HUMAN GENETICS

CLINIC . LABORATORY . COUNSELLING

AT

SAMAD IVF HOSPITALS

INFERTILITY/ART-IVF/GENETICS
ATTINGAL-TRIVADNRUM-KOLLAM-KOCHI

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GENETICS SERVICES AT SAMAD IVF HOSPITALS

1. INTRODUCTION:

Genetics, the science of heredity and variation in living organisms is playing an important role in the practice of clinical medicine. Medical genetics involves any application of genetics to medical practice. It thus includes studies of the inheritance of diseases in families, the mapping of disease genes to specific locations on chromosomes, analysis of the molecular mechanisms through which genes cause disease, and the diagnosis and treatment of genetic disease.

The Human Genome project (HGP) an international research programme begun in 1991with the primary goal of mapping all the genes to specific chromosomes and completed in the year 2005 and has now become a central focus in the field of medical genetics.

Genomics is the study of an organism's entire genome, which offers a unique opportunity of scanning the entire genome to understand the role of genetic factors in health and disease for its prevention, diagnosis and management. **Proteomics** is the study of proteins, produced inside the body under the influence of genes. Metabolomics is the systematic study of the unique chemical fingerprints that specific cellular processes leave behind.

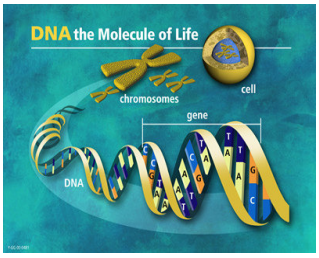
In recent years, an increasing number of genetic factors have been implicated in the development and function of male and female reproductive system. One of the important area of focus in medical genetics is infertility which is a major health problem affecting couples. There is an incidence of genetic disorders in 2-3% neonates, 50-60% pregnancy loss, 11% in mental retardation and many more.

2. HUMAN CELL:

Each human progress from a single cell Zygote to a complex organism containing trillions of cells. Zygote is formed by fertilization between 2 haploid cells – an ova from female and a sperm from male, which combines to form single diploid cell which contains DNA derived from both mother and father and this provides all the genetic information. Because few cells last for an individual's lifetime, new one must be generated continuously to replace those that die. Two types of cell division occur in humans namely mitosis, which occurs in somatic cells and give rise to diploid cells and meiosis, which occurs in germ cells or the sex cells and results in haploid cells.

Genes control the growth and function of cells in our body which is apolymer of deoxyribonucleotides (DNA) - basic unit of heredity composed of nitrogenous bases (A-Adenine, T-Thymine, C-Cytosine & G-Suanine) sugar and phosphate. Human cells have 23 pairs of nuclear chromosomes, giving a total of 46 per cell. The totoal length of DNA of all the 46 human chromosomes comes to about 2 meter consisiting of 3 billion base pairs

Males have 44 autosomes and two sex chromosomes X and Y. Females have 44 autosomes and two sex chromosomes XX. People have two copies of each gene, one copy inherited from the mother and the other copy inherited from the father. So the proper functioning of genes is vital to every process of living. Therefore, it is not surprising that changes in genes can sometimes lead to disease



3. DISORDERS:

Disorders are classified into:

A. Genetic Disorders

1. Chromosomal disorders
   Eg. Trisomies of chromosomes 13, 18 and 21
2. Single gene disorders
   Eg. Cystic fibrosis
3. Polygenic disorders
   Eg. Cancer
4. Mitochondrial DNA disorders
   Eg. Myoclonic Epilepsy and Ragged Red Fibre disease- MERRF

B. Epigenetic Disorders

Non-gene factors or epigenetic factors are features within the cell that can be inherited when cells divide but they don't change the genes themselves.

Among the several types of genetic disorders, incidence of chromosomal aberrations is observed more commonly and is responsible for a significant proportion of genetic diseases, occurring in approximately 1 in 150 live births. They are the leading known cause of both mental retardation and pregnancy loss. Chromosome abnormalities are seen in 50% and 20% of first and second trimester spontaneous abortions, respectively.

Recent technologies enable to diagnose genetic disorders at the following levels:

1. Preconception [Gamete level for sperms and oocytes]
2. Pre implantation [Embryo]
3. Prenatal [product of conception / foetal cells]
4. Postnatal / adults [cells]

These detections finally provide various reproductive options to infertile couples and thus increase the healthy take home baby rates.

