Post-Graduate Degree Programme (CBCS)
in
ZOOLOGY

SEMESTER-IV

SOFT CORE THEORY PAPER

MEDICAL EMBRYOLOGY
ZDSE(MN)T-409

SELF LEARNING MATERIAL

DIRECTORATE OFOPEN AND DISTANCE LEARNING
UNIVERSITY OF KALYANI
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Director's Message

Satisfying the varied needs of distance learners, overcoming the obstacle of distance and reaching the unreached students are the threefold functions catered by Open and Distance Learning (ODL) systems. The onus lies on writers, editors, production professionals and other personnel involved in the process to overcome the challenges inherent to curriculum design and production of relevant Self Learning Materials (SLMs). At the University of Kalyani, a dedicated team under the able guidance of the Hon'ble Vice-Chancellorhas invested its best efforts, professionally and in keeping with the demands of Post Graduate CBCS Programmes in Distance Mode to devise a self-sufficient curriculum for each course offered by the Directorate of Open and Distance Learning (DODL), University of Kalyani.

Development of printed SLMs for students admitted to the DODL within a limited time to cater to the academic requirements of the Course as per standards set by Distance Education Bureau of the University Grants Commission, New Delhi, India under Open and Distance Mode UGC Regulations, 2020 had been our endeavour. We are happy to have achieved our goal.

Utmost care and precision have been ensured in the development of the SLMs, making them useful to the learners, besides avoiding errors as far as practicable. Further suggestions from the stakeholders in this would be welcome.

During the production-process of the SLMs, the team continuously received positive stimulations and feedback from Professor (Dr.) Amalendu Bhunia, Hon'ble Vice- Chancellor, University of Kalyani, who kindly accorded directions, encouragements and suggestions, offered constructive criticism to develop it within proper requirements. We gracefully, acknowledge his inspiration and guidance.

Sincere gratitude is due to the respective chairpersons as well as each and everymember of PGBOS (DODL), University of Kalyani. Heartfelt thanks are also due to the Course Writers-faculty members at the DODL, subject-experts serving at University Post Graduate departments and also to the authors and academicians whose academic contributions have enriched the SLMs. We humbly acknowledge their valuable academic contributions. I would especially like to convey gratitude to all other University dignitaries and personnel involved either at the conceptual or operational level of the DODL of University of Kalyani.

Their persistent and coordinated efforts have resulted in the compilation of comprehensive, learner-friendly, flexible texts that meet the curriculum requirements of the Post Graduate Programme through Distance Mode.

All the Self Learning Materials are self-writing and collected from e-book, journals and websites.

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Directorate of Open and Distance Learning
University of Kalyani

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SOFT CORE THEORY PAPER (ZDSE(MN)T -409)

MEDICAL EMBRYOLOGY

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	III	Genetic errors of human development- Down syndrome, Fragile X syndrome, Turner's Syndrome.		
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	V	Techniques used in Medical Embryology: i) Amniocentesis ii) Chorionic villus sampling iii) Ultrasonography		
	VI	Techniques used in Medical Embryology iv) DNA Finger printing v) Karyotyping		
	Total counselling session hrs.			

Unit-I

Medical implications: Infertility- Diagnostic infertility, causes of infertility

Objective: In this unit we will discuss different aspects of male and female infertility including causes of infertility and how it can be diagnosed.

Infertility:

Infertility is a term that is defined as the inability of couples to become pregnant after one year of regular intercourse without contraception, or 6 months if a woman is 35 years or older. Infertility can occur in both females and males, and there are many causes. In order to get pregnant, a woman's body must go through three main steps: ovulation, fertilization, and implantation. In ovulation, an egg is released from one of her ovaries, which then travels down to one of the fallopian tubes and into the uterus. The egg must then be fertilized by the sperm during the process of fertilization. Finally, in implantation, the fertilized egg implants itself in the uterine wall. When there are problems in any of these steps, infertility can be diagnosed.

- **Types of Infertility:** Infertility can be primary or secondary.

i. **Primary infertility** is when a couple has not conceived after trying for at least 12 months without using birth control

ii. **Secondary infertility** is when they have previously conceived but are no longer able to.

- **Causes of Infertility:**

The causes of infertility may be physical, congenital, disease, drug, immunological or even psychological. In India, when a couple is childless, the female is usually blamed. But more often, the males are detected to be responsible. However, now, specialized health care units known as infertility clinics are available. They could identify the cause of infertility and take up treatment to remove the disorder.

A. <u>Male Infertility:</u>

Infertility in males may be due to the following causes:

a. Azoospermia:

Absence of sperms in the semen is known as azoospermia. This may occur because of lack

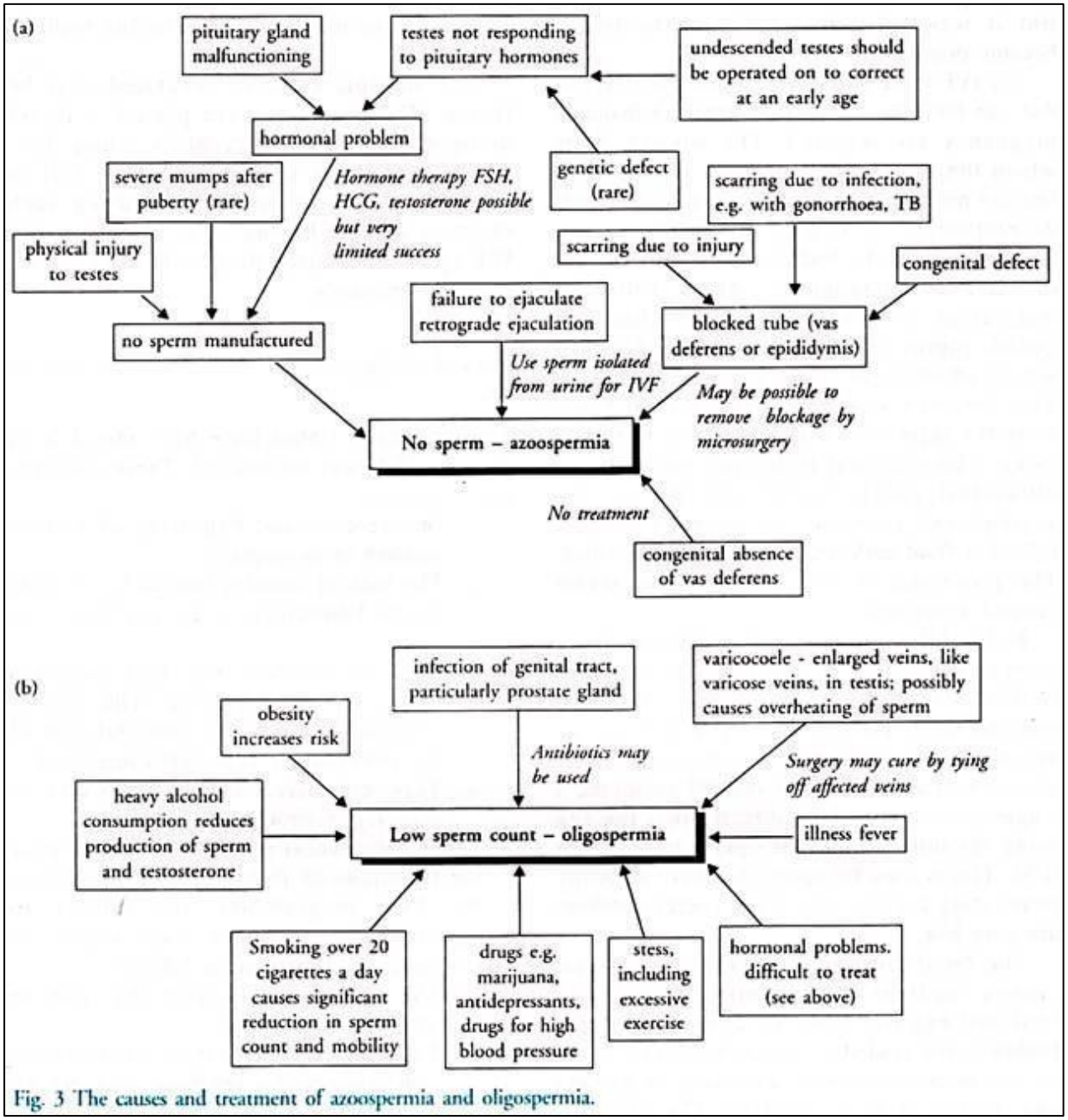


Fig. 3 The causes and treatment of azoospermia and oligospermia.

of sperm production or because of blocked tubes which does not permit the sperms to appear in the semen. Blockage can occur due to an infection or injury.

Failure of the ejaculation mechanism is another possible reason of azoospermia. Failure to produce sperms may result because of injury to the testes or as a result of infection such as mumps virus or due to hormonal reasons (Fig. 3).

b. Oligospermia:

Low sperm count is known as oligospermia. More than 90% males suffer from infertility due to low sperm count. The reasons of oligospermia are summarized in Fig. 3.

c. Abnormal Sperms:

Abnormal sperms may possess two heads, or no tail or may have abnormal shapes (Fig. 4). The

reasons are not known and may be because of hormonal malfunctions.

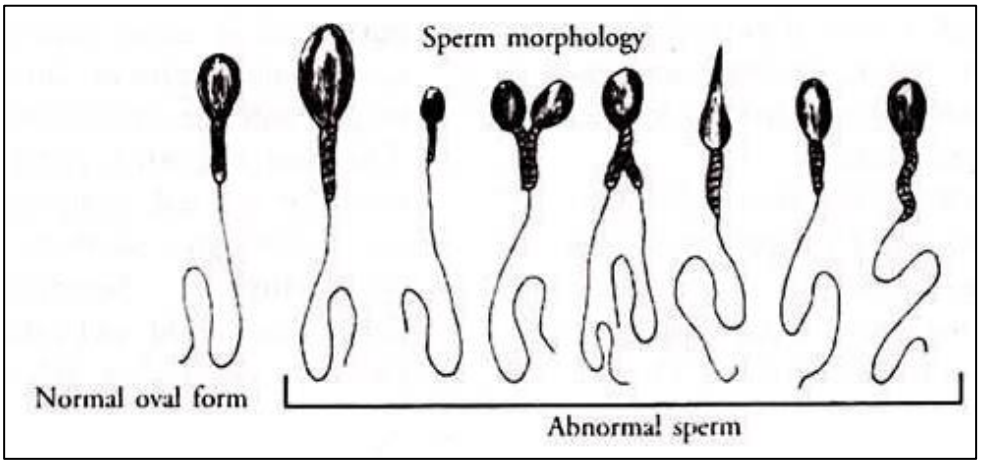


Fig 4: Semen analysis

d. Autoimmunity:

In some males, the immune system may attack the sperms and reduce the sperm numbers. Treatment is not usually possible.

e. Impotence and Premature Ejaculation:

The inability to achieve an erection of the penis is known as impotence. Psychological counselling may help in some cases. Premature ejaculation is a condition where the man releases the semen even before penetration into the vagina. This condition is treatable with psychological treatment.

f. Immotile cilia:

Absence of tail in sperm makes it immotile. Hence, sperms cannot move from vagina to upper portions of genital tract of female.

g. Absence of Y-chromosome:

Sometimes, deletion of Y-chromosomes in primordial germ cells leads to sperm production without Y-chromosome. Such sperms cannot form viable zygole.

h. Tubular blockage:

Blockage of vasa deferentia and vasa efferentia stops sperm transport.

i. Antisperm antibodies:

Such antibodies are IgG, IgM and IgA. Sometimes IgG is found in cervical mucous, serum and semen.

j. High scrotal temperature:

Due to development of dilated veins in testis (varicocela) scrotal temperature is raised and sperm production is minimized leading to oligospermia.

k. Low fructose content and high prostaglandin in seminal fluid led to sperm destruction.

l. Vasectomy leads to irreversible infertility in males.

Other causes of male sterility may include:

a. Genetic factors: A man should have an X and Y chromosome. If he has two X chromosomes and one Y chromosome, as in Klinefelter's syndrome, the testicles will develop abnormally and there will be low testosterone and a low sperm count or no sperm.

b. Mumps: If this occurs after puberty, inflammation of the testicles may affect sperm production.

c. Hypospadias: The urethral opening is under the penis, instead of its tip. This abnormality isusually surgically corrected in infancy. If the correction is not done, it may be harder for the sperm to get to the female's cervix. Hypospadias affects about 1 in every 500 newborn boys.

d. Cystic fibrosis: This is a chronic disease that results in the creation of a sticky mucus. Thismucus mainly affects the lungs, but males may also have a missing or obstructed vas deferens.The vas deferens carries sperm from the epididymis to the ejaculatory duct and the urethra.

e. Radiation therapy: This can impair sperm production. The severity usually depends on hownear to the testicles the radiation was aimed.

f. Some diseases: Conditions that are sometimes linked to lower fertility in males are anaemia,Cushing's syndrome, diabetes, and thyroid disease.

- ## Some medications increase the risk of fertility problems in men

a. Sulfasalazine: This anti-inflammatory drug can significantly lower a man's sperm count. Itis often prescribed for Crohn's disease or rheumatoid arthritis. Sperm count often returns to normal after stopping the medication.

b. Anabolic steroids: Popular with bodybuilders and athletes, long-term use can seriously reduce sperm count and mobility.

c. Chemotherapy: Some types may significantly reduce sperm count.

d. Illegal drugs: Consumption of marijuana and cocaine can lower the sperm count.

e. Age: Male fertility starts to fall after 40 years.

f. Exposure to chemicals: Pesticides, for example, may increase the risk.

g. Excess alcohol consumption: This may lower male fertility. Moderate alcohol consumptionhas not been shown to lower fertility in most men, but it may affect those who already have a low sperm count.

h. Mental stress: Stress can be a factor, especially if it leads to reduced sexual activity.

B. Female Infertility:

A woman may be infertile due to several causes.

Some important reasons are as follows:

a. Failure to Ovulate:

Failure to ovulate is one common cause of infertility in females. This is because the pituitary or hypothalamus fails to produce the FSH which is required for follicle development or LH required for release of the egg from the ovary. It may also be because the ovaries fail to produce oestrogen or progesterone. Hormonal imbalances may be corrected by administering synthetic hormones to the affected individual.

The most commonly used drug is Clomiphene, a synthetic oestrogen like drug which stimulates ovulation. Tamoxifen is another drug used. These pills are taken orally for five days soon after the menstrual cycle starts. Injection of HCG, which is chemically similar to LH is given at the middle of the cycle to stimulate ovulation. 'Fertility drugs' which contains FSH and LH or only FSH is also used. But these have the danger of multiple egg release and consequently multiple pregnancies. Advance techniques include small implants in the upper arm which releases smallamounts of GnRH mimicking the activity of the hypothalamus.

b. Damage to Oviducts:

The fallopian tubes may be blocked or narrowed in some women. This interferes with the movement of the eggs and fertilisation. This can be treated by laser surgery.

c. Damage to Uterus:

In about 5-10% cases, infertility problems are due to a damaged uterus. The uterus is unable tomaintain pregnancy, i.e., the fertilised zygote does not get implanted. Sometimes large non-malignant tumours called fibroids or smaller growths known as polyps which grow in the wallsof the uterus can cause infertility. These can be surgically removed. IUCD or PID also causes inflammation in the uterus and cause problems. This can be treated by using antibiotics. Adhesion in the uterus, i.e. sticking of parts of the uterus which occurs as a result of an abortionis another reason for infertility.

d. Damage to the Cervix:

The cervix is the neck of the uterus. The cervix may become damaged because of the abortion or difficult birth. A narrow cervix may interfere with sperm movement.

e. Antibodies to Sperm:

In some rare cases, women may produce antibodies against sperms. These are found in the cervix, uterus and oviducts. These may be treated using immunosuppressant drugs, but IVF isa better method of treatment.

f. Ovarian problem:

There may not be normal ovulation in ovary. Sometimes there is failure of corpus luteum

formation.

g. Hormonal cause:

Decreased level of FSH and LH, drug induced ovulation may not allow fertilization and development of the foetus.

h. Uterine factor:

Unfavourable endometrium for implantation, chronic endometritis, fibroid uterus etc. may be the cause of infertility.

i. Cervical factor: In effective sperm penetration, chronic cervicitis, presence of anti spermantibody and elongation of cervix may be the cause of infertility.

j. Fimbriae:

Fimbriae of Fallopian tube may not pick up secondary oocyte from ovary.

k. Dyspareunia:

Painful sexual intercourse experienced by female may be another cause of infertility.

l. Macrophages:

Increased sperm phagocytosis by macrophages may be the cause of infertility.

m. Miscarriage:

Early miscarriage before complete development of foetus due to various gyaenic problems maybe also the reason of infertility.

n. Tubectomy:

Like vasectomy in males, tubectomy in females causes permanent infertility.

