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REVIEW ARTICLE

Chromosomal Analysis of Pre-implantation Embryos: Its Place in Current IVF Practice

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Abstract

Background The intersection of ART and molecular genetic science is fast growing. It is now possible to utilize the advances in molecular genetics for clinical application to detect chromosomal aberrations in **preimplanting** embryos.
As molecular genetic techniques have improved, it is now possible to test the complete characterization of human genome variation with reasonable accuracy. In this article, we have tried to summarize the common current indications of chromosomal analysis of **preimplanting** embryos in couples having various chromosomal dominant or chromosomal recessive heritable disorders leading to the birth of a new born baby with chromosomal aberrations or leading to repeated miscarriage.
Conclusion The currently available techniques of embryo biopsy have their advantages and shortcomings. Today, preimplantation genetic testing to diagnose a euploid embryo is widely used in clinical practice in couples undergoing IVF ET treatment. By eliminating the transfer of aneuploid embryos, the pregnancy rate improves per embryo transfer and it shortens the time of conception from the start of IVF treatment. We have also discussed the current scenario of the place of PGT-A for routine use in IVF treatment procedure in view of the possible risk of losing euploid embryos due to the shortcoming of the embryo biopsy procedure.

Keywords Chromosomal analysis · PGT · Assisted reproductive technology · Embryo biopsy · Common indications

Introduction

In assisted reproductive technology (ART) procedures, preimplantation genetic diagnosis (PGD) was introduced in humans in the early 1990s. This was an alternative to prenatal diagnosis, to select genetically transferrable embryo in cases of couples having a history of known chromosomal abnormality in either or both partners [1]. Over the period, during 'In Vitro Fertilization' (IVF) treatment, became apparent that even in couples with normal genetic profile, a major proportion of the embryos created in vitro may be genetically incompetent [2]. This knowhow expanded the scope of chromosomal analysis to select euploid embryos for embryo transfer to initiate an early pregnancy by selecting the most appropriate embryo [3].

Historically, first animal experiments of chromosomal analysis through embryo biopsies were performed by German scientist F Seidel in 1952 [4]. First PGD was reported by Edwards and Gardner in 1968 with rabbit blastocysts using 1% acetoorcein with the intention of sex determination [5]. This concept was successfully applied to humans in the form of blastomere biopsy by Leeanda Wilton in 1989 [6] and in the form of trophectoderm biopsies by Audrey Muggleton-Harris [7].

In 1993, extensive research carried out by Munne et al. which led to the foundation of 'Fluorescent In Situ Hybridization' (FISH) technique [8]. However, this technique required probes for specific chromosomes to be analyzed. The limited availability of probes posed limitations on aberrations that could be detected in evaluating a vast number of chromosomes which need to be analyzed. In 1999,

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Comparative Genomic Hybridization (CGH) technology was introduced using blastomeres from cleavage stage human embryos to check for aneuploidies of all chromosomes [9].

Still there remained many situations where chromosomal variations could not be detected through available techniques. These shortcomings were overcome with NGS technology which has been validated and is now clinically applied to detect partial or segmental aneuploidies, chromosomal aberrations including imbalanced translocations, inversion, deletion, duplication insertion and mosaicism, triploidy and single gene disorder which are often responsible for infertility and can be associated with spontaneous abortions and fetal malformations or diseases.

The complete procedure of chromosomal analysis using embryonic cells is distinctly divided into two parts. 1- Obtaining cells from embryos through biopsy and 2- analyzing their genetic content for assessing chromosomal competency.

Because of the ongoing research in the field of molecular genetics, it is now possible to utilize this technology in clinical applications [10].

Indications of Chromosome Analysis in ART

In 2017, 'International Glossary for Infertility & fertility care' introduced new terminology of Pre-implantation genetic testing (PGT) which is a more expansive term.

Under the PGT umbrella, there are 3 categories-

1. PGT for monogenic/single gene defects (PGT-M).
2. PGT for chromosomal structural rearrangements (PGT-SR).
3. PGT for Aneuploidies (PGT-A).Currently, PGT-A is the most commonly used technique followed by PGT-M for specific circumstances.

Pre-Implantation Genetic Test for Single Gene Disorder (PGT-M) in ART.

PGT–M testing is indicated whenever the chromosomal location of a gene causing a disorder is known, namely.

- Couples with a family history of X-linked disorders (25% risk of having an affected embryo [half of the male embryos].)
- Carriers of autosomal recessive diseases (the risk an embryo may be affected is 25%).
- Carriers of autosomal dominant diseases (the risk an embryo may be affected is 50%).

