diagnostic test/ procedure by genetic clinic/ ultrasound clinic/ imaging centre.

FORM G: Form of consent (for invasive techniques)

And also all consent forms related to IVF procedure as per Clinic Protocol.



PART - 7

Learning Curve

LEARNING CURVE

As Embryo biopsy involves multiple steps like assisted hatching, biopsy , tubing...Each step is crutial for the results. The long learning curve for the embryologist in PGT, includes perfection in embryo biopsy techniques without harming the embryo and with good take home baby rate.

It is recommended that an experienced embryologist (i.e. general embryology and micromanipulation of embryos) performs the biopsy procedure after appropriate training [33] and follow standard operating procedures. Deviations to SOPs and protocols should be documented. 1.3. Training for biopsy personnel should be documented. It is recommended that at least 100 oocytes/embryos are successfully biopsied prior to clinical work resulting in the removal of 90% intact cells. Training for biopsy should be at least to the standard required for certification in routine embryology. It is essential to ensure that an adequate labelling system is used to identify the cell number and the oocyte/embryo from which it was biopsied and it is critical that all stages have appropriate and recorded witnessing. This must include documented matching of the cell and oocyte/embryo after biopsy, of the cell and slide/tube during preparation and finally of the embryos recommended for transfer on the PGD report prior to embryo transfer.

PART - 7
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Organised by

INDIAN FERTILITY SOCIETY

15th Annual Congress of Indian Fertility Society

FERTIVISION 2019

6-8 December

The Leela Ambience Hotel, Gurugram
New Delhi | India

Theme: Beyond Tomorrow

First Announcement

www.fertivision2019.com

Invitation

Dear Friends, Welcome to *FERTIVISION 2019*

On behalf of the Indian Fertility Society (IFS), we are extremely pleased to announce and cordially invite you to the much awaited academic event – the **15th National Annual Conference - Fertivision 2019**, to be held on **6th, 7th & 8th December 2019** at Hotel **The Leela Ambience, Gurugram, New Delhi / NCR, India**.

This conference is aimed to provide the most comprehensive academic platform in the field of Infertility and Assisted Reproductive Technology (ART)" befitting the theme of the meeting "Beyond Tomorrow"

Renowned and leading expert faculty from around the world would gather and deliver talks in our cutting edge scientific program which will not only enrich your current knowledge and clear all doubts faced in day-to-day clinical practice, but will also enlighten you about the latest innovations and ongoing research.

A large number of renowned international faculties have already confirmed their participations till date. The pre-congress workshops on 6th December are specially designed for informal in-depth training with hands on sessions on simulators and live, where ever feasible. There will be 4 simultaneous running streams on 7th & 8th December covering a wide variety of topics, enabling you to choose the deliberations specific to your area of interest and clinical practice. We are having a dedicated hall for the esteemed embryologist friends.

The best oral and poster presenters under various categories and the quiz winners will be honored with special awards and prizes. Do join us in large numbers and update your knowledge with most updated current standards in clinical practice, as well as get inspired to innovate further to overcome remaining enigmatic issues!

The three days of scientific program will encompass didactic lectures, keynote presentations, panel discussions and orations. There will be 9 Pre-conference workshops based on Ovulation Induction, Ultrasound, Andrology, Embryology, Hands on Embryo Transfer, Ovum Pickup and PGS and more. These workshops will be in addition to the special state of the art workshops by the faculty from IFFS and ESHRE. We expect delegates across India, Sri lanka, Bangladesh, Nepal, Middle - East Countries and African Nations and the arrangements are being made to accommodate more than 2500 delegates.

The exhibition area will be one of the highlights of the conference. Exhibiting provides tremendous benefits to both participating industry and the society. Tea, coffee and lunch will be served confluent with the trade area to allow optimal interaction between the trade companies and delegates during beverage and lunch breaks.

We invite you to participate in the Fertivision 2019 and exchange your expertise with more than 2500 specialists in the field of Assisted Reproduction.