Other causes of female sterility may include:

a. Age: The ability to conceive starts to fall around the age of 32 years.

b. Smoking: Smoking significantly increases the risk of infertility in both men and women, and it may undermine the effects of fertility treatment. Smoking during pregnancy increases the chance of pregnancy loss. Passive smoking has also been linked to lower fertility.

c. Alcohol: Any amount of alcohol consumption can affect the chances of conceiving.

d. Being obese or overweight: This can increase the risk of infertility in women as well asmen.

e. Eating disorders: If an eating disorder leads to serious weight loss, fertility problems mayarise.

f. Diet: A lack of folic acid, iron, zinc, and vitamin B-12 can affect fertility. Women who are at risk, including those on a vegan diet, should ask the doctor about supplements.

g. Exercise: Both too much and too little exercise can lead to fertility problems.

h. Sexually transmitted infections (STIs): Chlamydia can damage the fallopian tubes in a woman and cause inflammation in a man's scrotum. Some other STIs may also cause infertility.

i. Exposure to some chemicals: Some pesticides, herbicides, metals, such as lead, and solventshave been linked to fertility problems in both men and women. A mouse study has suggested that ingredients in some household detergents may reduce fertility.

j. Mental stress: This may affect female ovulation and male sperm production and can lead toreduced sexual activity.

- **Some medical conditions can affect female fertility:**

Ovulation disorders appear to be the most common cause of infertility in women. Ovulation is the monthly release of an egg. The eggs may never be released or they may only be releasedin some cycles.

- **Ovulation disorders can be due to:**

a. Premature ovarian failure: The ovaries stop working before the age of 40 years.

b. Polycystic ovary syndrome (PCOS): The ovaries function abnormally and ovulation maynot occur.

c. Hyperprolactinemia: If prolactin levels are high, and the woman is not pregnant orbreastfeeding, it may affect ovulation and fertility.

d. Poor egg quality: Eggs that are damaged or develop genetic abnormalities cannot sustain apregnancy. The older a woman is, the higher the risk.

e. Thyroid problems: An overactive or underactive thyroid gland can lead to a hormonal imbalance.

f. Chronic conditions: These include AIDS or cancer.

- **Problems in the uterus or fallopian tubes** can prevent the egg from traveling from the ovaryto the uterus, or womb. If the egg does not travel, it can be harder to conceive naturally.

Causes include:

a. Surgery: Pelvic surgery can sometimes cause scarring or damage to the fallopian tubes. Cervical surgery can sometimes cause scarring or shortening of the cervix. The cervix is the neck of the uterus.

b. Submucosal fibroids: Benign or non-cancerous tumours occur in the muscular wall of theuterus. They can interfere with implantation or block the fallopian tube, preventing sperm fromfertilizing the egg. Large submucosal uterine fibroids may make the uterus' cavity bigger,

increasing the distance the sperm has to travel.

c. Endometriosis: Cells that normally occur within the lining of the uterus start growing elsewhere in the body.

d. Previous sterilization treatment: In women who have chosen to have their fallopian tubesblocked, the process can be reversed, but the chances of becoming fertile again are not high.

Diagnosis of Infertility:

Most people will visit a physician if there is no pregnancy after 12 months of trying. If the woman is aged over 35 years, the couple may wish to see a doctor earlier, because fertility testing can take time, and female fertility starts to drop when a woman is in her 30s. A doctor can give advice and carry out some preliminary assessments. It is better for a couple to see the doctor together. The doctor may ask about the couple's sexual habits and make recommendations regarding these. Tests and trials are available, but testing does not always reveal a specific cause.

- ## Infertility tests for men:

The doctor will ask the man about his medical history, medications, and sexual habits and carry out a physical examination. The testicles will be checked for lumps or deformities, and the shape and structure of the penis will be examined for abnormalities.

a. Semen analysis: A sample may be taken to test for sperm concentration, motility, colour, quality, any infections, and whether any blood is present. Sperm counts can fluctuate, so that several samples may be necessary.

b. Blood test: The lab will test for levels of testosterone and other hormones.

c. Ultrasound: This may reveal issues such as ejaculatory duct obstruction or retrograde ejaculation.

d. Chlamydia test: Chlamydia can affect fertility, but antibiotics can treat it.

- ## Infertility tests for women:

A woman will undergo a general physical examination, and the doctor will ask about her medical history, medications, menstruation cycle, and sexual habits.

She will also undergo a gynaecologic examination and a number of tests:

a. Blood test: This can assess hormone levels and whether a woman is ovulating.

b. Hysterosalpingography: Fluid is injected into the woman's uterus and X-rays are taken todetermine whether the fluid travels properly out of the uterus and into the fallopian tubes. If ablockage is present, surgery may be necessary.

c. Laparoscopy: A thin, flexible tube with a camera at the end is inserted into the abdomen and pelvis, allowing a doctor to look at the fallopian tubes, uterus, and ovaries. This can reveal signs of endometriosis, scarring, blockages, and some irregularities of the uterus and fallopian tubes.

Other tests include:

a. ovarian reserve testing, to find out how effective the eggs are after ovulation

b. genetic testing, to see if a genetic abnormality is interfering with fertility

c. pelvic ultrasound, to produce an image of the uterus, fallopian tubes, and ovaries

d. Chlamydia test, which may indicate the need for antibiotic treatment

e. thyroid function test, as this may affect the hormonal balance.

• Ectopic pregnancy

This is when a fertilized egg implants outside the womb, usually in a fallopian tube. If it stays in there, complications can develop, such as the rupture of the fallopian tube. This pregnancy has no chance of continuing.

Immediate surgery is needed and, sadly, the tube on that side will be lost. However, future pregnancy is possible with the other ovary and tube. Women receiving fertility treatment havea slightly higher risk of an ectopic pregnancy. An ultrasound scan can detect an ectopic pregnancy.

Treatment:

Treatment will depend on many factors, including the age of the person who wishes to conceive, how long the infertility has lasted, personal preferences, and their general state of health.

• Fertility treatments for men:

Treatment will depend on the underlying cause of the infertility.

a. Erectile dysfunction or premature ejaculation: Medication, behavioural approaches, or both may help improve fertility.

b. Varicocele: Surgically removing a varicose vein in the scrotum may help.

c. Blockage of the ejaculatory duct: Sperm can be extracted directly from the testicles and injected into an egg in the laboratory.

d. Retrograde ejaculation: Sperm can be taken directly from the bladder and injected into anegg in the laboratory.

e. Surgery for epididymal blockage: A blocked epididymis can be surgically repaired. The

epididymis is a coil-like structure in the testicles which helps store and transport sperm. If theepididymis is blocked, sperm may not be ejaculated properly.

- ## Fertility treatments for women:

Fertility drugs might be prescribed to regulate or induce ovulation. They include:

a. Clomifene (Clomid, Serophene): This encourages ovulation in those who ovulate either irregularly or not at all, because of PCOS or another disorder. It makes the pituitary gland release more follicle-stimulating hormone (FSH) and luteinizing hormone (LH).

b. Metformin (Glucophage): If Clomifene is not effective, metformin may help women with PCOS, especially when linked to insulin resistance.

c. Human menopausal gonadotropin, or hMG (Repronex): This contains both FSH and LH. Patients who do not ovulate because of a fault in the pituitary gland may receive this drug as an injection.

d. Follicle-stimulating hormone (Gonal-F, Bravelle): This hormone is produced by the pituitary gland that controls oestrogen production by the ovaries. It stimulates the ovaries to mature egg follicles.

e. Human chorionic gonadotropin (Ovidrel, Pregnyl): Used together with clomiphene, hMG, and FSH, this can stimulate the follicle to ovulate.

f. Gonadotropin-releasing hormone (Gn-RH) analogs: These can help women who ovulatetoo early—before the lead follicle is mature—during hmG treatment. It delivers a constant supply of Gn-RH to the pituitary gland, which alters the production of hormone, allowing the doctor to induce follicle growth with FSH.

g. Bromocriptine (Parlodel): This drug inhibits prolactin production. Prolactin stimulates milk production during breastfeeding. Outside pregnancy and lactation, women with high levels of prolactin may have irregular ovulation cycles and fertility problems.

- ## Assisted conception:

The following methods are currently available for assisted conception.

a. Intrauterine insemination (IUI): At the time of ovulation, a fine catheter is inserted throughthe cervix into the uterus to place a sperm sample directly into the uterus. The sperm is washedin a fluid and the best specimens are selected. The woman may be given a low dose of ovary stimulating hormones. IUI is more commonly done when the man has a low sperm count, decreased sperm motility, or when infertility does not have an identifiable cause. It can also help if a man has severe erectile dysfunction.

b. In-vitro fertilization (IVF): Sperm are placed with unfertilized eggs in a petri dish, where fertilization can take place. The embryo is then placed in the uterus to begin a pregnancy. Sometimes the embryo is frozen for future use.

c. Intracytoplasmic sperm injection (ICSI): A single sperm is injected into an egg to achieve fertilization during an IVF procedure. The likelihood of fertilization improves significantly formen with low sperm concentrations.

d. Sperm or egg donation: If necessary, sperm or eggs can be received from a donor. Fertilitytreatment with donor eggs is usually done using IVF.

e. Assisted hatching: The embryologist opens a small hole in the outer membrane of the embryo, known as the zona pellucid. The opening improves the ability of the embryo to implant into the uterine lining. This improves the chances that the embryo will implant at, or attach to,the wall of the uterus. This may be used if IVF has not been effective, if there has been poor embryo growth rate, and if the woman is older. In some women, and especially with age, the membrane becomes harder. This can make it difficult for the embryo to implant.

f. Electric or vibratory stimulation to achieve ejaculation: Ejaculation is achieved with electric or vibratory stimulation. This can help a man who cannot ejaculate normally, for example, because of a spinal cord injury.

g. Surgical sperm aspiration: The sperm is removed from part of the male reproductive tract,such as the vas deferens, testicle, or epididymis.

- ## Surgical procedures for women:

If the fallopian tubes are blocked or scarred, surgical repair may make it easier for eggs to pass through. Endometriosis may be treated through laparoscopic surgery. A small incision is made in the abdomen, and a thin, flexible microscope with a light at the end, called a laparoscope, isinserted through it. The surgeon can remove implants and scar tissue, and this may reduce painand aid fertility.

Probable Questions:

1. Define infertility. What are the types of infertility?
2. Discuss the causes of male sterility.
3. What medicines induce male sterility.
4. Discuss the causes of female sterility.
5. How male sterility can be diagnosed?
6. How female sterility can be diagnosed?
7. What is ectopic pregnancy? Explain.
8. Discuss fertility treatment in females?
9. Discuss fertility treatments in males?

Suggested Readings:

1. Developmental Biology: Michael J.F. Barresi Scott F. Gilbert,(12 th Ed).
2. Principles of Development: Lewis Wolpert and Cheryll Tickle (4th Ed.).
3. iGenetics: A molecular approach. 3rd Ed. Peter J. Russell. Pearson International Edition.
4. Biology of human reproduction. Ramon Pinon Jr. University Science Book publishers
5. Embryology by N. Kumarsen
6. Developmental Biology by Veerbala Rastogi.
7. Embryology by M.P. Arora

Unit-II

Assisted Reproductive Technologies: Sperm and ova bank; Artificial Insemination donor (AID); in vitro fertilization (IVF), procedures, variations of IVF, Success rates and complications; Gamete Intrafallopian transfer (GIFT), Intracytoplasmic sperm Injection (ICSI), Surrogate mothers

Objective: In this unit we will discuss bout sperm and ova bank and their utility. We will also discuss about different methods of in vitro fertilization, their types, risk factors and success rate. We will also discuss about surrogacy.

Sperm Bank

A sperm bank can freeze and store sperm samples for patients in need of preserving their fertility or for use in fertility treatment. The second purpose is to store donor sperm for use by patients who cannot otherwise conceive children. The sperm bank offers fertility preservation for men who are undergoing either surgery or treatment that may cause permanent changes in their chances of conceiving children, either by affecting the sperm production process, i.e. chemotherapy, orchiectomy or testosterone medication; or where the ducts are cut or clogged, i.e. vasectomy. Short-term storage of semen is also offered to patients who have busy travelling schedules or who live abroad. The sperm can then be used for artificial inseminations, IVF or ICSI even though the husband/male partner is away. In some case where sperm counts are very low, sperm is stored as a back-up in case the count is even lower at the time of ICSI. Back-up storage is also done when a man struggles with passing a sample, then the pressure is off on the day when sperm are needed for ICSI or AI. The second purpose of a sperm bank is the accumulation of donated sperm. Sperm donation is most probably one of the oldest forms of fertility treatment. In some cases, a sperm donor is the only way of conception, e.g. for azoospermia males, i.e. no sperm production is present on confirmation of a testicular biopsy;and in homosexual female couples. In cases of a very low sperm count, ICSI would be the optimal treatment, but it might not be possible for the couple because of financial constraints or religious views. In such situations, artificial insemination with donor sperm can be considered an alternative treatment option.

Ova Bank

Egg donation is a process by which women donate eggs for the purposes of assisted reproduction. This process typically involves in vitro fertilization technology, with the eggs being fertilised in the laboratory. Unfertilised eggs can be frozen and stored for future use at an ova bank. The first child born from egg donation was reported at Monash IVF Clinic, Australia in 1983.

Need for egg donation: This may arise for a number of reasons such as,

a. Infertile couples may resort to egg donation when the female partner cannot have her own genetic children because her own eggs cannot generate a viable pregnancy; or because they could generate a viable pregnancy, but the chances are so low that it is not advisable to do IVFwith her own eggs. This situation is often, but not always, based on advanced reproductive age.

b. It can also be due to early onset of menopause, which can occur as early as in women's 20s.

c. In addition, some women are born without ovaries, while some women's reproductive organs have been damaged or surgically removed due to disease or other circumstances.

d. Another indication would be a genetic disorder that either renders her infertile or dangerousfor any offspring – problems that can be circumvented by using eggs from another woman.

Artificial Insemination donor (AID)

- ✓ Artificial insemination is when a partner's semen is injected directly into the neck of the womb, or into the womb itself, at a time when a woman is ovulating. Donor insemination is exactly the same process, but using a donor sperm instead of one's partners.
- ✓ Initially, the patient must undergo hormone treatment (although it may also be done in natural cycles) in order to achieve growth and follicular maturation (maturation of the egg).
- ✓ This treatment can last between 10 and 14 days and it is simple and slightly aggressive, since the dosages of medication given tend to be low.
- ✓ Once oocyte maturation has been achieved (proved by periodic ultrasound controls performed during the treatment), ovulation is provoked within the next 36 hours and the donor sperm sample is then prepared.
- ✓ Regarding donor selection, it is a complex and careful procedure which consists in carrying out a series of interviews and tests to candidates in order to rule out those with diseases of genetic or serological origin, physical examinations to verify that the reproductive organs are working well, as well as psychological tests to assess the intelligence quotient and the integrity of the donor.
- ✓ Donors tend to be young men between the ages of 18 and 30, who donate altruistically and voluntarily sperm samples to the center during a six-month period.
- ✓ These samples are treated and frozen in order to keep them until the moment when a couple with similar physical characteristics to the those of the donor require them.
- ✓ The sample preparation consists in washing it in order to remove any seminal plasma

and non-motile or morphologically altered sperm. In this way, there is only good motile sperm, capable of fertilizing the egg.

- ✓ Once the sample is thawed and prepared, it is placed into an insemination cannula and is left inside the patient's uterus. The procedure is simple, quick and little painful.
- ✓ Once finished, the patient will remain lying down for some minutes.
- ✓ Insemination is a technique which does require neither rest nor change of life habits of the patient.
- ✓ After treatment, within fifteen days, the patient will take a pregnancy test to know if pregnancy has been achieved.

In Vitro Fertilization (IVF)

In Vitro Fertilization is an assisted reproductive technology (ART) commonly referred to as IVF. IVF is the process of fertilization by extracting eggs, retrieving a sperm sample, and then manually combining an egg and sperm in a laboratory dish. The embryo(s) is then transferred to the uterus. Other forms of ART include gamete intrafallopian transfer (GIFT) and zygote intrafallopian transfer (ZIFT).

Why is IVF used? IVF can be used to treat infertility in the following patients:

a. Blocked or damaged fallopian tubes
b. Male factor infertility including decreased sperm count or sperm motility
c. Women with ovulation disorders, premature ovarian failure, uterine fibroids
d. Women who have had their fallopian tubes removed
e. Individuals with a genetic disorder
f. Unexplained infertility

The important techniques employed in assisted reproductive technology are listed below:

i. Intrauterine insemination (IUI).
ii. In vitro fertilization and embryo transfer (IVF and ET).
iii. Gamete intra-fallopian transfer (GIFT).
iv. Zygote intra-fallopian transfer (ZIPT).
v. Intra-vaginal culture (IVC).
vi. Cytoplasmic transfer (CT).

vii. Micromanipulation (Intra-cytoplasmic sperm injection (ICSI), sub-zonal insertion (SUZI).

viii. Cryopreservation.

ix. Assisted hatching (AH).

Among these techniques, the most commonly used procedure is in vitro fertilization and embryo transfer. Important features of different types of ART are briefly described.

- **In Vitro Fertilization and Embryo Transfer (IVF and ET):**

In vitro fertilization broadly deals with the removal of eggs from a women, fertilizing them in the laboratory, and then transferring the fertilized eggs (zygotes) into the uterus a few days later.

Indications for IVF:

Infertility due to the following causes may be considered for IVF.

i. Failed ovulation induction

ii. Tubal diseases

iii. Cervical hostility

iv. Endometriosis

v. Idiopathic infertility (in men and women).

Ideal Subjects for IVF:

Although it is not always possible to have a choice in the selection of subjects, the following criteria are preferred.

i. Woman below 35 years.

ii. Presence of at least one functional ovary.

iii. Husband with normal motile sperm count.

iv. The couple must be negative for HIV and hepatitis.

Methodology of IVF:

The in vitro fertilization broadly involves the following steps.