INDICATION FOR CYTOGENETIC STUDIES

Infants/Children/Adults

A. Ambiguous genetalia
B. abnormal growth, developmental delay
C. Deafness / blind

D. Dysmorphic features like cleft palate / lip, low set ears, bulbous nose
E. Primary or secondary amenorrhoea
F. Metabolic disorders
G. Congenital abnormalities

Preconception and prenatal

A. Consanguinity
B. Infertility
C. Single gene disorders (Hemophilia, Thalassemia, DMD)
D. Miscarriages
E. Positive triple test
F. Abnormal ultrasound findinsg (Nuchal cord Translucency, choroid plexus cyst)
G. Advanced maternal age

a. CYTOGENETICS TESTS: KARYOTYPING;

Cytogenetics tests involve the determination of chromosomal abnormalities including many structural and numerical abnormalities. Cytogenetic tests can be carried out prenatally and postnatally.

Chromosome are separated from cells, stained and arranges in order from largest to smallest so that their number and structure can be studied under a microscope and hence determined chromosome abnormalities. It can be carried out in various smaples – peripheral blood, amniotic fluid, bone marrow, cord blood etc

Karyotype of Peripheral blood

Samples: Approximately 5 ml of peripheral blood is collected in a sterile lab condition in a green top sodium heparin vacutainer and mixed well.

Storage**:** preferably for one day after storing at 4°C.

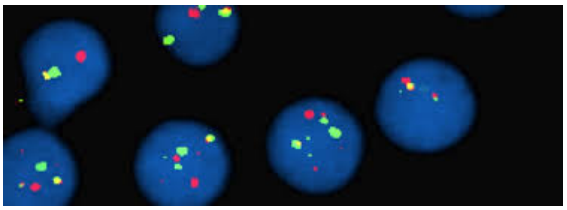
Reporting Time: 7-10 days

Sensitivity and Specificity of the Test**:** The sensitivity of all the cytogenetic tests depends on the quantity and quality of metaphases obtained and also the banding patterns. Metaphase chromosomes are obtained by the routine classical cytogenetic procedure which includes culturing and harvesting the cells at the metaphase stage.

Some chromosomal abnormalities remain undetectable as limited number of metaphases is normally scanned to generate a karyotyping report. The specificity of Cytogenetics tests is also limited to disease detections at the chromosome level as it can only determine chromosomal disorders (numerical and structural). It cannot detect abnormalities at the gene level.

b. MOLECULAR CYTOGENETICS TESTS: Fluorescent *In Situ* Hybridization (FISH):

A technique used to identify the presence of specific chromosomes or chromosomal regions in interphase or metaphase stage of cell division through hybridization of fluorescently labeled probes to denatured chromosomal DNA.



FISH Techniques:

1. **Metaphase FISH:** culturing of peripheral blood cells same as in routine cytogenetics protocol then subjecting the metaphase chromosomal preparation to FISH.

   Diseases detected: Microdeletions – Di George syndrome, Williams syndrome

   Samples: 5 ml of peripheral blood is collected in sterile lab condition in a green top sodium heparin vacutainer and mixed well.

   Reporting Time: 5-7 days

2. **Interphase FISH**: long term culturing of cells is not required. Interphase FISH involves rapid detection.

   Disease detected: Trisomies (chromosomes13, 18, 21)
   Autosomal and Sex chromosomal aneuploidies.

   Samples: 8 ml of amniotic fluid (avoid blood contamination) are collected through amniocentesis at the 15-17 weeks of gestation under ultrasound guidance in a sterile heparin vacutainer.

   Reporting time: 3days.

   Storage and Transport: Preferably at room temperature.

   Precautions: Make sure the amniotic fluid after collection is immediately transferred from the disposable syringe to the sterile heparin vacutainer. A delay of up to 24 hrs does not affect the results, provided the samples are kept in a clean atmosphere and transported at room temperature. The sample should never be frozen.

   Indications
   - Advanced maternal age
   - Previous baby with a chromosomal abnormality.
   - Family H/O genetic defect.
   - Spontaneous abortions.
   - Consanguineous marriage.

3. **Sperm FISH:** carried out in fresh semen samples to detect the aneuplodies involved in the sex chromosomes.

Indications

- Men referred for ICSI.
- Males with abnormal sperm motility, count or morphology.

c. MOLECULAR GENETICS TESTS:

Molecular Cytogenetics techniques involve detection of genetic disorders at the gene level. The technique of detection of these disorders can be achieved by *Polymerase Chain Reactions (PCR) with Gel Documentation System and DNA Sequencing methods*

Molecular basis of male genetic disorders play a role in about 10% of male infertility, Human Y Chromosome consists of three azoospermic factor regions (AZFa, AZFb and AZFc).There are large number of genes in these regions and they exist in multiple copies.