We look forward to your active participation and suggestions for successful conduct of the conference.

With Our Best Regards

Dr. M Gouri Devi
Organizing Chairperson
FERTIVISION 2019

Dr. Pankaj Talwar
Organizing Secretary
FERTIVISION 2019

and All Executive Committee of Current IFS team

Scientific Highlights

1. Fertivision 2019 Would be One of the Most Comprehensive Coverage on "Best Practices, Innovations and Progress in the Field of Infertility and ART" Being Conducted in India.
2. We Promise You Cutting Edge Academic Deliberations Delivered by Leading Renowned Expert Faculty from Around the world befitting the Theme of the Meeting "Beyond tomorrow"
3. In the Conference There Would Be 4 Simultaneous Halls Running with Legendary Faculty in Lead Interacting With You, Covering a Wide Variety of Topics, and Enabling You to Tailor the Program Especially to Your Area of Interest and Clinical Practice. We are Having a Dedicated Hall for the Esteemed Embryologist Friends.
4. Along With the Main Conference We are Having 9 Pre -Congress Workshops on 6th Dec 2019 Pertaining to the Burning Issue in ART.
5. We Welcome You and Offer You This Opportunity to Showcase Your Research Work on a Prestigious National Platform and Enhance Your CV.
6. Scientific Quiz for sharp talented young minds with primary rounds conducted by various IFS Chapters across the country and abroad
7. Several Prizes and Awards for Best Paper, Poster and Quiz winners
8. Enjoy the Evenings With Exciting Social and Cultural Program
9. Sightseeing Tours in and Around Delhi Organized Professionally by Leading Event Management Teams.
10. A Great Opportunity for All of Us to Amalgamate the Most Updated Current Standards in Our Clinical Practice and Look Beyond Tomorrow

Limited Seats

Choose from 10 Pre Conference Workshops | 6 December

1)	*IFFS Workshop on Do's and Don'ts in Ovarian Stimulation*
2)	*Reproductive Surgery*
3)	*Ultrasonography Imaging In Infertility*
4)	*Andrology & Semenology*
5)	*Ovum Pickup and Embryo Transfer (With Simulators)*
6)	*Cryobiology*
7)	*Total Quality Management*
	Pre Lunch Workshop (0900 - 1300 Hrs)
8 A)	*Holistic Medicine and Patient Counselling*
	Post Lunch Workshop (1400 - 1700 Hrs)
8 B)	*Publish or Perish*
9)	*PGT and Genomics*