1. Induction of superovulation.
2. Monitoring of ovarian response.
3. Oocyte retrieval.
4. Fertilization in vitro.

5. Embryo transfer.

Induction of Superovulation:

It is well known that the success rate IVF is much higher when more embryos (3-5) are transferred. This is possible only with controlled ovarian hyper-stimulation (COH). The otheradvantages of COH include improvement in the quality of oocyte, control of ovulation timing,besides overcoming the ovulatory dysfunction. The following drug regimens are in use to induce superovulation.

i. Clomiphene citrate (CC).

ii. CC + human menopausal gonadotrophin (hMG).

iii. CC + follicle stimulating hormone (FSH).

iv. Human menopausal gonadotrophin.

v. Follicle stimulating hormone.

vi. Gonadotrophin releasing hormone agonists (GnRHa) + hMG (or FSH).

It is now common to use GnRH agonists to induce ovulation. These compounds act through aprocess called down regulation of the physiologic hypothalamic- pituitary-ovarian feedback mechanism to effectively suppress spontaneous ovulation.

Monitoring of Ovarian Response:

The follicular growth or ovarian response can be monitored by increase in serum oestradiol level, increase in follicular diameter and thickening of endometrial bed.

Oocyte Retrieval:

The most common method for oocyte retrieval is carried out through vaginal route under ultrasound guidance. This method is simple and less invasive, and can be performed with analgesics only. It is easy to recognize the oocyte as a single cell surrounded by a mass of cumulus cells. The recovered oocytes are maintained in vitro culture for 4-6 hours.

Fertilization in Vitro:

The semen specimens are collected (just prior to oocyte retrieval) via masturbation, processed,and incubated in protein-supplemented media for 3-4 hours prior to fertilization. The incubation results in sperm capacitation.

The retrieved oocytes are also cultured in protein-supplemented media for about 6-8 hours. Forthe purpose of IVF, 50,000-1, 00,000 capacitated sperms are placed in culture with a single oocyte. The signs of fertilization may be demonstrated 16-20 hours later by the presence of twopronuclei within the developing embryo. There is no need to change the

regime for a single failure of IVF. Many a times, success occurs in the subsequent cycles. The two most importantcriteria for the success of IVF are sperm density and motility.

Embryo Transfer:

Embryo at a stage between pronuclei and blastocyst stage are transferred. Conventionally, 4-8 cell stage embryos are transferred between 48-60 hours following insemination. The transfer procedure is carried out by use of a catheter.

Not more than three embryos are transferred (per cycle) to minimize multiple pregnancies. However, in the women above the age of 40 years, higher number of embryos may betransferred. (Note: Excess oocytes and embryos are cryopreserved for further use. This will reduce the cost, besides the risk of ovarian hyper stimulation).

Luteal phase support is given by administration of progesterone for about two weeks. By this time, the diagnosis of pregnancy can be assessed by estimating human chorionic gonadotrophin(hCG).

Success Rates of IVF:

Success of IVF varies from programme to programme and within the same programme, the success rate is dependent on the correct diagnosis of the patient, and age. The overall pregnancyrate in IVF is in the range of 25-35% per oocyte retrieval. The take home baby rate is about 15-20% per procedure.

- ## Side effects of in vitro fertilization

Although you may need to take it easy after the procedure, most women can resume normal activities the following day.

Some side effects after IVF may include:

1. Passing a small amount of fluid (may be clear or blood-tinged) after the procedure
2. Mild cramping
3. Mild bloating
4. Constipation
5. Breast tenderness

Some side effects of fertility medications may include:

a. Headaches
b. Mood swings
c. Abdominal pain
d. Hot flashes
e. Abdominal bloating

f. Rare: Ovarian hyperstimulation syndrome (OHSS)

- **Risks associated with in vitro fertilization:**

As with most medical procedures, there are potential risks. More severe symptoms, typically from OHSS, include the following:

a. Nausea or vomiting

b. Decreased urinary frequency

c. Shortness of breath

d. Faintness

e. Severe stomach pains and bloating

f. Ten-pound weight gain within three to five days

If you experience any of these symptoms above, contact your doctor right away.

- **Additional risks of IVF include the following:**

a. Egg retrieval carries risks of bleeding, infection, and damage to the bowel or bladder.

b. The chance of a multiples pregnancy is increased with the use of fertility treatment. There are additional risks and concerns related to multiples during pregnancy including the increasedrisk of premature delivery and low birth weight.

c. Though the rates of miscarriage are similar to unassisted conception, the risk does increase with maternal age.

d. The Mayo Clinic reports that the risk of ectopic pregnancy with IVF is 2-5%. An ectopic pregnancy is when a fertilized egg implants anywhere outside the uterus and is not viable.

e. Assisted reproductive technology (ART) involves a significant physical, financial, and emotional commitment on the part of a couple. Psychological stress and emotional problems are common, especially if in vitro fertilization (IVF) is unsuccessful.

f. IVF is expensive, and many insurance plans do not provide coverage for fertility treatment. The cost for a single IVF cycle can range from at least $12,000-$17,000.

- **The success rate of IVF is rather low due to the following reasons:**

i. Increased risk of abortion

ii. Multiple pregnancy

iii. Ectopic pregnancy

iv. Low birth weight baby

v. Premature delivery.

The success rate of IVF clinics depends on a number of factors including reproductive history, maternal age, the cause of infertility, and lifestyle factors. It is also important to understand that pregnancy rates are not the same as live birth rates.

In the United States, the live birth rate for each IVF cycle started is approximate:

a. **41-43%** for women under age 35

b. **33-36%** for women ages 35 to 37

c. **23-27%** for women ages 38 to 40

d. **13-18%** for women ages over 40

- ## The World's Picture of Test Tube Babies:

By employing in vitro fertilization and embryo transfer, the world's first test tube baby (Louise Brown) was born in UK on 28th July 1978. The world's second test tube baby (Kanupriya alias Durga) was born in Kolkata on 3rd October 1978. A team led by Subhash Mukherjee carried IVF and ET in India. Scientists responsible for the "birth of test tube babies were severely criticized then. In fact, IVF turned out to be one of the major achievements of medical sciences in the last century. It has become a novel way of treating infertility. Today, there are more than a million test tube babies born all over the world. In 2003, the world celebrated the silver jubileeof IVF with much fanfare.

Gamete Intra-Fallopian Transfer (GIFT)

Gamete intra-fallopian transfer involves the transfer of both sperm and unfertilized oocyte intothe fallopian tube. This allows the fertilization to naturally occur in vivo. The prerequisite for GIFT procedure is that the woman should have at least one normal fallopian tube.

The induction of ovulation and the monitoring procedures for GIFT are almost the same as described for IVF. A couple of hours prior to oocyte retrieval, semen specimens are collected.Two oocytes along with 2-5 lakhs motile sperms for each fallopian tube are placed in a plastictube container. It is then inserted (by laparoscopy) 4 cm into the distal end of the fallopian tube,and the oocyte sperm combination is injected. The overall pregnancy rate is as high as 30- 40%.The take home baby rate is about 25%. This is much higher when compared to IVF. But the major limitation is the requirement of laparoscopy (a major surgical procedure) to transfer oocytes and sperms into the fallopian tubes.

Zygote Intra-Fallopian Transfer (ZIFT)

ZIFT is suitable when the infertility lies in men, or in case of failure of GIFT.

The wife's oocytes are exposed to her husband's sperms in the laboratory. The fertilized eggs (zygotes) within 24 hours are transferred to the fallopian tube by using laparoscopy.

ZIPT has an advantage over GIFT with male factor infertility. Further, it can be known whether the wife's oocytes have been fertilized by her husbands' sperms.

Surrogacy

Surrogacy is an arrangement, often supported by a legal agreement, whereby a woman (the surrogate mother) agrees to bear a child for another person or persons, who will become the child's parent(s) after birth. People may seek a surrogacy arrangement when pregnancy is medically impossible, when pregnancy risks are too dangerous for the intended mother, or when a single man or a male couple wish to have a child. Surrogacy is considered one of many assisted reproductive technologies. In surrogacy arrangements, monetary compensationmay or may not be involved. Receiving money for the arrangement is known as commercial surrogacy. The legality and cost of surrogacy varies widely between jurisdictions, sometimes resulting in problematic international or interstate surrogacy arrangements. Couples seeking a surrogacy arrangement in a country where it is banned sometimes travel to a jurisdiction that permits it. In some countries, surrogacy is only legal if money does not exchange hands.

Where commercial surrogacy is legal, couples may use the help of third-party agencies to assistin the process of surrogacy by finding a surrogate and arranging a surrogacy contract with her.These agencies often screen surrogates' psychological and other medical tests to ensure the bestchance of healthy gestation and delivery. They also usually facilitate all legal matters concerning the intended parents and the surrogate.

- ## Indications for surrogacy:

Opting for surrogacy is often a choice made when women are unable to carry children on their own. This can be for a number of reasons, including an abnormal uterus or a complete absence of a uterus either congenitally (also known as Mayer-Roakitansky-Kuster-Hauser syndrome) or post-hysterectomy. Women may have a hysterectomy due to complications in childbirth such as heavy bleeding or a ruptured uterus. Medical diseases such as cervical cancer or endometrial cancer can also lead to surgical removal of the uterus. Past implantationfailures, history of multiple miscarriages, or concurrent severe heart or renal conditions that can make pregnancy harmful may also prompt women to consider surrogacy. The biological impossibility of single men and same-sex couples having a baby also may indicate surrogacy as an option.

- ## Types of Surrogacy

There are two types of surrogacy — traditional surrogacy and gestational surrogacy. In traditional surrogacy, a surrogate mother is artificially inseminated, either by the intended father or an anonymous donor, and carries the baby to term. The child is thereby genetically related to both the surrogate mother, who provides the egg, and the intended father oranonymous donor.

In gestational surrogacy, an egg is removed from the intended mother or an anonymous donorand fertilized with the sperm of the intended father or anonymous donor. The fertilized egg, orembryo, is then transferred to a surrogate who carries the baby to term. The child is thereby genetically related to the woman who donated the egg and the intended father or

sperm donor, but not the surrogate. Some lesbian couples find gestational surrogacy attractive because it permits one woman to contribute her egg and the other to carry the child. Traditional surrogacyis more controversial than gestational surrogacy, in large part because the biological relationship between the surrogate and the child often complicates the facts of the case if parental rights or the validity of the surrogacy agreement are challenged. As a result, most states prohibit traditional surrogacy agreements. Additionally, many states that permit surrogacy agreements prohibit compensation beyond the payment of medical and legal expenses incurred as a result of the surrogacy agreement.

- **Qualification of a surrogate mother:**

Most surrogacy agencies and fertility clinics require surrogates to meet the following general qualifications:

a. Be in good physical and mental health;

b. Have carried and delivered at least one child;

c. Have had pregnancies that were all free of complications and were full-term;

d. Be less than 43 years of age (some clinics will accept older woman in certain circumstances; others have younger age cut-offs for all surrogates);

e. Be in a stable living situation; and

f. Not smoke or abuse alcohol.

- **Risks of surrogacy**

The embryo implanted in gestational surrogacy faces the same risks as anyone using IVF would. Preimplantation risks of the embryo include unintentional epigenetic effects, influence of media which the embryo is cultured on, and undesirable consequences of invasive manipulation of the embryo. Often, multiple embryos are transferred to increase the chance ofimplantation, and if multiple gestations occur, both the surrogate and the embryos face higherrisks of complications.

Gestational surrogates have a smaller chance of having hypertensive disorder during pregnancy compared to mothers pregnant by oocyte donation. This is possibly because surrogate mothers tend to be healthier and more fertile than women who use oocyte donation. Surrogate mothers also have low rates of placenta praevia / placental abruptions (1.1-7.9%). Children born through singleton IVF surrogacy have been shown to have no physical or mental abnormalities compared to those children born through natural conception. However, children born through multiple gestation in surrogate mothers often result in preterm labour and delivery, resulting in prematurity and physical and/or mental anomalies.

Probable Questions:

1. What is Ova bank. State its utility.
2. What is sperm bank? State its utility.
3. Define IVF? Why it is needed?
4. Discuss different steps of IVF.
5. What is intrauterine insemination. Discuss its advantages.
6. Why success rate of IVF is low?
7. Discuss Gamete Intra-Fallopian Transfer.
8. Discuss Zygote Intra-Fallopian Transfer.
9. What are the two methods of cytoplasmic transfer?
10. What is Intra-Cytoplasmic Sperm Injection?
11. Discuss Round Spermatid nucleus Injection.
12. Wat is assisted hatching?
13. What are the side effects of In vitro fertilization?
14. Define surrogacy? What are the qualification of a surrogate mother should be?
15. Discuss the types of surrogacy.
16. Discuss about risks of surrogacy.

Suggested Readings:

1. Biotechnology by P.K. Gupta
2. Gene Cloning by T. Brown.
3. Biotechnology by N. Kumarsen.
4. Biotechnology by B.D. Singh

Unit-III

Genetic errors of human development- Down syndrome, Fragile X syndrome, Turner's Syndrome

Objective: In this unit we will discuss about some genetic errors of human development suchas Down syndrome, Fragile X syndrome and Turner's syndrome.

Down Syndrome:

The first autosomal abnormality described in man by John Langdon Down (1966) was knownas Down's syndrome, more commonly used as mongolism. In this case, there is simple trisomyof chromosome 21, i.e. each cell containing three chromosomes of 21 rather than two. The term"Mongolism" has been applied due to their facial characteristics (round, full face with upper eyelids turned downwards), very similar to that of the Mongolian race. It is impossible to be certain whether the trisomy involves chromosome 21 or 22 (of Gr. G) since they are morphologically identical. For this, it is sometimes called G-trisomy.

There are three types of Down syndrome: trisomy 21, translocation Down syndrome, and mosaic Down syndrome.

I. Trisomy 21

This is the most common type of Down syndrome, with 95% of people with DS having trisomy 21. Here, all your body's cells have three copies of chromosome 21 instead of two.

II. Translocation Down syndrome

About 3% of people with Down syndrome have this type, where there's an extra part or whole chromosome 21, but it's attached to another chromosome instead of being a separate chromosome 21.

III. Mosaic Down syndrome

This type of Down syndrome, which affects about 2% of people with the condition, happens when only some cells in your body have an extra chromosome 21.

You can't tell what type of Down syndrome someone has by how they look. The effects of all three types are very similar, but someone with mosaic Down syndrome may not have as many symptoms because fewer cells have the extra chromosome. So it's possible to have undiagnosed mosaic Down syndrome.

• Chromosomal Findings in Down's Syndrome:

There are 4 types of Karyotypes associated with Down's Syndrome. Richards (1967) collecteddata from 1103 cases. Majority are primary 21 trisomies with no great risk of recurrence, but the other 3 types may all be associated with familial Down's syndrome — in siblings and otherrelatives if there is an inherited translocation and in the offspring of

the patient if he or she is amosaic.

1. Trisomic — 94.4% (47, XX or XY, G+)
2. t(Dq. Gq) —1.5% 46, XX or XY — D, t (Dq. Gq) +3.
46(XX or XY) G—, t(Gq. Gq) +1.7%
4. Myxoploid/Mosaic -2.3 to 2.5% (46 XX or XY/47, XX or XY + G)

• Physical and Physiological Features of Down's Syndrome:

1. Mentally retarded child.2. I Q ≤ 50[3])
2. Dull and happy looking.
3. Less sensitive to external stimuli.
4. Individuals having very low birth-weight (mean = 2.83 kg.)
5. Skull is brachycephalic (short from front to back).
6. A round, full face with epicanthic folds in their eyes. Here upper eyelid covers the lowereyelid. Brush filled spots i.e., light speckles around the margin of the iris, are present.
7. Nose from root to tip is short as well as flat and mouth with the lips in the shape of a Cupid'sbow.
8. Small, rotated ear.
9. A creased tongue.
10. Short stature, stubby hands and feet.
11. On the palm, the two normal more or less diagonal main creases may be replaced by asingle transverse crease — "the so-called Simian crease".
12. Dermatoglyphics increased ulnar loops on fingertips (except the index finger and thumb).
13. Usually loops in the fingertips.
14. Little finger short, flexion is usually absent, incurved.
15. Feet normal but with wide gap between the 1st and 2nd toes.
16. A few babies have serious intestinal malformations, such as duodenal atresia.
17. Congenital heart defects.
18. No sexual maturity.
19. In males, testicular degeneration and infertility seem to be the rule, with usually small,undescended testis.
20. In females the labia majora tend to be large and cushion-like and the labia minora are smallor absent.