More than 600 different disorders can be detected by this methodology. Following are the common clinical conditions.

Identifying Human Leukocyte Antigen—Compatible Embryos for Stem Cell Transplantation Indication

PGT-M will allow obtaining embryo whose cord blood at birth can be used for stem cell transplantation to β thalassemia major sibling or sibling with somatic cell origin of leukemia [11].

Adult-Onset Heritable Disorder

Heritable cancer, cardiac disorders and neurodegenerative disorders can be prevented by PGT-M.

Breast Cancer

Seventy-two percentage of women with pathogenic BRACA1 mutation, especially 187 del NG will manifest neoplasm by age 80 years, and 69% in BRACA2, especially those women with 617 delT mutation will get neoplasm at the same age [12].

Autosomal Dominant Conditions

Multiple endocrine neoplasias (MEN) familial adenomatous polyposis and retinoblastoma can also be detected and prevented.

Heritable cardiac disorders Marfan syndrome, hypertrophic cardiac myopathy and hypertrophic cardiomyopathy [13].

Heritable neurodegenerative disorder Huntington disease, early-onset (less than 50 years) of Alzheimer syndrome.

Before PGT-M, the options for couples having a risk of transmitting a genetic disease were prenatal diagnosis either by amniocentesis or chorion villus biopsy, reproductive roulette, i.e., taking a chance without undergoing any diagnostic evaluations, gamete donation, adoption or remain childless. The main advantage of PGT-M is it avoids most difficult decision of termination of pregnancy in affected couples.

Pre-implantation Genetic Testing For Structural Chromosomal Abnormalities (PGT-SR)

In a couple where one partner has balanced translocation, there is an increased risk for unbalanced gametes. This causes a high proportion of abnormal gametes due to meiotic segregation. Only 10–20% of tested embryos are normal in such a couple. In absence of infertility, the

cumulative chance of normal offspring is 65–75%, similar to the couple without translocation. But, the meantime to achieve a natural live born pregnancy is about 5 years in translocation couples. PGT-SR is advisable if the couple has balanced translocation and is of advanced age in order to shorten the time of conception.

Balanced translocation can lead to repeated implantation failure or recurrent pregnancy loss. PGT-SR is also indicated in such couples [14].

Pre-implantation Genetic Tests for Aneuploidy (PGT-A)

Currently, PGT-A is widely used to improve the pregnancy rate in IVF ET cycle by identifying euploid embryo for transfer [15]. By transferring euploid embryo, the IVF success rate/ovum pickup is not increased but the pregnancy rate per embryo transfer increases. This shortens the time taken for the patient to become pregnant and may help reduce the dropout rate during IVF treatment as shown by BEST trial [16].

PGT-A allows the transfer of single euploid embryo without compromising pregnancy rate, thus reducing multiple pregnancy rates and its complications following ART treatment.

IVF and Embryo Selection

The original equation that determined the ART success rate, namely the number of follicles stimulated to obtain many embryos for transfer, has now changed. For many years, the quality of the embryo was assessed based on only its morphological appearance and its growth pattern. Transfer of many embryos per cycle would then result in multiple pregnancies which were associated with increased maternal and perinatal morbidity and perinatal mortality. Currently, we prefer to transfer fewer embryos (preferably single embryo). Unfortunately, not all embryos produced in the laboratory have successful development through cleavage stage and give live births. Optimal development to blastocyst stage allows the elimination of cleavage arrested embryos and gives a better pregnancy rate allowing the transfer of single blastocyst. The availability of extended culture medium and better culture conditions, as well as improved embryo freezing technology, have helped embryologist to select morphological superior embryo for transfer. Unfortunately, morphological appearance of embryo neither through its static nor through its dynamic state can diagnose chromosomal contents of embryo [17].

IVF and Aneuploidy

The high incidence of chromosome aneuploidy in human gametes and embryos is a major cause of IVF failure. Most aneuploidies arise in maternal meiosis which increases with woman's age more so after 35 years. A rapid decline in the IVF success rate is therefore noted in women with advanced maternal age. SART data of 2016 showed cumulative birth rate per ovum pick up decreases from 54.5% in young patient to 13.4% in women of 41–42 years, and similarly increase in aneuploidy is noticed from 30 to 50% in women under 35 years to 80% in women 42 years or older [18].