Early Bird Reg. Ends 1st September

Registration Details

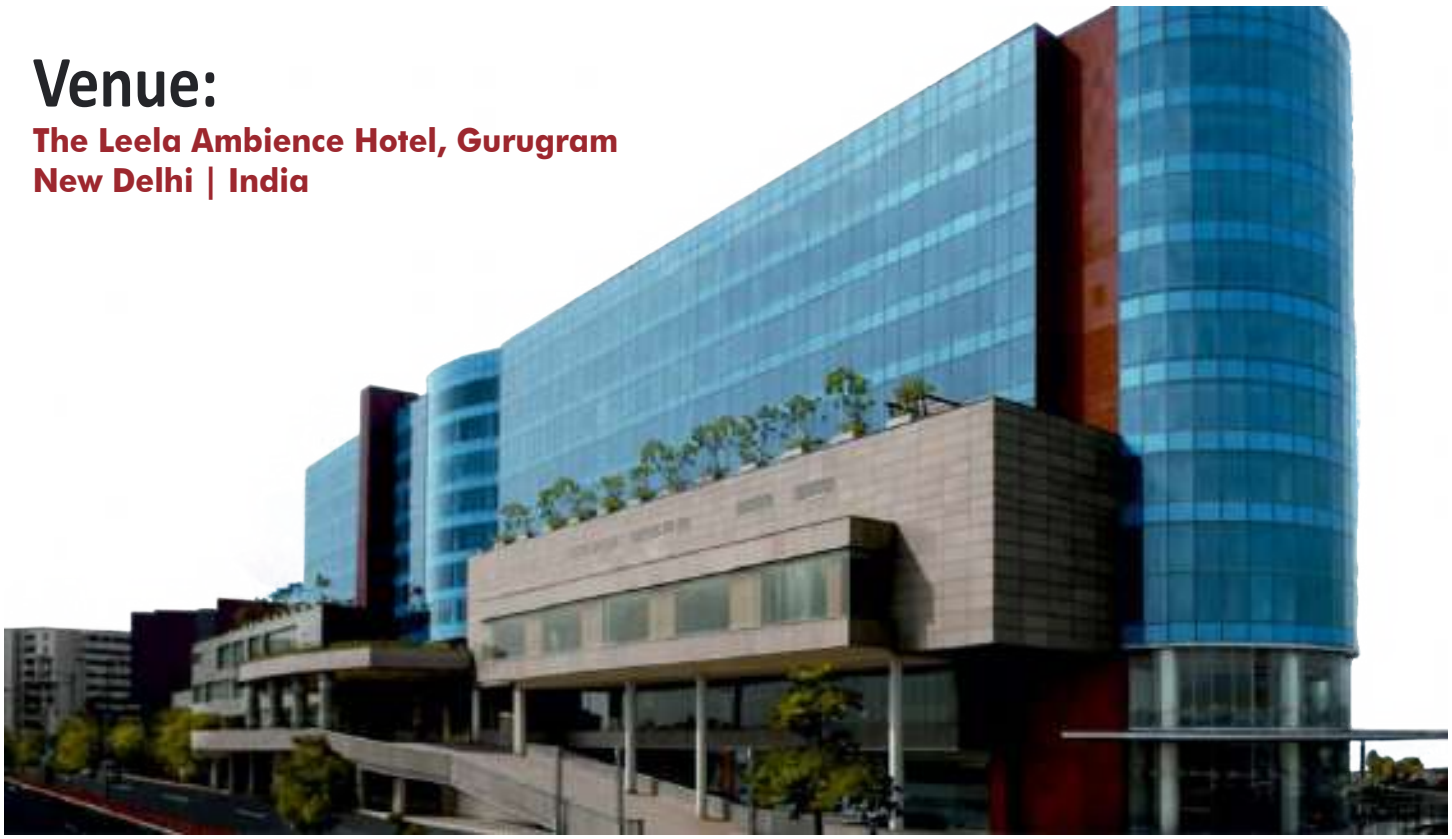
Category	Early Bird Fees Till 1st September 2019		Regular Fees Till 15th October 2019		Onspot	
IFS Member	INR 10500		INR 12500		INR 14500	
Non IFS Member	INR 12500		INR 14500		INR 16500	
Conference Registration plus Life Time IFS Membership	Embryologist	INR 14500	Embryologist	INR 16500	Embryologist	INR 18500
	Gynaecologist	INR 15500	Gynaecologist	INR 17500	Gynaecologist	INR 19500
PG Students (No Dinner)	INR 6000		INR 7000		INR 8000	
Accompanying Person	INR 10500		INR 11500		INR 12500	
Foreign Delegates	$ 350		$ 400		$ 500	

Inclusive of 18% GST

Register at www.fertivision2019.com

Venue:

The Leela Ambience Hotel, Gurugram
New Delhi | India



The Leela Ambience Hotel & Residences is located on the fringe of the Gurgaon Central Business District, fifteen minutes from Delhi's International Airport and thirty minutes from Central Delhi.

In addition to 322 contemporary and world class five star deluxe rooms and suites, The Leela Ambience Gurgaon also features 90 residencies (with one, two and three bedroom) fully serviced luxury Residences.

Multi Award winning restaurants include; Multi-cuisine all day dining– Spectra, Italian – Zanotta, cuicina Italiana, North Indian – Diya and whisky bar- Rubicon. The "Royal Club" is located on the 6th floor of the Hotel. The Royal Club features are 24 hour butler service, with evening cocktails and a Boardroom.