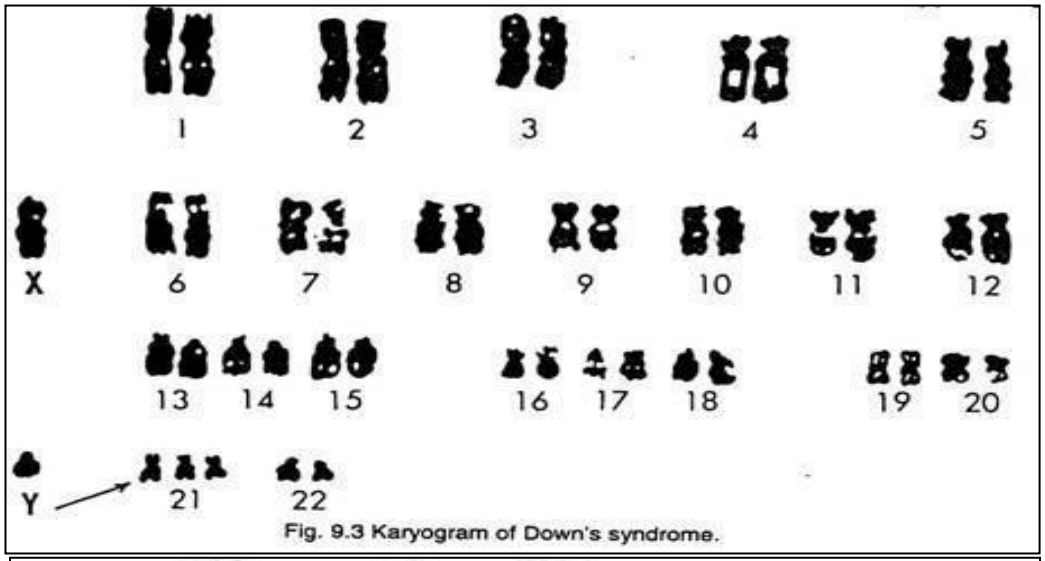


Fig. 9.3 Karyogram of Down's syndrome.

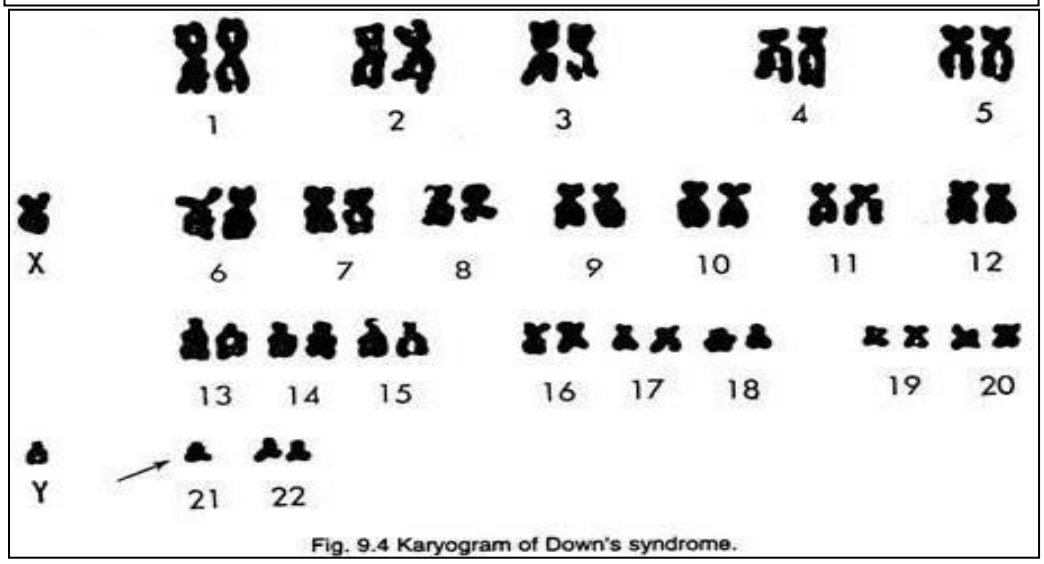


Fig. 9.4 Karyogram of Down's syndrome.

- **Causes of Down's Syndrome:**

After much research on these cell division errors, researchers know that:

- In the majority of cases, the extra copy of chromosome 21 comes from the mother through the egg.
- In a small percentage (less than 5%) of cases, the extra copy of chromosome 21 comes from the father through the sperm.
- In the remaining cases, the error occurs after fertilization, as the embryo grows

Incidence of Down's Syndrome:

Mongoloid idiocy occurs once in each 500 or 600 births, being more frequent when mothers are older than the average. So, the incidence of mongolism in a mongoloid family depends onthe maternal age and not on paternal age.

Age of Mother	Frequency of mongoloid child
20 years	1/30,000
40 years	1/40

- **Diagnosis**

There are two basic types of tests available to detect Down syndrome during pregnancy: screening tests and diagnostic tests. A screening test can tell a woman and her healthcare provider whether her pregnancy has a lower or higher chance of having Down syndrome. Screening tests do not provide an absolute diagnosis, but they are safer for the mother and the developing baby. Diagnostic tests can typically detect whether or not a baby will have Down syndrome, but they can be riskier for the mother and developing baby. Neither screening nor diagnostic tests can predict the full impact of Down syndrome on a baby; no one can predict this.

i. Screening Tests

Screening tests often include a combination of a blood test, which measures the amount of various substances in the mother's blood (e.g., MS-AFP, Triple Screen, Quad-screen), and an ultrasound, which creates a picture of the baby. During an ultrasound, one of the things the technician looks at is the fluid behind the baby's neck. Extra fluid in this region could indicate a genetic problem. These screening tests can help determine the baby's risk of Down syndrome. Rarely, screening tests can give an abnormal result even when there is nothing wrong with the baby. Sometimes, the test results are normal and yet they miss a problem that does exist.

ii. Diagnostic Tests

Diagnostic tests are usually performed after a positive screening test in order to confirm a Down syndrome diagnosis. Types of diagnostic tests include:

- Chorionic villus sampling (CVS)—examines material from the placenta
- Amniocentesis—examines the amniotic fluid (the fluid from the sac surrounding the baby)
- Percutaneous umbilical blood sampling (PUBS)—examines blood from the umbilical cord

These tests look for changes in the chromosomes that would indicate a Down syndrome diagnosis.

- **Epidemiology:**

Down syndrome is the most common chromosomal abnormality in humans. Globally, as of 2010, Down syndrome occurs in about 1 per 1,000 births and results in about 17,000 deaths. More children are born with Down syndrome in countries

where abortion is not allowedand in countries where pregnancy more commonly occurs at a later age. About 1.4 per 1,000 live births in the United States and 1.1 per 1,000 live births in Norway are affected. In the 1950s, in the United States, it occurred in 2 per 1000 live births with the decrease since then due to prenatal screening and abortions. The number of pregnancies with Down syndrome is more than two times greater with many spontaneously aborting.[9] It is the cause of 8% of all congenital disorders.

Maternal age affects the chances of having a pregnancy with Down syndrome. At age 20, the chance is 1 in 1,441; at age 30, it is 1 in 959; at age 40, it is 1 in 84; and at age 50 it is 1 in 44. Although the probability increases with maternal age, 70% of children with Down syndrome are born to women 35 years of age and younger, because younger people have more children. The father's older age is also a risk factor in women older than 35, but not in womenyounger than 35, and may partly explain the increase in risk as women age

- **Prognosis:**

Between 5 and 15% of children with Down syndrome in Sweden attend regular school. Some graduate from high school; however, most do not. Of those with intellectual disability in the United States who attended high school about 40% graduated. Many learn to read and write and some are able to do paid work. In adulthood about 20% in the United States do paid workin some capacity. In Sweden, however, less than 1% have regular jobs. Many are able to live semi-independently, but they often require help with financial, medical, and legal matters. Those with mosaic Down syndrome usually have better outcomes.

Individuals with Down syndrome have a higher risk of early death than the general population. This is most often from heart problems or infections. Following improved medical care, particularly for heart and gastrointestinal problems, the life expectancy has increased. This increase has been from 12 years in 1912, to 25 years in the 1980s, to 50 to60 years in the developed world in the 2000s. Currently between 4 and 12% die in the first yearof life. The probability of long-term survival is partly determined by the presence of heart problems. In those with congenital heart problems, 60% survive to 10 years and 50% survive to 30 years of age. In those without heart problems, 85% survive to 10 years and 80% surviveto 30 years of age. About 10% live to 70 years of age. The National Down Syndrome Society provide information regarding raising a child with Down syndrome

- **Treatment**

There’s no cure for Down syndrome, but treatment is available to help your child reach their full potential. Treatment focuses on helping your child thrive physically and mentally. Treatment options could include:

- Physical or occupational therapy.
- Speech therapy.

- Participating in special education programs in school.
- Treating any underlying medical conditions.
- Wearing glasses for vision problems or assisted hearing devices for hearing loss.

Fragile X syndrome (FXS)

Fragile X syndrome is one of the most common forms of inherited mental retardation with theestimated incidence of 1 in 4000 males and 1 in 8000 females. The syndrome is transmitted as an X-linked dominant trait and with reduced penetrance (80% in males and 30% in females). Fragile X syndrome is associated with a fragile site, designated FRAXA (Fragile site, X chromosome, A site), at Xq27.3 near the end of the long arm. The clinical presentations of fragile X syndrome include mild to severe mental retardation, with IQ between 20 and 60, mildly abnormal facial features of a prominent jaw and large ears, mainly in males, and macroorchidism in post-pubescent males. Many patients also display subtle connective tissue abnormalities, hyperactive and attention deficit disorder and autistic-like behaviour. In 1991, the molecular basis of fragile X syndrome was revealed by positioning cloning and shown to be associated with a massive trinucleotide repeat expansion within the gene *fragile X mental retardation-1* (*FMR1*). This was one of the first identified human disorders caused by dynamic mutation, trinucleotide repeat expansion.

Fragile X syndrome has an X-linked dominant inheritance. It is typically caused by an expansion of the CGG triplet repeat within the *FMR1* (fragile X mental retardation 1) gene onthe X chromosome. This results in silencing (methylation) of this part of the gene and a deficiency of the resultant protein (FMRP), which is required for the normal development of connections between neurons. Diagnosis requires genetic testing to determine the number of CGG repeats in the *FMR1* gene. Normally, there are between 5 and 40 repeats; fragile X syndrome occurs with more than 200. A premutation is said to be present when the gene has between 40 and 200 repeats; women with a premutation have an increased risk of having an affected child. Testing for premutation carriers may allow for genetic counselling.

There is no cure. Early intervention is recommended, as it provides the most opportunity for developing a full range of skills. These interventions may include special education, speech therapy, physical therapy, or behavioural therapy. Medications may be used to treat associated seizures, mood problems, aggressive behaviour, or ADHD. Fragile X syndrome is estimated to occur in 1.4 in 10,000 males and 0.9 in 10,000 females.

▪ Signs and symptoms

People with Fragile X do not all have the same signs and symptoms, but they do have some things in common. Symptoms are often milder in females than in males.

- **Intelligence and learning.** Many people with Fragile X have problems with intellectual functioning.
    - These problems can range from the mild, such as learning disorders or problems with mathematics, to the severe, such as an intellectual or developmental disability.
    - The syndrome may affect the ability to think, reason, and learn.
    - Because many people with Fragile X also have attention disorders, hyperactivity, anxiety, and language-processing problems, a person with Fragile X may have more capabilities than his or her IQ (intelligence quotient) score suggests.
- **Physical.** Most infants and younger children with Fragile X don't have any specific physical features of this syndrome. When these children start to go through puberty, however, many will begin to develop certain features that are typical of those with Fragile X.
    - These features include a narrow face, large head, large ears, flexible joints, flat feet, and a prominent forehead.
    - These physical signs become more obvious with age.
- **Behavioral, social, and emotional.** Most children with Fragile X have some behavioral challenges.
    - They may be afraid or anxious in new situations.
    - They may have trouble making eye contact with other people.
    - Boys, especially, may have trouble paying attention or be aggressive.
    - Girls may be shy around new people. They may also have attention disorders and problems with hyperactivity.
- **Speech and language.** Most boys with Fragile X have some problems with speech and language.
    - They may have trouble speaking clearly, may stutter, or may leave out parts of words. They may also have problems understanding other people's social cues, such as tone of voice or specific types of body language.
    - Girls usually do not have severe problems with speech or language.
    - Some children with Fragile X begin talking later than typically developing children. Most will talk eventually, but a few might stay nonverbal throughout their lives.
- **Sensory.** Many children with Fragile X are bothered by certain sensations, such as bright light, loud noises, or the way certain clothing feels on their bodies.

- These sensory issues might cause them to act out or display behavior problems.

Cause of Fragile X Syndrome

Fragile X syndrome is a genetic disorder which occurs as a result of a mutation of the *fragile X mental retardation 1* (*FMR1*) gene on the X chromosome, most commonly an increase in thenumber of CGG trinucleotide repeats in the 5' untranslated region of *FMR1*. Mutation at that site is found in 1 out of about every 2000 males and 1 out of about every 259 females. Incidenceof the disorder itself is about 1 in every 3600 males and 1 in 4000–6000 females. Although thisaccounts for over 98% of cases, FXS can also occur as a result of point mutations affecting *FMR1*.

In unaffected individuals, the *FMR1* gene contains 5–44 repeats of the sequence CGG, most commonly 29 or 30 repeats. Between 45-54 repeats is considered a "grey zone", with a premutation allele generally considered to be between 55 and 200 repeats in length. Individualswith fragile X syndrome have a full mutation of the *FMR1* allele, with over 200 CGG repeats. In these individuals with a repeat expansion greater than 200, there is methylation of the CGG repeat expansion and *FMR1* promoter, leading to the silencing of the *FMR1* gene anda lack of its product.

this methylation of *FMR1* in chromosome band Xq27.3 is believed to result in constriction ofthe X chromosome which appears 'fragile' under the microscope at that point, a phenomenon that gave the syndrome its name. One study found that FMR1 silencing is mediated by the FMR1 mRNA. The FMR1 mRNA contains the transcribed CGG-repeat tract as part of the 5' untranslated region, which hybridizes to the complementary CGG-repeat portion of the FMR1gene to form an RNA·DNA duplex. A subset of people with intellectual disability and symptoms resembling fragile X syndrome are found to have point mutations in *FMR1.* This subset lacked the CGG repeat expansion in *FMR1* traditionally associated with fragile xsyndrome.

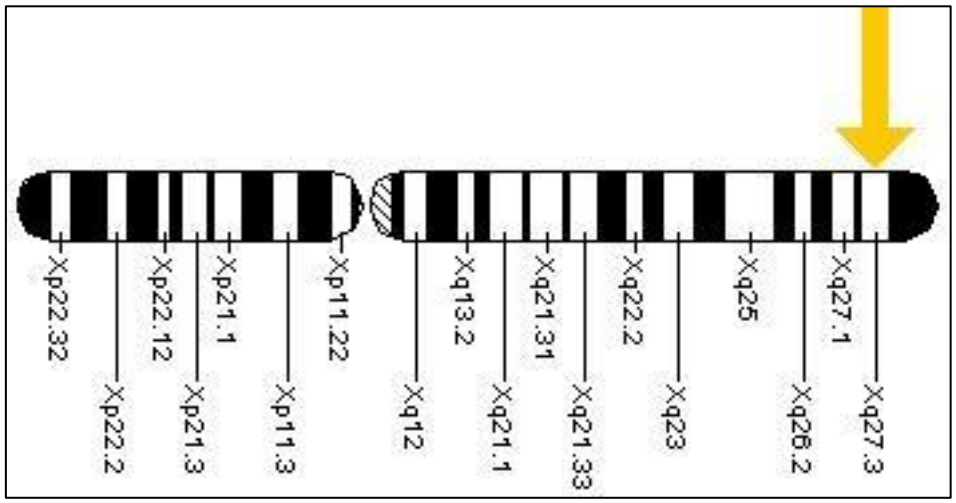


Figure: Location of the FMR1 gene on the X chromosome

- **Inheritance:**

Fragile X syndrome has traditionally been considered an X-linked dominant condition with variable expressivity and possibly reduced penetrance. However, due to genetic anticipation and X-inactivation in females, the inheritance of Fragile X syndrome does not follow the usual pattern of X-linked dominant inheritance, and some scholars have suggested discontinuing labelling X-linked disorders as dominant or recessive. Females with full FMR1 mutations may have a milder phenotype than males due to variability in X-inactivation.

Before the *FMR1* gene was discovered, analysis of pedigrees showed the presence of male carriers who were asymptomatic, with their grandchildren affected by the condition at a higherrate than their siblings suggesting that genetic anticipation was occurring. This tendency for future generations to be affected at a higher frequency became known as the Sherman paradox after its description in 1985. Due to this, male children often have a greater degree ofsymptoms than their mothers.

The explanation for this phenomenon is that male carriers pass on their premutation to all of their daughters, with the length of the *FMR1* CGG repeat typically not increasing during meiosis, the cell division that is required to produce sperm. Incidentally, males with a full mutation only pass on pre-mutations to their daughters. However, females with a full mutation are able to pass this full mutation on, so theoretically there is a 50% chance that a child will be affected. In addition, the length of the CGG repeat frequently does increase duringmeiosis in female premutation carriers due to instability and so, depending on the length of their premutation, they may pass on a full mutation to their children who will then be affected. Repeat expansion is considered to be a consequence of strand slippage either during DNA replication or DNA repair synthesis.

- **Diagnosis:**

These tests can be done during pregnancy to see if an unborn baby has fragile X:

- **Amniocentesis** -- doctors check a sample of amniotic fluid for the FMR1 gene change
- **Chorionic villus sampling (CVS)** -- doctors test a sample of cells from the placenta to check for the FMR1 gene

After the child is born, a blood test can diagnose fragile X syndrome. This test looks for the FMR1 gene change.

Babies born with fragile X syndrome don't always show signs of it. The doctor might notice that the baby's head is larger than usual. As the child gets older, learning and behavior problems can start.