Y chromosome microdeletion assay using RT-PCR, multiplex PCR and DNA sequencing techniques. The Y chromosome deletion detection system provides a rapid method for the detection of specific regions of the human Y chromosome.

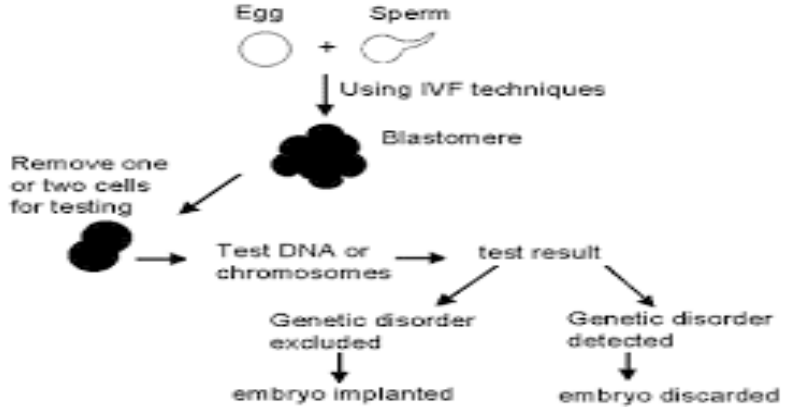
Indications

a. Men referred for ICSI
b. Males with abnormal sperm motility, count or morphology

d. PREIMPLANTATION GENETIC DIAGNOSIS (PGD):

The embryo selection for ET is done by morphology [embryo grading] & metabolism [physiology].By PGD, genetic selection is also applied for good quality.

The goal of every successful IVF Clinic is to increase the success rate of infertility treatment with the ART techniques with single normal embryo transfer (SET) to avoid multiple pregnancies and thus providing infertile couples with healthy babies.



The procedure which involves the removal of one or more nuclei from oocytes or embryos (blastomere – 8 cell stage) to test for mutations in gene sequence or aneuploidy prior to implantation. Therefore existing genetic problem will not be transferred to offspring and healthy children could be born. Preimplantation genetics is the latest development of genetic selection of embryos to identify chromosomal aberrations prior to embryo in couples that have opted for IVF.

Genetic test: FISH
Embryo handling: Cryopreservation or Blastocyst formation
ET: After FISH results

Indications:-

1. Couples carrying abnormal karyotypes
    1. Translocations
    2. Mosaicism
    3. Sex chromosome abnormalities
2. Recurrent implantation failure
    1. Recurrent pregnancy losses
    2. Inherited genetic syndromes

Advantages of PGD

- Increased chance of having a normal baby.
- If PGD becomes widespread, the incidence of many genetic diseases would be reduced.
- Since, PGD is 90% accurate, it is recommended that CVS or amniocentesis be performed with Karyotyping at the appropriate time for confirmation that the developing fetus has normal chromosomal make-up.
- If genetically embryos are transferred, the pregnancy rate increases and the miscarriage rate decreases. The chance of inducing a pregnancy termination following a CVS or amniocentesis is decreased.

e. PRENATAL SCREENING TESTS/DIAGNOSIS:

Prenatal diagnosis or prental screening is testing for diseses or conditions in a fetus or embryo before it is born. The aim is to detect birth defects such as neural tube defects, Down syndrome, chromosome abnormalities, genetic diseases etc.

Purpose of Prenatal diagnosis

- To enable timely medical or surgical treatment of a condition before or after birth
- To give the parents the chance to abort a fetus with the diagnosed condition
- To give parents the chance to prepare psychologically, socially, financially, and medically for a baby with a health problem or diability , or for the likelihood of a still birth

- To counsel the parents and avoid the recurrence of the disorder in the next day

Indications for prenatal diagnosis

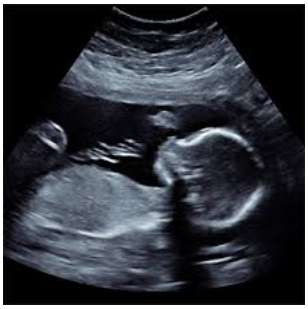
- Advanced maternal age
- Previous child with a chromosome abnormality
- Family history of a chromosome abnormality, single gene disorder, neural tube defect, other congenital structural abnormalities
- Abnormalities identified in pregnancy
- Other high risk factors (consanguinity, poor obstetrics, history, maternal illness)

Tests involved in prental diagnosis

- Invasive
  - Amniocentesis
  - Chorionic villus sampling
  - Cordocentesis
  - Premimplantation genetic diagnosis
  - fetoscopy
- Noninvasive
  - Maternal serum AFP
  - Maternal serums creen
  - Ultrasonogrpahy
  - Isolation of fetal cells from maternal circulation.