The recent introduction of next-generation sequence (NGS)-based methods have increased sensitivity and reduction of copy number variation genome-wide to diagnose euploid embryo for transfer in IVF cycle.

Is PGT-A Indicated for Every IVF Cycle?

STAR study by Munne et al. in 2017 from 34 IVF centers showed that PGT only improved pregnancy rate in elderly patients above 35 years [19].

Similarly, SART data 2015 showed that the implantation rate was 50% for PGT-A irrespective of maternal age, but pregnancy rates decreased without PGT-A after 35 years [20]. It is also suggested that PGT-A does not help young patients because some IVF centers may be losing euploid embryo during biopsy procedure due to current limitations of PGT-A.

Technical Aspects of Embryo Biopsy

Three types of cells can be used for genetic screening. Polar body, blastomere and trophectoderm

Polar Body Biopsy

Polar body (PB) is a by-product of Meiosis that does not have any role as such in embryogenesis. PB provides information of the maternal chromosomal structure (Fig. 1).

Blastomere Biopsy

Still, the most common approach for PGD/PGS is to biopsy a single or occasionally two blastomeres from day three embryos before the compaction begins. It allows the detection of maternal, paternal and early post-fertilization defects.

It also gives enough time for the genetic diagnosis if the biopsied embryo is to be transferred on day 5 (Fig. 2).

Blastocyst Biopsy

Blastocyst formation begins on day 5 of post-ovum pickup. While selecting for biopsy, it should have distinct inner cell mass and a healthy, well-defined trophectoderm. The removal of trophectoderm is technically a more challenging procedure than a polar body or blastomere biopsy.

Hatching blastocyst is the most preferred stage for biopsy for two reasons. (1) It makes the procedure easier due to already protruding trophectoderm, and (2) it reduces the probability of monozygotic twinning as we do not create an additional hatching site other than blastocyst's natural preference (Fig. 3). However, a biopsy can also be performed on expanded, non-hatching blastocyst (Fig. 3).

For the biopsy of an expanded, un-hatched blastocyst, the already thin zona pellucida is ruptured using tiny laser shots, and 4–6 cells are removed from the trophectoderm using a fine biopsy pipette. The inner cell mass is left undisturbed.

Irrespective of the stage of embryo, it is necessary to retain the intact nucleus in the aspirated cell to get proper results. If the nuclear membrane gets disintegrated, the chromosomal content will be lost and geneticists will not be able to give dependable results (Table 1).