Treatments

There is no cure for FXS. However, treatment services can help people learn important skills. Services can include therapy to learn to talk, walk, and interact with others. In addition, medicine can be used to help control some issues, such as behavior problems. To develop the best treatment plan, people with FXS, parents, and health care providers should work closely with one another, and with everyone involved in treatment and support—which may include teachers, childcare providers, coaches, therapists, and other family members. Taking advantage of all the resources available will help guide success.

Pathophysiology

Pathophysiology (or physiopathology) is a branch of study, at the intersection of pathology and physiology, concerning disordered physiological processes that cause, result from, or are otherwise associated with a disease or injury.

FMRP is found throughout the body, but in highest concentrations within the brain and testes. Itappears to be primarily responsible for selectively binding to around 4% of mRNA in mammalian brains and transporting it out of the cell nucleus and to the synapses of neurons. Most of these mRNA targets have been found to be located in the dendrites of neurons, and brain tissue from humans with FXS and mouse models shows abnormal dendritic spines, which are required to increase contact with other neurons. The subsequent abnormalities in the formation and function of synapses and development of neural circuits result in impaired neuroplasticity, an integral part of memory and learning. Connectome changes have long been suspected to be involved in the sensory pathophysiology and most recently a range of circuit alterations have been shown, involving structurally increased local connectivity andfunctionally decreased long-range connectivity.

In addition, FMRP has been implicated in several signalling pathways that are being targeted by a number of drugs undergoing clinical trials. The group 1 metabotropic glutamate receptor (mGluR) pathway, which includes mGluR1 and mGluR5, is involved in mGluR-dependent long term depression (LTD) and long term potentiation (LTP), both of which are important mechanisms in learning.[11][13] The lack of FMRP, which represses mRNA production and thereby protein synthesis, leads to exaggerated LTD. FMRP also appears to affect dopamine pathways in the prefrontal cortex which is believed to result in the attention deficit, hyperactivity and impulse control problems associated with FXS. The downregulation of GABA pathways, which serve an inhibitory function and are involved in learning and memory, may be a factor in the anxiety symptoms which are commonly seen in FXS.

- **Prognosis:**

A 2013 review stated that life expectancy for FXS was 12 years lower than the general population and that the causes of death were similar to those found for the general population.

Turner's Syndrome

Turner's Syndrome, a condition that affects only females, results when one of the X chromosomes (sex chromosomes) is missing or partially missing. Turner's Syndrome can cause a variety of medical and developmental problems, including short height, failure of the ovaries to develop and heart defects.

Turner's Syndrome may be diagnosed before birth (prenatally), during infancy or in early childhood. Occasionally, in females with mild signs and symptoms of Turner's Syndrome, the diagnosis is delayed until the teen or young adult years.

Girls and women with Turner syndrome need ongoing medical care from a variety of specialists. Regular checkups and appropriate care can help most girls and women lead healthy, independent lives.

Frequency

This condition occurs in about 1 in 2,000 newborns worldwide who were assigned female at birth. However, this condition is much more common among pregnancies that do not survive to term (miscarriages and stillbirths).

Symptoms

Signs and symptoms of Turner's Syndrome may vary among girls and women with the disorder. For some girls, the presence of Turner syndrome may not be readily apparent, but in other girls, several physical features are apparent early. Signs and symptoms can be subtle, developing slowly over time, or significant, such as heart defects.

i. Before birth

Turner's Syndrome may be suspected prenatally based on prenatal cell-free DNA screening — a method to screen for certain chromosomal abnormalities in a developing baby using a blood sample from the mother — or prenatal ultrasound. Prenatal ultrasound of a baby with Turner syndrome may show:

- Large fluid collection on the back of the neck or other abnormal fluid collections (edema)
- Heart abnormalities
- Abnormal kidneys

ii. At birth or during infancy

Signs of Turner's Syndrome at birth or during infancy may include:

- Wide or weblike neck

- Low-set ears
- Broad chest with widely spaced nipples
- High, narrow roof of the mouth (palate)
- Arms that turn outward at the elbows
- Fingernails and toenails that are narrow and turned upward
- Swelling of the hands and feet, especially at birth
- Slightly smaller than average height at birth
- Slowed growth
- Cardiac defects
- Low hairline at the back of the head
- Receding or small lower jaw
- Short fingers and toes

iii. In childhood, teens and adulthood

The most common signs in almost all girls, teenagers and young women with Turner's Syndrome are short stature and ovarian insufficiency due to ovarian failure. Failure of the ovaries to develop may occur at birth or gradually during childhood, the teen years or young adulthood. Signs and symptoms of these include:

- Slowed growth
- No growth spurts at expected times in childhood
- Adult height significantly less than might be expected for a female member of the family
- Failure to begin sexual changes expected during puberty
- Sexual development that "stalls" during teenage years
- Early end to menstrual cycles not due to pregnancy
- For most females with Turner syndrome, inability to conceive a child without fertility treatment

Causes

Most people are born with two sex chromosomes. Males inherit the X chromosome from their mothers and the Y chromosome from their fathers. Females inherit one X chromosome from each parent. In females who have Turner syndrome, one copy of the X chromosome is missing, partially missing or changed.

The genetic changes of Turner's Syndrome may be one of the following:

- **Monosomy.** The complete absence of an X chromosome generally occurs because of an error in the father's sperm or in the mother's egg. This results in every cell in the body having only one X chromosome.
- **Mosaicism.** In some cases, an error occurs in cell division during early stages of fetal development. This results in some cells in the body having two complete copies of the X chromosome. Other cells have only one copy of the X chromosome.
- **X chromosome changes.** Changed or missing parts of one of the X chromosomes can occur. Cells have one complete and one altered copy. This error can occur in the sperm or egg with all cells having one complete and one altered copy. Or the error can occur in cell division in early fetal development so that only some cells contain the changed or missing parts of one of the X chromosomes (mosaicism).
- **Y chromosome material.** In a small percentage of Turner syndrome cases, some cells have one copy of the X chromosome and other cells have one copy of the X chromosome and some Y chromosome material. These individuals develop biologically as female, but the presence of Y chromosome material increases the risk of developing a type of cancer called gonadoblastoma.

Risk factors

The loss or alteration of the X chromosome occurs randomly. Sometimes, it's because of a problem with the sperm or the egg, and other times, the loss or alteration of the X chromosome happens early in fetal development.

Family history doesn't seem to be a risk factor, so it's unlikely that parents of one child with Turner syndrome will have another child with the disorder.

Complications

Turner’s Syndrome can affect the proper development of several body systems, but this varies greatly among individuals with the syndrome. Complications that can occur include:

- **Heart problems.** Many infants with Turner syndrome are born with heart defects or even slight abnormalities in heart structure that increase their risk of serious complications. Heart defects often include problems with the aorta, the large blood vessel that branches off the heart and delivers oxygen-rich blood to the body.
- **High blood pressure.** Turner syndrome can increase the risk of high blood pressure — a condition that increases the risk of developing diseases of the heart and blood vessels.
- **Hearing loss.** Hearing loss is common with Turner syndrome. In some cases, this is due to the gradual loss of nerve function. An increased risk of frequent middle ear infections can also result in hearing loss.

- **Vision problems.** An increased risk of weak muscle control of eye movements (strabismus), nearsightedness and other vision problems can occur with Turner syndrome.
- **Kidney problems.** Turner syndrome may be associated with malformations of the kidneys. Although these abnormalities generally don't cause medical problems, they may increase the risk of urinary tract infections.
- **Autoimmune disorders.** Turner syndrome can increase the risk of an underactive thyroid (hypothyroidism) due to the autoimmune disorder Hashimoto's thyroiditis. There is also an increased risk of diabetes. Sometimes Turner syndrome is associated with gluten intolerance (celiac disease) or inflammatory bowel disease.
- **Skeletal problems.** Problems with the growth and development of bones increase the risk of abnormal curvature of the spine (scoliosis) and forward rounding of the upper back (kyphosis). Turner syndrome can also increase the risk of developing weak, brittle bones (osteoporosis).
- **Learning disabilities.** Girls and women with Turner syndrome usually have normal intelligence. However, there is increased risk of learning disabilities, particularly with learning that involves spatial concepts, math, memory and attention.
- **Mental health issues.** Girls and women with Turner syndrome may have challenges functioning in social situations, may experience anxiety and depression, and may have an increased risk of attention-deficit/hyperactivity disorder (ADHD).
- **Infertility.** Most females with Turner syndrome are infertile. However, a very small number may become pregnant spontaneously, and some can become pregnant with fertility treatment.
- **Pregnancy complications.** Because women with Turner syndrome are at increased risk of complications during pregnancy, such as high blood pressure and aortic dissection, they should be evaluated by a heart specialist (cardiologist) and a high-risk pregnancy doctor (maternal-fetal medicine specialist) before pregnancy.

Diagnosis

Healthcare providers may diagnose Turner syndrome at any stage of a child's development after birth. Sometimes, they can detect the condition before birth with the following tests:

Noninvasive prenatal testing (NIPT): This is a screening blood test for the pregnant parent. It checks for signs that show an increased chance of a chromosomal issue with the fetus. Not every pregnant person gets this type of screening.

Ultrasound during pregnancy: An ultrasound may show that the fetus has some physical features of TS, like heart problems or fluid around the neck. They'll likely request amniocentesis or chorionic villus sampling to help confirm the diagnosis.

Amniocentesis and chorionic villus sampling: These tests check the amniotic

fluid or tissue from the placenta. Providers perform a genetic test with karyotype analysis on the fluid or tissue, which can confirm if the fetus has Turner syndrome.

Treatment

There's no cure for Turner syndrome. But certain medications and therapies can help manage its symptoms.

Besides care for related medical problems (like heart conditions), Turner syndrome treatment often focuses on hormones. Treatments may include:

Human growth hormone therapy: Injections of human growth hormone help with vertical growth. If treatment starts early enough, these shots can increase your child's final height by several inches.

Estrogen therapy: Often, people with TS have low levels of estrogen, a sex hormone, which impacts their sexual development. Estrogen can help with breast development and menstruation. Estrogen replacement improves brain development, heart function, liver function and bone health, too.

Cyclic progestins: These medications induce regular menstrual periods. Healthcare providers typically start them around the age of 11 or 12.

Probable Questions:

1. Discuss the causes of Down Syndrome.
2. How Down syndrome can be diagnosed before birth?
3. How Down syndrome can be diagnosed after birth?
4. Discuss about prognosis of Down Syndrome?
5. Discuss about epidemiology of Down syndrome.
6. What are the causes of Fragile X syndrome?
7. Discuss about signs and symptoms of Fragile X syndrome.
8. Discuss epidemiology of Fragile X syndrome.
9. Discuss prognosis of Fragile X syndrome.
10. What is the pathophysiology of Fragile X syndrome.
11. Discuss about diagnosis of Fragile X syndrome.
12. Define amniocentesis? How it is done?
13. Discuss about signs and symptoms of Turner's Syndrome.
14. Discuss epidemiology of Turner's Syndrome.

15. Discuss prognosis of Turner's Syndrome.

Suggested Readings:

1. Principles of Genetics. Snustad and Simmons.
2. Genetics . Verma and Agarwal.
3. Principles of Genetics by Tamarin.
4. Biotechnology by V. KumaresanEmbryology by N. Kumarsen

Unit-IV

Future of medicine: Differentiation therapy, gene therapy (Ex Vivo and In vivo), germ line gene therapy

Objective: In this unit we will discuss about Future of medicines which includes differentiation therapy, ex vivo and in vivo gene therapy and also germ line gene therapy.

Differentiation therapy

An approach to the treatment of advanced or aggressive malignancies in which the malignant cells are treated so that they can resume the process of maturation and differentiation into mature cells. Differentiation therapy is based on the conceptthat cancer cells are normal cells that have been arrested at or have gone back to an immature or less differentiated state, lack the ability to control their own growth, and so multiply at an abnormally fast rate. Differentiation therapy aims to force the cancer cell to resume the processof maturation. Although differentiation therapy does not destroy the cancer cells, it restrains their growth and allows the application of more conventional therapies (such as chemotherapy)to eradicate the malignant cells. Differentiation agents tend to have less toxicity than conventional cancer treatments.

The first differentiation agent found to be successful was all-trans-retinoic acid (ATRA) in thetreatment of acute promyelocytic leukaemia (APL). APL is the result of a translocation (an exchange of chromosome material) between chromosomes 15 and 17. There are two chromosome breaks: one in chromosome 15 and the other in chromosome 17. The break in chromosome 15 disrupts the promyelocytic leukaemia (PML) gene which encodes a growth suppressing transcription factor. And the break in chromosome 17 interrupts the retinoic acid receptor alpha (RARa) gene which regulates myeloid differentiation. The translocation creates a PML/RARa fusion gene. It produces an abnormal protein referred to as a chimeric protein that causes an arrest of maturation in myeloid cell maturation at the promyelocytic stage. (It reduces terminal cell differentiation.) And this causes the increased proliferation of promyelocytes.

Traditional chemotherapy or radiotherapy generally involves killing tumour cells . However, cancer cells may instead be coaxed into becoming normal cells by differentiation therapy, which aims to reactivate endogenous differentiation programs in cancer cells to resume the maturation process and eliminate tumour phenotypes (Figure below). Generally, differentiation agents tend to have less toxicity than conventional cancer treatments. A prototype differentiation therapy is all-trans-retinoic acid (ATRA), which induces complete remission inpatients with acute promyelocytic leukaemia (APL). ATRA induces terminal cell differentiation by disrupting the promyelocytic leukaemia/retinoic acid receptor α (PML/RARα) fusion protein that arrests the maturation of myeloid cells at the promyelocytic stage. Subsequently, emerging studies have focused on elucidating the mechanisms of action of differentiation therapy in cancers, particularly in solid tumours.

Most APL patients are now treated first with all-trans-retinoic acid (ATRA). It causes the promyelocytes to differentiate (to mature) and so deters them from proliferating. ATRA induces a complete remission in about 70% of cases. ATRA is the prototype of a differentiationtherapy agent.

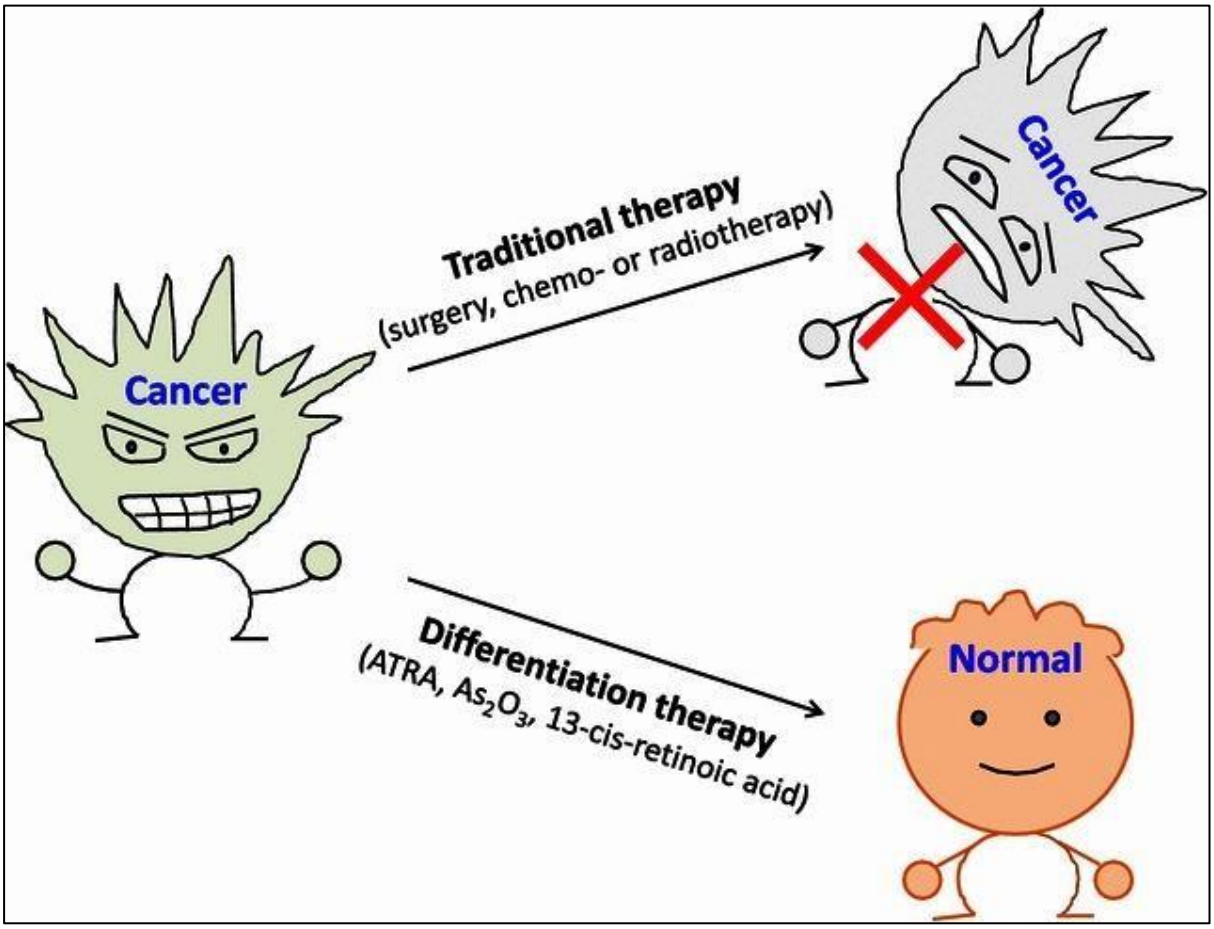


Figure: Diagram of differentiation therapy. Compared with traditional cancer treatments, such as surgery, chemotherapy, and radiotherapy that aim to kill tumour cells, differentiation therapy has opened a new door for the treatment of malignant tumours. Differentiation therapy is based on the concept that a neoplasm is a differentiation disorder or a dedifferentiation disease. In response to the induction of differentiation, tumour cells can revert to normal or nearly normal cells, thereby alteringtheir malignant phenotype and ultimately alleviating the tumour burden or curing the malignant disease without damaging normal cells. ATRA, all-trans-retinoic acid

Gene Therapy

Gene therapy is a novel treatment method which utilizes genes or short oligonucleotide sequences as therapeutic molecules, instead of conventional drug compounds. This technique is widely used to treat those defective genes which contribute to disease development. Gene therapy involves the introduction of one or more foreign genes into an organism to treat hereditary or acquired genetic defects. In gene therapy,

DNA encoding a therapeutic protein is packaged within a "vector", which transports the DNA inside cells within the body. The disease is treated with minimal toxicity, by the expression of the inserted DNA by the cell machinery. In 1990 FDA for the first time approved a gene therapy experiment on ADA-SCID in the United States after the treatment of Ashanti DeSilva. After that, approximately 1700 clinical trials on patients have been performed with various techniques and genes for numerous diseases. Gene therapy is the process of inserting genes into cells to treat diseases. The newly introduced genes will encode proteins and correct the deficiencies that occur in genetic diseases. Thus, gene therapy primarily involves genetic manipulations in animals or humans tocorrect a disease, and keep the organism in good health. The initial experiments on gene therapyare carried out in animals, and then in humans. Obviously, the goal of the researchers is to benefit the mankind and improve their health.