a. <u>Ultrasonography:</u>

Ultrasonography has become the most widely used form of fetal visualization and is used in the detection of many fetal malformations. Many ultrasound soft markers are associated with genetic abnormalities. Ex: presence of nuchal translucency thickness seen in trisomy 21 fetuses.



b. Maternal Blood estimation

1.α Feto Protein

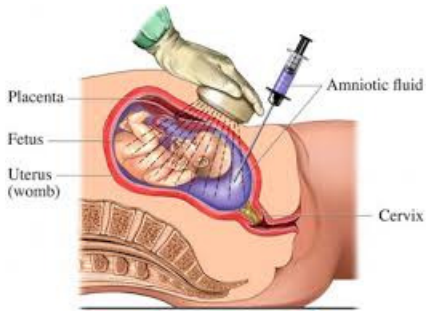
2.Estriol

3.Total HCG

4.PAPA-A

In the maternal blood estimation three tests are usually carried out to look for the levels of these hormones during pregnancy referred to as Triple test .

SAMPLING



1. Amniotic fluid

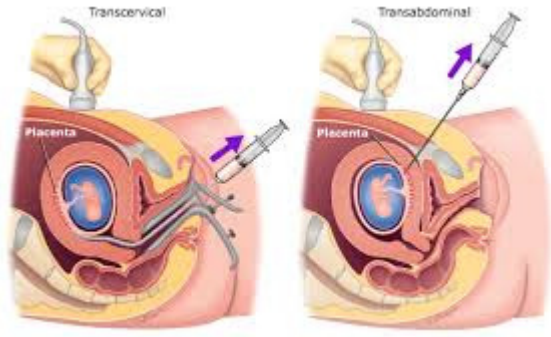
   Performed during 15-20 weeks of gestation

   Sampling:- 20 ml of amniotic fluid in sterile tube

2. Chorionic Villi

   Performed between 10-12 weeks of pregnancy

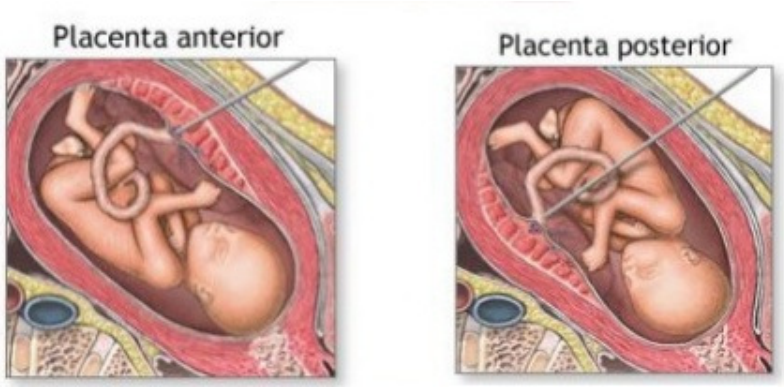
   1. Trans abdominal
   2. Trans cervical



3. Cord Blood

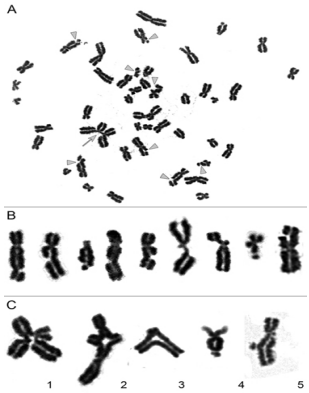
   Cord blood is drawn by visualization of the umbilical vessel by transabdominal ultrasound. The test generally done is17-20 weeks

Sampling: 2 ml of cord blood in sodium heparinised vial. Tests have to be done to avoid maternal blood contamination with the fetal blood.



f. GENOTOXICITY STUDIES

Cytogenetic studies used for detecting chromosome damage caused by a range of environmental and occupational calstogens, mutations and radiations. This includes sturucturla chromosome aberration.



1. Chromosome breakage studies
   Eg:- Fanconi's anaemia
2. Sister chromatid exchange
   Eg:- Bloom syndrome
3. Micronucleus tests

g. GENETIC COUNSELING PROGRAMME:

Genetic counseling programme helps to understand the consequences and nature of the disorder, the probability of developing or transmitting it, and the options open to them in management and family planning in order to prevent, avoid or ameliorate it. The complex process can be seen from diagnostic (the actual estimation of risk) and supportive aspects.

Receivers of Genetic Counseling

1. Couples affected with infertility
2. Pregnant Women
3. Persons, after the birth of a child with genetic condition

Importance of Genetic Counseling

1. To comprehend the medical facts; the diagnosis, probable course of the disorder, and the available management
2. To understand the alternatives to provide various reproductive options.