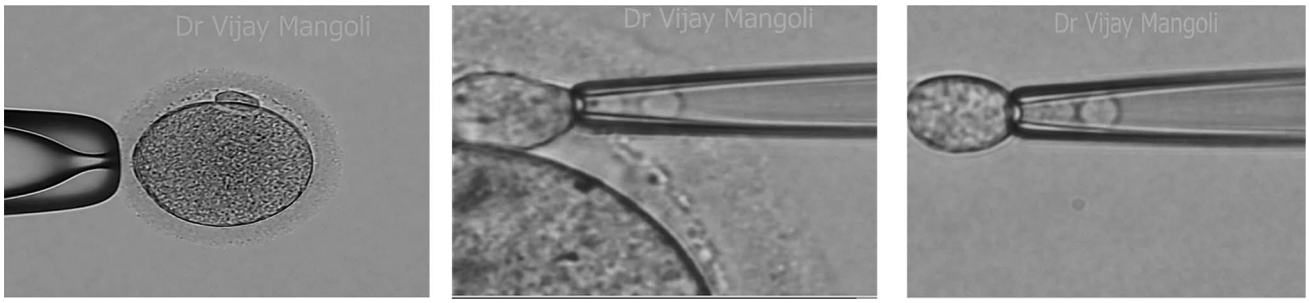


Fig.1 Polar body biopsy

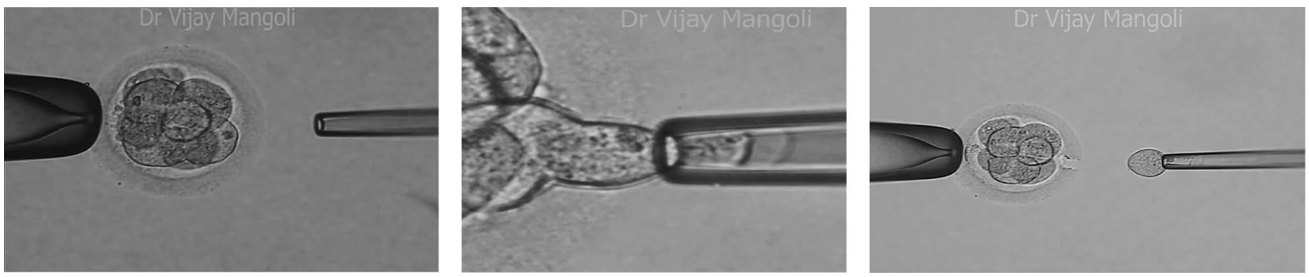


Fig.2 Blastomere biopsy

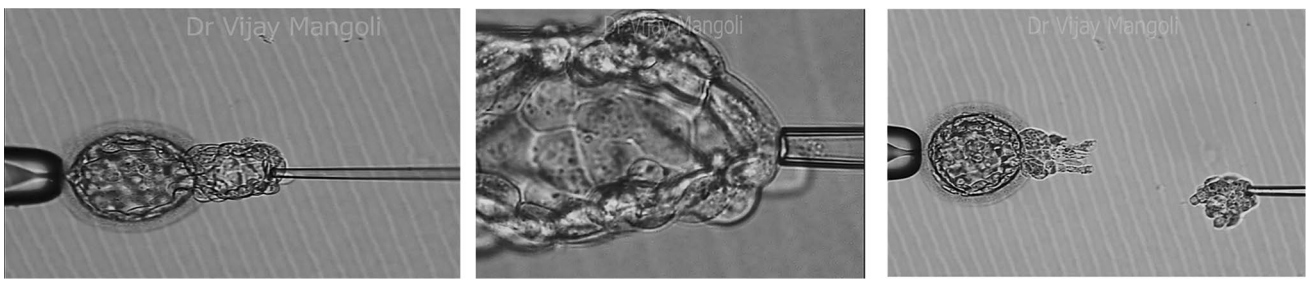


Fig.3 Blastocyst biopsy

Table 1 Stages of embryo biopsy (EB) and their limitations

Stage of EB	Limitations
Polar body biopsy	It does not provide any information regarding the chromosomal constitution of the embryo. Hence used in only rare cases of X linked disorders
Blastomere biopsy	The acquired blastomere may not represent the entire embryo; hence, interpretation will be cell dependent in mosaicism Ploidy status may change between D3 and D5. Possibility of discarding eventual euploid embryo based on day three analyses exists
Blastocyst biopsy	Cells from the trophectoderm are not representative of the developing embryo (inner cell mass). Mosaic pattern may misinterpret results. Centre should have a very efficient vitrification program as biopsy results may take up to 48 h

Mosaicism

This is one of the most significant limitations in accepting PGT-A as a routine and dependable application to assess the chromosomal anomaly of a **pre-implantation** embryo. Mosaicism is a condition of possessing cells of two or more different genetic constitutions. Mosaic embryo (ME) has both normal and abnormal cells in it. In humans, up to 80% of the embryos produced in vitro can be mosaic [21].

As presence of abnormal cells in a blastocyst has unpredictable distribution patterns, to locate them precisely at every biopsy is practically difficult to achieve.

As illustrated in Table 2, mosaic cells may get distributed as part of trophectoderm or inner cell mass. Depending upon pattern of mosaic cell distribution, during biopsy procedure, there can be nine various probabilities to extract normal or mosaic cells from euploid and aneuploid embryos. As during biopsy it is not possible to differentiate between the two types of cells, the geneticist will interpret results based on type of cells provided to him. It is scary to see that seven out of nine combinations of embryo biopsy may lead to incorrect interpretations. Currently, as per ASRM guidelines, embryos with more than 20% mosaicism in embryos cannot be considered for transfer [22].

Several studies have reported live births following mosaic blastocyst transfer, and resulting newborns appear to be healthy. These data should be interpreted with caution because there is a lack of accompanying postnatal correlation of the chromosomal studies [23, 24].

As blastocyst biopsy involves removal of few cells as representation from TE, practically, there can be nine combinations in which cells can be removed and seven of the nine results can be misleading as far as the euploid status of a blastocyst is concerned. Currently, this is unavoidable. Therefore, it is necessary for the clinician to understand possible circumstances and counsel the patient accordingly.