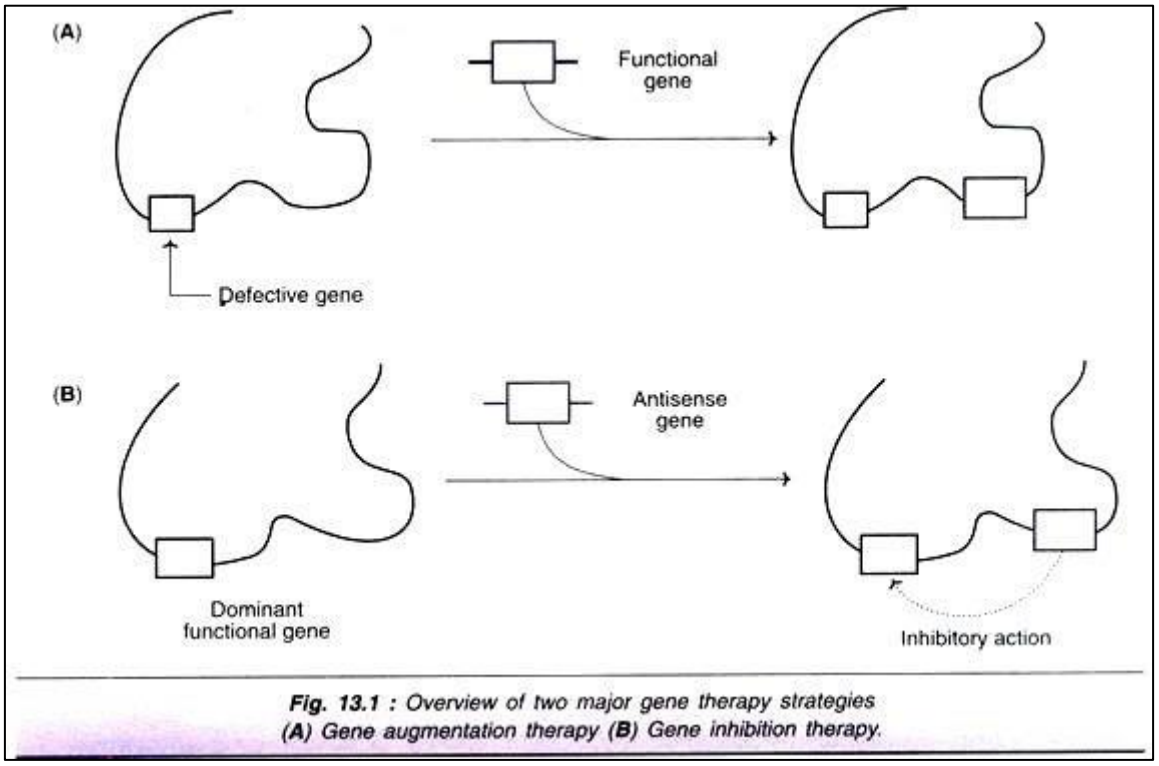


***Fig. 13.1** : Overview of two major gene therapy strategies (A) Gene augmentation therapy (B) Gene inhibition therapy.*

An overview of gene therapy strategies is depicted in Fig. 13.1. In gene augmentation therapy,a DNA is inserted into the genome to replace the missing gene product. In case of gene inhibition therapy, the antisense gene inhibits the expression of the dominant gene.

I. General gene therapy strategies

a. Gene Augmentation Therapy (GAT):

For diseases caused by loss of function of a gene, introducing extra copies of the normal gene may increase the amount of normal gene product to a level where the normal phenotype is restored (see Fig. 23.1). As a result GAT is targeted at clinical disorders where the pathogenesis is reversible. It also helps to have no precise requirement for expression levels of the

introducedgene and a clinical response at low expression levels. GAT has been particularly applied to autosomal recessive disorders where even modest expression levels of an introduced gene maymake a substantial difference.

Dominantly inherited disorders are much less amendable to treatment; gain-of-function mutations are not treatable by this approach and, even if there is a loss-of- function mutation, high expression efficiency of the introduced gene is required: individuals with 50% of normal gene product are normally affected, and so the challenge is to increase the amount of gene product towards normal levels.

b. Targeted Killing of Specific Cells:

This general approach is popular in cancer gene therapies. Genes are directed to the target cellsand then expressed so as to cause cell killing. Direct cell killing is possible if the inserted genesare expressed to produce a lethal toxin (suicide genes), or a gene encoding a pro drug is inserted, conferring susceptibility to killing by a subsequently administered drug. Indirect cell killing uses immunostimulatory genes to provoke or enhance an immune response against thetarget cell.

c. Targeted Mutation Correction:

If an inherited mutation produces a dominant-negative effect, gene augmentation is unlikely tohelp. Instead, the resident mutation must be corrected. Because of practical difficulties, this approach has yet to be applied but, in principle, it can be done at different levels: at the gene level (e.g. by gene targeting methods based on homologous recombination); or at the RNA transcript level (e.g. by using particular types of therapeutic ribozymes — or therapeutic RNA editing).

d. Targeted Inhibition of Gene Expression:

If disease cells display a novel gene product or inappropriate expression of a gene (as in the case of many cancers, infectious diseases, etc.), a variety of different systems can be used specifically to block the expression of a single gene at the DNA, RNA or protein levels. Allele-specific inhibition of expression may be possible in some cases, permitting therapies for some disorders resulting from dominant negative effects.

II. Approaches for Gene Therapy:

There are two approaches to achieve gene therapy.

1. Somatic Cell Gene Therapy:

The non- reproductive (non-sex) cells of an organism are referred to as somatic cells. These are the cells of an organism other than sperm or eggs cells, e.g., bone marrow cells, blood cells,skin cells, intestinal cells. At present, all the research on gene therapy is directed to correct thegenetic defects in somatic cells. In essence, somatic cell gene therapy involves the insertion ofa fully functional and expressible gene into a target somatic cell to correct a genetic disease permanently.

2. Germ Cell Gene Therapy:

The reproductive (sex) cells of an organism constitute germ cell line. Gene therapy involving the introduction of DNA into germ cells is passed on to the successive generations. For safety,ethical and technical reasons, germ cell gene therapy is not being attempted at present.

The genetic alterations in somatic cells are not carried to the next generations. Therefore, somatic cell gene therapy is preferred and extensively studied with an ultimate objective of correcting human diseases. Development of gene therapy in humans for any specific disease involves the following steps. In fact, this is a general format for introducing any therapeutic agent for human use.

a. In vitro experiments and research on laboratory animals (pre-clinical trials).

b. Phase I trials with a small number (5-10) of human subjects to test safety of the product.

c. Phase II trials with more human subjects to assess whether the product is helpful.

d. Phase III trials in large human samples for a final and comprehensive analysis of the safetyand efficacy of the product.

III. Methods of gene therapy:

There are mainly two approaches for the transfer of genes in gene therapy:

1. Transfer of genes into patient cells outside the body (ex vivo gene therapy)

2. Transfer of genes directly to cells inside the body (in vivo).

1. Ex vivo gene therapy:

The ex vivo gene therapy can be applied to only selected tissues (e.g., bone marrow)

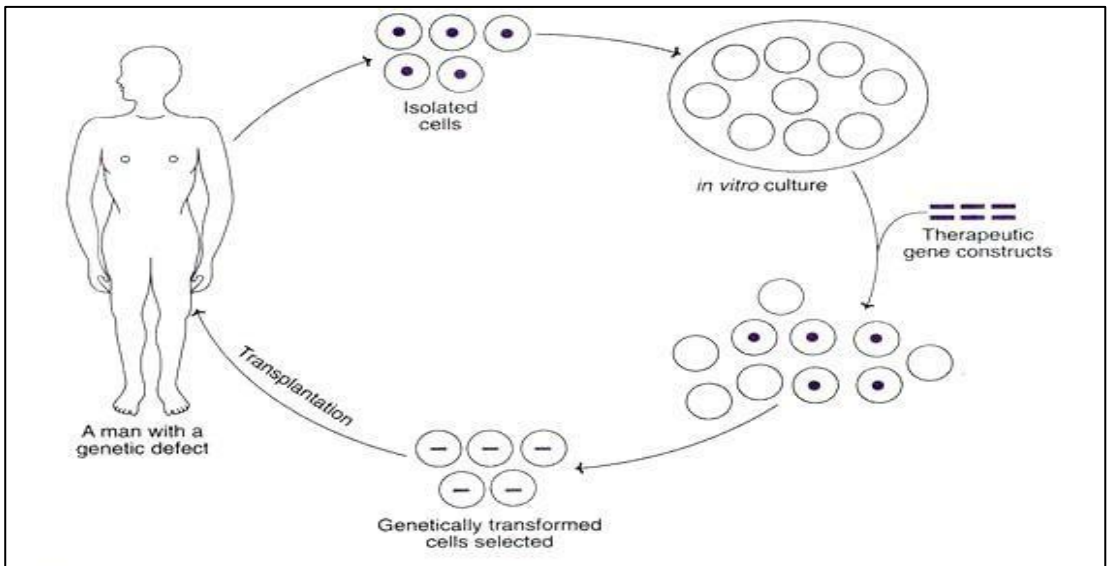


Fig. 13.2 : The procedure for ex vivo gene therapy.

whose cells can be cultured in the laboratory. The technique of ex vivo gene therapy involves the following steps (Fig. 13.2).

1. Isolate cells with genetic defect from a patient.
2. Grow the cells in culture.
3. Introduce the therapeutic gene to correct gene defect.
4. Select the genetically corrected cells (stable trans-formants) and grow.
5. Transplant the modified cells to the patient.

The procedure basically involves the use of the patient's own cells for culture and genetic correction, and then their return back to the patient. This technique is therefore, not associatedwith adverse immunological responses after transplanting the cells. Ex vivo gene therapy is efficient only, if the therapeutic gene (remedial gene) is stably incorporated and continuously expressed. This can be achieved by use of vectors.

Vectors in Gene Therapy:

The carrier particles or molecules used to deliver genes to somatic cells are referred to as vectors. The important vectors employed in ex vivo gene therapy are listed below and briefly described next.

i. Viruses

ii. Human artificial chromosome

iii. Bone marrow cells.

i. Viruses:

The vectors frequently used in gene therapy are viruses, particularly retroviruses. RNA is the genetic material in retroviruses. As the retrovirus enters the host cell, it synthesizes DNA from RNA (by reverse transcription). The so formed viral DNA (referred to as provirus) gets incorporated into the DNA of the host cell.

AIDS virus in gene therapy?

It is suggested that the human immunodeficiency virus (HIV) can be used as a vector in gene transfer. But this is bound to create public uproar. Some workers have been successful in creating a harmless HIV (crippled HIV) by removing all the genes related to reproduction. Atthe same time, the essential genes required for gene transfer are retained. There is a distinct advantage with HIV when compared with MLV. MLV is capable of bringing out gene transferonly in dividing cells. HIV can infect even non-dividing cells (e.g., brain cells) and do the jobof gene transfer effectively.

However, it is doubtful whether HIV can ever be used as a vector.

ii. Human Artificial Chromosome:

The details of human artificial chromosome (HAC) are described elsewhere .HAC is a synthetic chromosome that can replicate with other chromosomes, besides encoding a human protein. As already discussed above, use of retroviruses as vectors in gene therapy is associatedwith a heavy risk. This problem can be overcome if HAC is used. Some success has been achieved in this direction.

iii. Bone Marrow Cells:

Bone marrow contains totipotent embryonic stem (ES) cells. These cells are capable of dividing and differentiating into various cell types (e.g., red blood cells, platelets, macrophages, osteoclasts, B- and T-lymphocytes). For this reason, bone marrow transplantation is the most widely used technique for several genetic diseases.

- **Selected Examples of Ex Vivo Gene Therapy:**

a. Therapy for Adenosine Deaminase Deficiency:

The first and the most publicized human gene therapy was carried out to correct the deficiencyof the enzyme adenosine deaminase (ADA).

b. Severe combined immunodeficiency (SCID):

This is rare inherited immune disorder associated with T-lymphocytes, and (to a lesser extent)B-lymphocytes dysfunction. About 50% of SCID patients have a defect in the gene (located onchromosome 20, and has 32,000 base pairs and 12 exons) that encodes for adenosine deaminase. In the deficiency of ADA, deoxyadenosine and its metabolites (primarily deoxyadenosine 5′-triphosphate) accumulate and destroy T-lymphocytes.

c. Therapy for Familial Hypercholesterolemia:

The patients of familial hypercholesterolemia lack the low-density lipoprotein (LDL) receptorson their liver cells. As a result, LDL cholesterol is not metabolised in liver. The accumulatedLDL- cholesterol builds up in the circulation, leading to arterial blockage and heart diseases. Attempts are being made by gene therapists to help the victims of familial hypercholesterolemia. In fact, there is some success also. In a woman, 15% of the liver was removed. The hepatocytes were transduced with retroviruses carrying genes for LDL receptors.These genetically modified hepatocytes were infused into the patient's liver.

The hepatocytes established themselves in the liver and produced functional LDL-receptors. Asignificant improvement in the patient's condition, as assessed by estimating the lipid parameters in blood, was observed. Further, there were no antibodies produced against the LDL-receptor molecules, clearly showing that the genetically modified liver cells were accepted.

d. Therapy for Lesch-Nyhan Syndrome:

Lesch-Nyhan syndrome is an inborn error in purine metabolism due to a defect in a gene that encodes for the enzyme hypoxanthine-guanine phosphoribosyl transferase (HCPRT). In the absence of HGPRT, purine metabolism is disturbed and uric acid level builds up, resulting in severe gout and kidney damage. The victims of Lesch- Nyhan syndrome exhibit symptoms ofmental retardation, besides an urge to bite lips and fingers, causing self-mutilation.

By using retroviral vector system, HGPRT producing genes were successfully inserted into cultured human bone marrow cells. The major problem in humans is the involvement of brain. Experiments conducted in animals are encouraging. However, it is doubtful whether good success can be achieved by gene therapy for Lesch-Nyhan syndrome in humans, in the near future.

e. Therapy for Haemophilia:

Haemophilia is a genetic disease due lack of a gene that encodes for clotting factor IX. It is characterized by excessive bleeding. By using a retroviral vector system, genes for thesynthesis of factor IX were inserted into the liver cells of dogs. These dogs no longer displayedthe symptoms of haemophilia.

2.In Vivo Gene Therapy:

The direct delivery of the therapeutic gene (DNA) into the target cells of a particular tissue ofa patient constitutes in vivo gene therapy (Fig. 13.6). Many tissues are the potential candidatesfor this approach. These include liver, muscle, skin, spleen, lung, brain and blood cells. Gene delivery can be carried out by viral or non- viral vector systems. The success of in vivo gene therapy mostly depends on the following parameters

i. The efficiency of the uptake of the remedial (therapeutic) gene by the target cells.

ii. Intracellular degradation of the gene and its uptake by nucleus.

iii. The expression capability of the gene.

In vivo gene therapy with special reference to gene delivery systems (viral, non-viral) with suitable example is described.

Therapy for cystic fibrosis:

Cystic fibrosis (CF) is one of the most common (frequency 1: 2,500) and fatal genetic diseases.It is characterized by the accumulation of sticky, dehydrated mucus in the respiratory tract an

lungs. Patients of CF are highly susceptible to bacterial infections in their lungs and most of them die before reaching the age of thirty.

Biochemical basis:

In the normal persons the chloride ions of the cells are pushed out through the participation ofa protein called cystic fibrosis trans membrane regulator (CFTR). In the patients of cystic fibrosis, the CFTR protein is not produced due to a gene defect. Consequently, the chloride ions concentrate within the cells which draw water from the surroundings. As a result, the respiratory tract and the lungs become dehydrated with sicky mucus, an ideal environment forbacterial infections.