Team of Genetic Counselors:

1. Dr. Sathy M Pillai MD, Chief Medical Officer
2. Ms. Neethu M R, M Sc., Medical Geneticist
3. Ms. Sanjuna Rani D Y, M Sc., Medical Geneticist

5. TARIFF:

Sl.No.	Test Name	Nature of Sample	Tariff*
1	Karyotyping	Blood	2,000
2	Karyotyping	Amniotic Fluid	2,500
3	FISH for Chromosome 13	Blood/ Amniotic Fluid	4,200
4	FISH for Chromosome 18	Blood/ Amniotic Fluid	4,200
5	FISH for Chromosome 21	Blood/ Amniotic Fluid	4,200
6	FISH for X/Y	Blood/ Amniotic Fluid	4,200
7	FISH for 13,18,21,XY	Amniotic Fluid	7,500
8	Molecular Detection of Y Microdeletion	Blood	3,500
9	Chromosomal breakage studies for Fanconi's anaemia	Blood	3,500
10	Di- George syndrome	Blood	4,000
11	Cri-Du-Chat syndrome	Blood	4,000
12	Prader-Willi/Angelman Syndrome	Blood	4,000

- Subjected to change

6. GLOSSARY:

AZF: Azoospermic factor namely three factors; associated with spematogenesis.
Anueploidy: The condition in which the number of chromosome is not a multiple of 23.
Autosome: 22 pairs of chromosome excluding the sex chromosome(X & Y)
Banding: The process of applying specific stains to chromosomes.
Base Pair: Two nitrogenous bases, one in each strand of double helical DNA, which interacts through Hydrogen bonds. The base pairs are adenine and thymine [A=T] and guanine and cytosine [G≡C].
CVS: Chorionic Villi Sampling; A prenatal diagnostic technique in which a small sample of chorionic villi is aspirated.

Chromosome: Thread like structure consisting of chromatin, Genes are arranged along the chromosomes.
Cell Culture: Culture of dispersed cells as monolayers or as suspension of cells.
Cryopreservation: Storage of cells, tissues or organs in liquid nitrogen at -196°C.
Diploid: Cells having 2 copies of each chromosome, in human the diploid number is 46.
DNA: Deoxyribo Nucleic Acid; a double stranded molecule. DNA is the genetic material of all the human beings.
DNA Sequencing: Determination of the base sequence of a DNA segment.
FISH: Fluorescent *In Situ* Hybridization; a molecular cytogenetic technique in which labeled probes are hybridized with chromosomes and then visualized under a florescent microscope.
Gel Documentation: Software enables visualization and comparison of electrophorised DNA sample according to bands .
Gene: Genes are the unit of hereditary. A segment of DNA which governs some trait.
Genetic Counseling: The delivery of information about genetic disorders [risks, natural history and management] to patients and their families.
Genome: The complete set of chromosomal genes and DNA present in an organism.
Genomics: Determination of the structure and function of the genome of an organism.
Gonosome: sex chromosomes (X & Y)
hCG: Human Chorionic Gonadotrophin.
Haploid: Cells having one copy of each chromosome.
HGP: Human Genome Project; a project to acquire knowledge of the organization, structure and function of the human genome .
ICSI: Intra Cytoplasmic Sperm Injection; ICSI is the most successful and viable technique in assisted fertilization for the treatment of male infertility.
Interphase: The state of the eukaryotic nucleus when it is not engaged in mitosis or meiosis, consist of G1, S & G2.
IVF: *In Vitro* Fertilization; union between oocyte and sperm outside the body of female, resulting embryo transferred into the uterus of female.
Metaphase: this is the mitotic stage at which the chromosomes are highly condensed and most easily visualized.
PCR: Polymerized Chain Reaction; a technique for amplifying a specific DNA sequence in the test sample.
RT-PCR: Real time PCR; this variation can be used to amplify RNA sequences into DNA duplexes by process known as Reverse transcription
Probe: Is labeled DNA or RNA used in a hybridization experiment, for gene identification.
PGD: Preimplantation Genetic Diagnosis for the screening of chromosomal abnormalities in preimplantation embryos in couples undergoing IVF-ET.
RNA: Ribo Nucleic Acid: a single stranded molecule, three basic types; messenger RNA, ribosomal RNA, transfer RNA.
SET: Single Embryo Transfer.
SRY: Sex Determining Region on the Y chromosome; SRY gene for growth and function of gonads.

Vacutainer: Heparin coated blood collection tubes.