The 27,000 square feet, beautifully finished convention facilities, meeting and boardrooms were recently awarded the prestigious 5-Star deluxe "Best Luxury Hotel and Conference Centre – India".

This venue has been chosen with a lot of care and thought keeping in mind the comfort and also enjoyment of the delegates when visiting Delhi.

Conference Secretariat

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15th Annual Congress of Indian Fertility Society

FERTIVISION 2019

6-8 December The Leela Ambience Hotel Gurugram, New Delhi, NCR | India

Organised by IFS Indian Fertility Society

Registration Form

Title Prof/ Dr/ Mr/ Ms _____ Gender : Male ☐ Female ☐

First Name _____ Last Name _____

Institution _____ IFS Member No. _____

Correspondence Address _____

_____

City _____ Pin Code _____ State _____

Mobile No. _____ Email _____

(All the above fields are mandatory)

Limited Seats

Choose from 9 Pre Conference Workshops | 6 December — Choose Any 1 Workshop

1)	☐	*IFFS Workshop on Do's and Don'ts in Ovarian Stimulation*	7)	☐ *Total Quality Management*
2)	☐	*Reproductive Surgery*		*Pre Lunch Workshop (0900 - 1300 Hrs)*
3)	☐	*Ultrasonography Imaging In Infertility*	8 A)	☐ *Holistic Medicine and Patient Counselling*
4)	☐	*Andrology & Semenology*		*Post Lunch Workshop (1400 - 1700 Hrs)*
5)	☐	*Ovum Pickup and Embryo Transfer (With Simulators)*	8 B)	☐ *Publish or Perish*
6)	☐	*Cryobiology*	9)	☐ *PGT and Genomics*

Registration Fees

Inclusive of 18% GST

Please tick the appropriate checkbox

Category	Early Bird Fees Till 1st September 2019	Regular Fees Till 15th October	Onspot
IFS Member	INR 10500 ☐	INR 12500 ☐	INR 14500 ☐
Non IFS Member	INR 12500 ☐	INR 14500 ☐	INR 16500 ☐
Conference Registration plus Life Time IFS Membership	Embryologist INR 14500 ☐ Gynaecologist INR 15500 ☐	Embryologist INR 16500 ☐ Gynaecologist INR 17500 ☐	Embryologist INR 18500 ☐ Gynaecologist INR 19500 ☐
PG Students (No Dinner)	INR 6000 ☐	INR 7000 ☐	INR 8000 ☐
Accompanying Person	INR 10500 ☐	INR 11500 ☐	INR 12500 ☐
Foreign Delegates	$ 350 ☐	$ 400 ☐	$ 500 ☐

Inclusive of 18% GST

Conference Registration Fees Includes

- 18 Hrs of World Class Academic Program with Access to Best & Brightest International & National Faculty
- 3 Lunches and 6 Tea / Coffee Served During the Conference on 6, 7 & 8 December
- Banquet Dinner on 7 December
- Conference Kit (Including Bag, Badge, Notepad, Certificate & Pen)
- 1 Pre Conference Workshop
- Accompanying Person is Entitled for Food Coupons Only

Mode of Payment

1. **Bank Draft/Cheque - To be made in favor of "INDIAN FERTILITY SOCIETY"**
2. **Bank Transfer Details**
   IFS Account Name : Indian Fertility Society
   Account Number: 50562010067180
   IFSC Code : ORBC0100179
   Bank Name: Oriental Bank of Commerce
   Branch: Connaught place, New Delhi- 110001

Cancellation Policy

- Cancellation till 31st October, 2019 – 50% Refund.
- Cancellation from 1st November, 2019 – No Refund.
- All refunds will be made after the congress.

Cheque / Draft No. _____ **Total Amount** _____

Note: Kindly email us bank deposit slip / UTR number once you made the payment for our record.
Payment confirmation will take 7-10 working days post deposit of cheque, DD or RTGS

3. To Register online log on to www.fertivision2019.com

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