Gene therapy for Cystic Fibrosis:

As the defective gene for cystic fibrosis was identified in 1989, researchers immediately startedworking on gene therapy for this disease. Adenoviral vector systems have been used, althoughthe success has been limited. The major drawback is that the benefits are short-lived, since theadenoviruses do not integrate themselves into host cells. Multiple administration of recombinant adenovirus caused immunological responses that destroyed the cells.

By using adeno-associated virus vector system, some encouraging results were reported in thegene therapy of CF. In the phase I clinical trials with CF patients, the vector persisted for about 70 days and some improvement was observed in the patients. Some researchers are trying to insert CF gene into the developing fetal cells (in experimental animals such as mice) to produce CFTR protein. But a major breakthrough is yet to come.

Gene Delivery by Non-Viral Systems:

There are certain limitations in using viral vectors in gene therapy. In addition to the prohibitive cost of maintaining the viruses, the viral proteins often induce inflammatory responses in the host. Therefore, there is a continuous search by researchers to find alternatives to viral vector systems.

a. Pure DNA constructs:

The direct introduction of pure DNA constructs into the target tissue is quite simple. However,the efficiency of DNA uptake by the cells and its expression are rather low. Consequently, large quantities of DNA have to be injected periodically. The therapeutic genes produce the proteins in the target cells which enter the circulation and often get degraded.

b. Lipoplexes:

The lipid-DNA complexes are referred to as lipoplexes or more commonly liposomes.

They have a DNA construct surrounded by artificial lipid layers. A large number of lipoplexes havebeen prepared and used. They are non-toxic and non-immunogenic.

The major limitation with the use of lipoplexes is that as the DNA is taken up by the cells, mostof it gets degraded by the lysosomes. Thus, the efficiency of gene delivery by lipoplex is verylow. Some clinical trials using liposome-CFTR gene complex showed that the gene expressionwas very short-lived.

c. DNA-molecular conjugates:

The use of DNA-molecular conjugates avoids the lysosomal breakdown of DNA. Another advantage of using conjugates is that large-sized therapeutic DNAs (> 10 kb) can be delivered to the target tissues. The most commonly used synthetic conjugate is poly-L-lysine, bound to a specific target cell receptor. The therapeutic DNA is then made to combine with the conjugateto form a complex (Fig. 13.7).

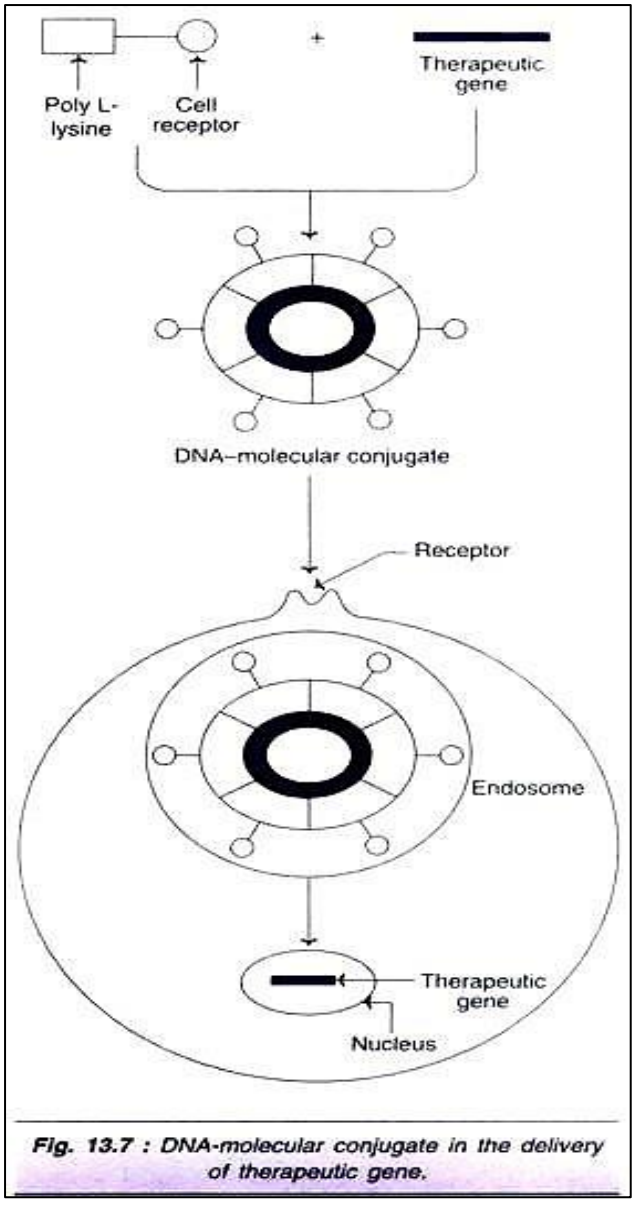


Fig. 13.7 : DNA-molecular conjugate in the delivery of therapeutic gene.

This DNA molecular conjugate binds to specific cell receptor on the target cells. It is engulfed by the cell membrane to form an endosome which protects the DNA from being degraded. TheDNA released from the endosome enters the nucleus where the therapeutic gene is expressed.

d. Human artificial chromosome:

Human artificial chromosome (HAC) which can carry a large DNA one or more therapeutic genes with regulatory elements is a good and ideal vector. Studies conducted in cell cultures using HAC are encouraging. But the major problem is the

delivery of the large-sized chromosome into the target cells. Researchers are working to produce cells containing genetically engineered HAC. There exists a possibility of encapsulating and implanting these cells in the target tissue. But a long way to go!

Gene Therapy Strategies for Cancer:

Cancer is the leading cause of death throughout the world, despite the intensive treatment strategies (surgery, chemotherapy, radiation therapy). Gene therapy is the latest and a new approach for cancer treatment. Some of the developments are briefly described hereunder.

Tumour necrosis factor gene therapy:

Tumour necrosis factor (TNF) is a protein produced by human macrophages. TNF provides defence against cancer cells. This is brought out by enhancing the cancer-fighting ability of tumour- infiltrating lymphocytes (TILs), a special type of immune cells.

The tumour-infiltrating lymphocytes were transformed with a TNF gene (along with a neomycin resistant gene) and used for the treatment of malignant melanoma (a cancer of melanin producing cells usually occurs in skin). TNF as such is highly toxic, and fortunately no toxic side effects were detected in the melanoma patients injected with genetically altered TILs with TNF gene. Some improvement in the cancer patients was observed.

- ## Advantages of Gene Therapy

Gene therapy can cure genetic diseases by addition of gene or by removal of gene or byreplacing a mutated gene with corrected gene.

Gene therapy can be used for cancer treatment to kill the cancerous cells.Gene expression can be controlled.

Therapeutic protein is continuously produced inside the body which also reduces the cost oftreatment in long ter

- ## The Future of Gene Therapy:

Theoretically, gene therapy is the permanent solution for genetic diseases. But it is not as simple as it appears since gene therapy has several inbuilt complexities. Gene therapy broadlyinvolves isolation of a specific gene, making its copies, inserting them into target tissue cells to make the desired protein. The story does not end here.

It is absolutely essential to ensure that the gene is harmless to the patient and it is appropriatelyexpressed (too much or too little will be no good). Another concern in gene therapy is the body's immune system which reacts to the foreign proteins produced by the new genes. The public, in general, have exaggerated expectations on gene therapy. The researchers, at least for the present, are unable to satisfy them. As per the records, by

1999 about 1000 Americans had undergone clinical trials involving various gene therapies.

Unfortunately, the gene therapists are unable to categorically claim that gene therapy has permanently cured any one of these patients. Some people in the media (leading newspapers and magazines) have openly questioned whether it is worth to continue research on gene therapy. It may be true that as of now, gene therapy due to several limitations, has not progressed the way it should, despite intensive research. But a breakthrough may come anytime, and of course, this is only possible with persistent research. And a day may come (it might take some years) when almost every disease will have a gene therapy, as one of the treatment modalities. And gene therapy will revolutionize the practice of medicine.

Germ line gene therapy

A piece of DNA is transferred to cells that create eggs or sperm. Gene therapy's effects will be passed on to the patient's offspring and future generations.

This inserts 'normal' human genes into the parent's eggs or sperm, as well as the offspring's fertilised egg or early embryo. The objective would be to alter the genetic inheritance of the future kid.

This might be done to avert a hereditary ailment or to add a genetic mutation that is 'enhancing'. There have been no human germline gene therapy studies, and there is an unofficial ban on such research in people among the scientific community. Its practicality and value are both unknown.

Other mammals' germlines have been successfully inserted with new genes, although with poor effectiveness. Pre-implantation genetic diagnosis, on the other hand, permits parents to choose embryos based on their genetic differences, as long as the parents developed the desired variants themselves.

Donated eggs or sperm, rather than somatic cell gene therapy, would be a safer and simpler means to transfer the desired genes. Germline gene therapy might be the most significant impediment to somatic cell gene therapy.

Examples- This type of gene therapy is still in its early stage of human medical trials, although instances exist, such as cows changed to produce more milk or release human hormones, or "knockin" and "knockout" mice models, which have been used to understand gene function for decades.

- ### Process

There are various techniques to perform gene therapy:

- **Gene augmentation therapy –** Here a functional gene is introduced, which produces sufficient levels of proteins to compensate for non-functional genes.

This is used to treat disease where there is a loss of function. E.g. cystic fibrosis, ADA deficiency, etc.

- **Gene inhibition therapy –** This is used when a gene activity is altered and needs to be suppressed, e.g. cancer, infectious diseases. Here a gene is introduced, which either inhibits the gene expression of another gene or interferes with the activity of another gene product. E.g. Gene inhibition therapy can be used to eliminate the activity of oncogenes and prevent further uncontrolled growth of cells.
- **Killing Specific Cells –** This is used to destroy a group of cells such as in cancer. Here a specific DNA called suicide DNA is inserted into diseased cells inorder to destroy it. Here the inserted DNA produces a product that helps the immune system to identify and attack those cells. It is important to target only diseased cells so that functional cells do not die

Conclusion

So, in this article, we have read about germline gen, germline gene therapy, its diagram.

It is very controversial to use this therapy a lot of countries have banned it as it violates the right of the ones that are yet to be born as they might get something from the therapy which they may not want when born, also it has huge risks by editing the genes of the future generations as we currently treat it for people who have some issue and this therapy might help them, but same does not go yet to be born ones.

Why is germline gene therapy illegal?

Germline gene therapy has been outright banned in many nations (and others impose heavy regulations) because of ethical concerns, uncertainty of consequences, and safety risks. A few of the reasons why the relatively new practice is ethically questioned relate to concerns over diversity, inclusivity, how it is controlled by parents and doctors, cost, and forensic analysis. Germline gene therapy can also lead to an increased risk of genetic disorders or even death, relating why studies in the field remain ongoing.

Probable questions:

1. Define Gene therapy.
2. What is ex vivo gene therapy and in vivo gene therapy?
3. How gene therapy is used in treatment of Cystic fibrosis?
4. What is are the advantages of gene therapy?
5. Write about the future of gene therapy?
6. How differentiation therapy an be applied in cancer treatment?

Suggested Readings:

1. Principles of Genetics. Snustad and Simmons.
2. Genetics . Verma and Agarwal.
3. Principles of Genetics by Tamarin.
4. Biotechnology by V. Kumaresan

Unit V

Techniques used in Medical Embryology: i) Amniocentesis ii) Chorionic villus sampling iii) Ultrasonography

Objective: In this unit we will learn about some techniques which are used in Medical Embryology like i) Amniocentesis ii) Chorionic villus sampling iii) Ultrasonography

Techniques used in Medical Embryology

A. Amniocentesis:

- **Definition:**

Amniocentesis is a procedure used to diagnose foetal defects in the early second trimester of pregnancy. A sample of the amniotic fluid, which surrounds a foetus in the womb, is collectedthrough a pregnant woman's abdomen using a needle and syringe. Tests performed on foetal cells found in the sample can reveal the presence of many types of genetic disorders, thus allowing doctors and prospective parents to make important decisions about early treatment and intervention.

- **Purpose:**

Since the mid-1970s, amniocentesis has been used routinely to test for Down syndrome , by far the most common, nonhereditary, genetic birth defect, afflicting about one in every 1,000 babies. By 1997, approximately 800 different diagnostic tests were available, most of them forhereditary genetic disorders such as Tay-Sachs disease, sickle cell anaemia, haemophilia, muscular dystrophy, and cystic fibrosis **.**

Amniocentesis, often called amnio, is recommended for women who will be older than 35 on their due-date. It is also recommended for women who have already borne children with birth defects, or when either of the parents has a family history of a birth defect for which a diagnostic test is available. Another reason for the procedure is to confirm indications of Down syndrome and certain other defects which may have shown up previously during routine maternal blood screening. The risk of bearing a child with a nonhereditary genetic defect suchas Down syndrome is directly related to a woman's age—the older the woman, the greater therisk. Thirty-five is the recommended age to begin amnio testing because that is the age at whichthe risk of carrying a foetus with such a defect roughly equals the risk of miscarriage caused by the procedure–about one in 200. At age 25, the risk of giving birth to a child with this typeof defect is about one in 1,400; by age 45 it increases to about one in 20. Nearly half of all pregnant women over 35 in the United States undergo amniocentesis and many younger womenalso decide to have the procedure. Notably, some 75% of all Down syndrome infants born in the United States

each year are to women younger than 35.

One of the most common reasons for performing amniocentesis is an abnormal alpha-fetoprotein (AFP) test. Alpha-fetoprotein is a protein produced by the foetus and present in the mother's blood. A simple blood screening, usually conducted around the 15th week ofpregnancy, can determine the AFP levels in the mother's blood. Levels that are too high or toolow may signal possible foetal defects. Because this test has a high false-positive rate, anothertest such as amnio is recommended whenever the AFP levels fall outside the normal range. Amniocentesis is generally performed during the 16th week of pregnancy, with results usuallyavailable within three weeks. It is possible to perform an amnio as early as the 11th week, butthis is not usually recommended because there appears to be an increased risk of miscarriage when done at this time. The advantage of early amnio and speedy results lies in the extra timefor decision making if a problem is detected. Potential treatment of the foetus can begin earlier.Important, also, is the fact that elective abortions are safer and less controversial the earlier they are performed.

- **Precautions**:

As an invasive surgical procedure, amnio poses a real, although small, risk to the health of a foetus. Parents must weigh the potential value of the knowledge gained, or indeed thereassurance that all is well, against the small risk of damaging what is in all probability a normalfoetus. The serious emotional and ethical dilemmas that adverse test results can bring must alsobe considered. The decision to undergo amnio is always a matter of personal choice.

- **Description:**

The word amniocentesis literally means "puncture of the amnion," the thin-walled sac of fluidin which a developing foetus is suspended during pregnancy. During the sampling procedure,the obstetrician inserts a very fine needle through the woman's abdomen into the uterus and amniotic sac and withdraws approximately one ounce of amniotic fluid for testing. The relatively painless procedure is performed on an outpatient basis, sometimes using local anaesthesia.

The physician uses ultrasound images to guide needle placement and collect the sample, thereby minimizing the risk of foetal injury and the need for repeated needle insertions. Once the sample is collected, the woman can return home after a brief observation period. She may be instructed to rest for the first 24 hours and to avoid heavy lifting for two days. The sample of amniotic fluid is sent to a laboratory where foetal cells contained in the fluid are isolated andgrown in order to provide enough genetic material for testing. This takes about seven to 14 days. The material is then extracted and treated so that visual examination for defects can be made. For some disorders, like Tay-Sachs, the simple presence of a telltale chemical compoundin the amniotic fluid is enough to confirm a diagnosis. Depending on the specific tests ordered,and the skill of the lab conducting them, all the results are available between one and four weeks after the sample is taken. Cost of the procedure depends on the doctor, the lab, and the tests

ordered. Most insurers provide coverage for women over 35, as a follow-up to positive maternal blood screening results, and when genetic disorders run in the family. An alternative to amnio, now in general use, is chorionic villus sampling, or CVS, which can be performed as early as the eighth week of pregnancy. While this allows for the possibility of a first trimester abortion, if warranted, CVS is apparently also riskier and is more expensive. The most promising area of new research in prenatal testing involves expanding the scope and accuracy of maternal blood screening as this poses no risk to the foetus.

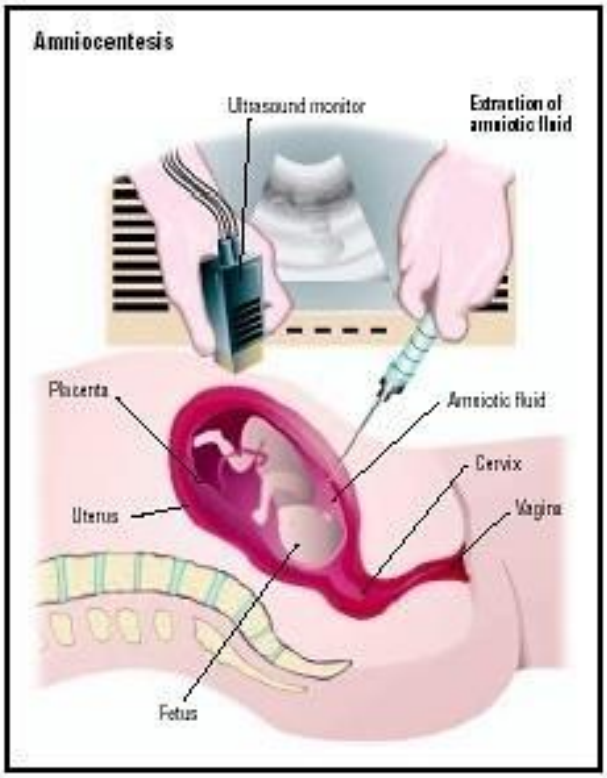


Figure: To perform amniocentesis, a physician uses an ultrasound monitor to visualize the foetus while inserting a syringe to extract amniotic fluid for analysis.