Self-repair mechanism

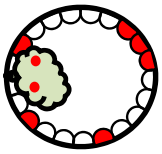
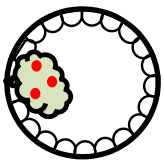
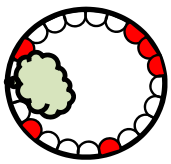
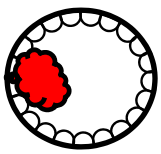
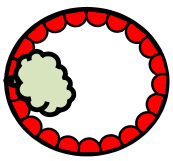
Dr. Bolton induced aneuploidy in mouse embryos using drug 'reversine' and observed the growth pattern of these embryos [25]. They observed aneuploid cells in the fetal lineage are eliminated by apoptosis, whereas those in the placental lineage show severe proliferative defects. Mosaic embryos have full developmental potential, provided they contain sufficient euploid cells in animal models. Even in humans, the birth of normal babies is reported after transferring day three aneuploid embryos when no other option was available and the patient gave consent for the transfer.

To summarize, following are some of the shortcomings of current PGT-A for improving pregnancy rate in IVF [26].

1. Expertise required for embryo biopsy may not be available at all centers. With this result, loss of normal euploid embryos can occur due to damage during the embryo biopsy procedure.
2. Euploid embryo may be lost because it may not reach blastocyst stage during long-term culture required for embryo biopsy especially when embryos formed are few.
3. Non-reliability of the genetic laboratory in diagnosing of mosaicism.
4. Occasionally unable to get sufficient DNA from the biopsied cells for chromosomal analysis.
5. No standardization of genetic laboratories performing chromosomal analysis of embryos [27].
6. Increased cost.

Due to shortcomings of PGT procedure, the current indication for PGT-A to improve pregnancy rate in IVF treatment is limited only when there is increased risk of aneuploidy namely in elderly women, H/o. repeated IVF failure, H/o. repeated miscarriage and severe male factor of infertility [28].

Table 2 Mosaicism patterns and PGT-A interpretations in blastocysts

Mosaic cell distribution patterns	Type of cells that can be obtained during biopsy	Diagnosis accuracy
 Both TE & ICM have few abnormal cells	Euploid	Misdiagnosis
	Mosaic	Correct Diagnosis
	Aneuploid	Misdiagnosis
 Only ICM has few abnormal cells.	Euploid	Misdiagnosis (Mosaicism goes unnoticed)
 Only TE has few abnormal cells	Euploid	Misdiagnosis
	Mosaic	Correct Diagnosis
	Aneuploid	Misdiagnosis
 Only ICM has only abnormal cells	Euploid	Misdiagnosis (Mosaicism goes unnoticed)
 Only TE has only abnormal cells	Aneuploid	Misdiagnosis (Mosaicism goes unnoticed)

Future prospects of PGT-A

Blastocentecis or removal of blastocoel fluid for chromosomal analysis is a new possibility being explored to replace invasive cell removal techniques which has shown comparable results [29].

Scientists are also trying niPGT-A (non-invasive PGT-A) to extract DNA from the medium droplet in which embryo is cultured till blastocyst stage [30]. Studies show that niPGT-A is less prone to errors associated with mosaicism and is more reliable than TE biopsy PGT-A. The concordance rate was found to be 84% with a false-positive rate of 8.6% and a false-negative rate of 2.5% [31].

Though more controlled uniform trials are needed to confirm the validity of such non-invasive techniques, with comparable outcomes, blastocentecis may eventually replace cell biopsies.

Summary

Chromosomal analysis of **pre-implantation** embryo has proven to be a great asset to many couples who are either carriers of a genetic disease condition or are facing recurrent implantation failure during ART treatment or when there is a H/o. recurrent miscarriage suspected to be due to chromosomal aberration.

With the advancement in molecular technology with targeted analysis and exclusion of aneuploid embryos for embryo transfer in ART treatment, the selection of best embryo for transfer to improve the pregnancy rate has become a feasible option.

The technical breakthrough in the field of vitrification and long-term culture media allows embryos to grow up to blastocyst stage this has helped embryologists to perform trophectoderm biopsy of an embryo without causing significant injuries to the embryo. Proper implementation of this technique in clinical IVF set up requires skilled molecular biologists, fully equipped standardized genetic laboratory and experienced embryologists who are well-trained in embryo biopsy procedure.

Active research is ongoing for non-invasive assessment of chromosomal content of embryo without embryo biopsy. With the fast advancing research in the field of assisted reproductive technology, a time has come when PGT-A may be routinely used by clinical IVF centers to improve pregnancy rate.

Compliance with ethical standards

Conflict of interest Authors have no conflict of interest.

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