- **Preparation:**

It is important for a woman to fully understand the procedure and to feel confident in the obstetrician performing it. Evidence suggests that a physician's experience with the procedure reduces the chance of mishap. Almost all obstetricians are experienced in performing amniocentesis. The patient should feel free to ask questions and seek emotional support before,during and after the amnio is performed.

Aftercare: Necessary aftercare falls into two categories, physical and emotional.

Physical after care: During and immediately following the sampling procedure, a woman mayexperience dizziness, nausea, a rapid heartbeat, and cramping. Once past these immediate hurdles, the physician will send the woman home with instructions to rest and to report any complications requiring immediate treatment, including.

- **Vaginal bleeding**: The appearance of blood could signal a problem.
- **Premature labour**: Unusual abdominal pain and/or cramping may indicate the onset of premature labour. Mild cramping for the first day or two following the procedure isnormal.
- **Signs of infection**: Leaking of amniotic fluid or unusual vaginal discharge,

and fever could signal the onset of infection.

- **Emotional aftercare:** Once the procedure has been safely completed, the anxiety of waiting for the test results can prove to be the worst part of the process. A woman should seek and receive emotional support from family and friends, as well as from her obstetrician and family doctor. Professional counselling may also prove necessary, particularly if a foetal defect is discovered.

- **Risks of amniocentesis:**

Most of the risks and short-term side effects associated with amniocentesis relate to the sampling procedure and have been discussed above. A successful amnio sampling results in nolong-term side effects. Risks include:

a. **Maternal/foetal haemorrhaging:** While spotting in pregnancy is fairly common, bleedingfollowing amnio should always be investigated.

b. **Infection:** Infection: although rare, can occur after amniocentesis. An unchecked infection can lead to severe complications.

c. **Foetal injury:** A very slight risk of injury to the fetus resulting from contact with the amnioneedle does exist.

d. **Miscarriage:** The rate of miscarriage occurring during standard, second trimester amnio appears to be approximately 0.5%. This compares to a miscarriage rate of 1% for CVS. Manyfoetuses with severe genetic defects miscarry naturally during the first trimester.

e. **The trauma of difficult family-planning decisions:** The threat posed to parental and familymental health from the trauma accompanying an abnormal test result can not be underestimated.

B. <u>Chorionic villus sampling:</u>

- **Definition:**

Chorionic villus sampling (CVS) is a form of prenatal diagnosis to determine genetic abnormalities in the fetus. It entails getting a sample of the chorionic villus (placental tissue) and testing it. It is generally carried out only on pregnant women over the age of 35 and those who have a higher risk of down syndrome and other chromosomal conditions. Chorionic villussampling (CVS) is a prenatal test that is used to detect birth defects, genetic diseases, and otherproblems during pregnancy. During the test, a small sample of cells (called chorionic villi) is taken from the placenta where it attaches to the wall of the uterus. Chorionic villi are tiny partsof the placenta that are formed from the fertilized egg, so they have the same genes as the baby.

The advantage of CVS is that it can be carried out at 10-12 weeks of pregnancy, earlier

than amniocentesis (which is carried out at 15-18 weeks). However, it is more risky than amniocentesis, with a 1 in 100 to 200 risk that it will cause a miscarriage.

- **What Diseases or Disorders Can CVS Identify?**

CVS can help identify such chromosomal problems as Down syndrome or other genetic diseases such as cystic fibrosis, Tay-Sachs disease, and sickle cell anemia. CVS is consideredto be 98% accurate in the diagnosis of chromosomal defects. The procedure also identifies thesex of the fetus, so it can identify disorders that are linked to one sex (such as certain types of muscular dystrophy that occur most often in males). CVS does not detect open neural tube defects like spina bifida.

- **Benefits of CVS:**

CVS can be done early in pregnancy (earlier than amniocentesis), and results are usually obtained within 10 days. Getting this kind of information early allows a woman to make choices in the beginning stage of her pregnancy. If a woman chooses to terminate the pregnancyafter receiving abnormal test results, the termination will be safer than if she waits until later for amniocentesis results.

- **What kind of problems does CVS diagnose?**

Like amniocentesis, CVS can identify:

i. Nearly all chromosomal abnormalities, including Down syndrome, trisomy 13, trisomy 18, andsex chromosome abnormalities (such as Turner syndrome). The test can diagnose these conditions, but it can't measure their severity.

ii. Several hundred genetic disorders, such as cystic fibrosis, sickle cell disease, and Tay-Sachs disease. The test is not used to look for all of them, but if your baby is at increased risk for oneor more of these disorders, CVS can usually tell you whether he has the disease.

iii. Unlike amniocentesis, CVS cannot detect neural tube defects, such as spina bifida. If you opt forCVS, you'll be offered a blood screening test in your second trimester to determine whether you're at increased risk for neural tube defects. Most neural tube defects can be detected by a detailed second-trimester ultrasound done at a state-of-the-art academic center.

Be aware that if you have CVS, there's a 1 to 2 percent chance of getting an unclear result. Thisis called a confined placental mosaicism, in which some of the cell lines cultured from the placenta contain abnormal chromosomes and some are normal. If your CVS detects a mosaicism,you'll have to have amniocentesis and possibly other testing to determine whether your baby isaffected.

- **Risks of CVS:**

There's no consensus on the risk of miscarriage due to CVS, although it's often estimated to bebetween one in 100 and one in 200 — which is higher than the usual estimates for

amniocentesis.But recent research suggests that the risk of miscarriage from CVS is really much lower. One center that does a lot of CVS found the miscarriage rate from the procedure was down to about1 in 360 — similar to the center's miscarriage rate from amniocentesis. This is most likely due toimprovements in ultrasound imaging and the doctors' increased experience in doing CVS. Because a certain percentage of women will end up miscarrying at this point in pregnancy anyway, there's no way of knowing for sure whether a miscarriage following CVS was actuallycaused by the procedure. Your particular risk depends in large part on the skill and experience of the doctor performing the procedure.

Some older studies found that CVS may have caused defects in a baby's fingers or toes, but thiswas mostly seen in tests done on women before 9 weeks of pregnancy. Current research suggeststhat there is no increased risk for this problem in women who have CVS at 11 weeks or later.

- **Who Should Be Tested With CVS?**

The American College of Obstetrics and Gynecology recommends offering CVS when there isan increased risk for a genetic disorder in the baby. This may include:

a. **Pregnant women who will be age 35 or older** on their due date (the risk of having a babywith a chromosomal problem such as Down syndrome increases with the age of the woman)

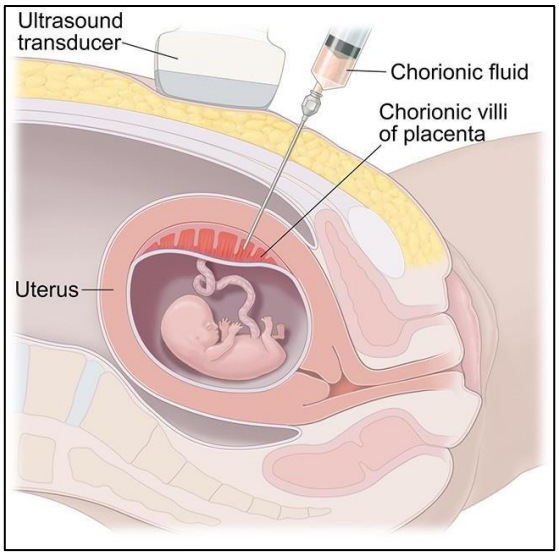
b. **Couples who already have had a child** with a birth defect or have a family history of certainbirth defects.

c. **Couples with a parent known to carry a chromosomal abnormality** or genetic diseasePregnant women with other abnormal genetic test results

- **How Is the CVS Test Performed?**

Before undergoing a CVS prenatal test, appropriate genetic counselling, including a detailed discussion regarding the risks and benefits of the procedure, are recommended. At the time ofinitial consultation and counselling, an ultrasound exam will be performed to confirm gestational age (the development stage of the embryo) and the location of the placenta. This isdone so that CVS can be performed at the appropriate gestational age (which is usually 10 to 12 weeks from the woman's last menstrual period). There are two ways to collect chorionic villi from the placenta: through the vagina or through the abdomen.

cells through the vagina, a speculum is inserted (in the same way as a Pap test). Thena very thin, plastic tube is inserted up the vagina and into the cervix. Using ultrasound images, the tube is guided up to the placenta, where a small sample is removed.



To collect cells through the abdomen, a slender needle is inserted through the woman's abdomen to the placenta, much like in amniocentesis. The sample of chorionic villi is then sentto a lab, where the cells are grown in a special fluid and tested a few days later. Culture resultswill be available within two weeks. Your doctor will notify you of the results.

- **What Happens After the CVS Test?**

You'll need to take it easy immediately after a CVS test, so arrange for someone to drive you home. For the rest of the day, you'll need to rest. Generally, women are advised to abstain fromstrenuous physical activity, sex, and exercise for three days following the procedure. You mayhave some cramping and bleeding, which is normal, but do tell your doctor or midwife. If younotice fluid leaking from your vagina, call your health care provider immediately.

- **How would woman decide between CVS and amniocentesis?**

Both tests can tell you whether your baby has a chromosomal problem or certain genetic disorders. CVS is done earlier in pregnancy (usually between 10 and 13 weeks), so you can findout sooner about your baby's condition. If everything's okay, your mind will be put at ease thatmuch sooner. Or, if there is a serious problem and you opt to terminate the pregnancy, you'll be able to do so while you're still in the first trimester.

On the other hand, you may prefer to wait for the results from second-trimester screening before subjecting yourself to an invasive test. At that point, amniocentesis would be your only option. Other considerations may influence your decision as well. For example, if you're at high risk forhaving a baby with a neural tube defect, you may want to have amniocentesis, as CVS cannotdiagnose these defects.

CVS is generally thought to have a slightly higher miscarriage rate than amniocentesis, but thismay not be the case everywhere. Medical centers that perform a lot of these procedures may havesimilar miscarriage rates for both. However, only a small percentage of doctors perform CVS, so in some areas it may be difficult to find an experienced specialist who does the procedure.

Women who choose to have CVS or amniocentesis are often those at increased risk

for geneticand chromosomal problems, in part because these tests are invasive and carry a small riskof miscarriage. The main advantage of CVS over amniocentesis is that you can have it done earlier — generally between 10 and 13 weeks of pregnancy. For an amnio, you'll have to waituntil you're at least 16 weeks pregnant.

C. Ultrasonography:

- **Definition:**

An ultrasound scan is a medical test that uses high-frequency sound waves to capture live images from the inside of your body. It's also known as sonography. The technology is similar to that used by sonar and radar, which help the military detect planes andships. An ultrasound allows your doctor to see problems with organs, vessels, and tissues without needing to make an incision. Unlike other imaging techniques, ultrasound uses no radiation. For this reason, it's the preferred method for viewing a developing foetus during pregnancy.

- **Why an ultrasound is performed:**

Most people associate ultrasound scans with pregnancy. These scans can provide an expectantmother with the first view of her unborn child. However, the test has many other uses. Doctormay order an ultrasound if you're having pain, swelling, or other symptoms that require an internal view of your organs. An ultrasound can provide a view of the:

- bladder
- brain (in infants)
- eyes
- gallbladder
- kidneys
- liver
- ovaries
- pancreas
- spleen
- thyroid
- testicles
- uterus
- blood vessels

An ultrasound is also a helpful way to guide surgeons' movements during certain medical procedures, such as biopsies.

- **Procedure of an ultrasound**

The steps you will take to prepare for an ultrasound will depend on the area or organ that is being examined. Your doctor may tell you to fast for eight to 12 hours before your ultrasound,especially if your abdomen is being examined. Undigested food can block the sound waves, making it difficult for the technician to get a clear picture.

For an examination of the gallbladder, liver, pancreas, or spleen, you may be told to eat a fat- free meal the evening before your test and then to fast until the procedure. However, you can continue to drink water and take any medications as instructed. For other examinations, you may be asked to drink a lot of water and to hold your urine so that your bladder is full and better visualized. Be sure to tell your doctor about any prescription drugs, over-the-counter medications, or herbal supplements that you take before the exam.

It's important to follow your doctor's instructions and ask any questions you may have beforethe procedure. An ultrasound carries minimal risks. Unlike X-rays or CT scans, ultrasounds use no radiation. For this reason, they are the preferred method for examining a developing foetus during pregnancy.

Before the exam, you will change into a hospital gown. You will most likely be lying down on a table with a section of your body exposed for the test. An ultrasound technician, called a sonographer, will apply a special lubricating jelly to your skin. This prevents friction so they can rub the ultrasound transducer on your skin. The transducer has a similar appearance to a microphone. The jelly also helps transmit the sound waves.

The transducer sends high-frequency sound waves through your body. The waves echo as theyhit a dense object, such as an organ or bone. Those echoes are then reflected back into a computer. The sound waves are at too high of a pitch for the human ear to hear. They form a picture that can be interpreted by the doctor. Depending on the area being examined, you mayneed to change positions so the technician can have better access.

After the procedure, the gel will be cleaned off of your skin. The whole procedure typically lasts less than 30 minutes, depending on the area being examined. You will be free to go aboutyour normal activities after the procedure has finished.

After an ultrasound:

Following the exam, your doctor will review the images and check for any abnormalities. Theywill call you to discuss the findings, or to schedule a follow-up appointment. Should anythingabnormal turn up on the ultrasound, you may need to undergo other diagnostic techniques, such as a CT scan, MRI, or a biopsy sample of tissue depending on the area examined. If your doctoris able to make a diagnosis of your condition based on your ultrasound, they may begin your treatment immediately.

Probable Questions:

1. Define amniocentesis? How it is done?
2. Describe the method of amniocentesis?
3. Discuss the risks of amniocentesis.
4. Define chorionic villus sampling.
5. What are the benefits of chorionic villus sampling.
6. What kind of problem chorionic villus sampling can identify?
7. What are the risks of chorionic villus sampling?
8. How chorionic villus sampling is done?
9. Compare chorionic villus sampling and amniocentesis.
10. What is the utility of ultrasonography?
11. How an ultrasound is performed?

Suggested Readings:

1. Principles of Genetics. Snustad and Simmons.
2. Genetics . Verma and Agarwal.
3. Principles of Genetics by Tamarin.
4. Biotechnology by V. Kumaresan
5. Embryology by N. Kumarsen
6. Developmental Biology by Veerbala Rastogi.
7. Embryology by M.P. Arora
8. Developmental Biology by Gilbert.
9. http://www.healthofchildren.com/A/Amniocentesis.html#ixzz6HsOA9h3x

Unit VI

Techniques used in Medical Embryology iv) DNA Finger printing v) Karyotyping

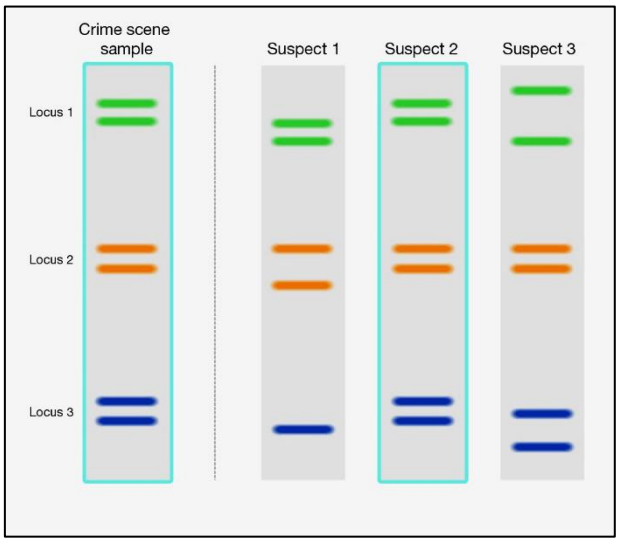
Objective: In this unit we will learn about some other techniques used in Medical Embryology like DNA Finger printing and Karyotyping.

Techniques used in Medical Embryology

A. DNA Finger printing

- **Definition**

DNA fingerprinting is a laboratory technique used to determine the probable identity of a person based on the nucleotide sequences of certain regions of human DNA that are unique to individuals. DNA fingerprinting is used in a variety of situations, such as criminal investigations, other forensic purposes and paternity testing. In these situations, one aims to "match" two DNA fingerprints with one another, such as a DNA sample from a known person and one from an unknown person.



- **DNA Fingerprinting Steps**

Alec Jeffreys developed this technique in which he used satellite DNAs also called VNTRs (Variable Number of Tandem Repeats) as a probe because it showed the high level of polymorphism.

DNA fingerprinting involves a number of intensive and important steps in order to fully complete and develop and DNA fingerprint of a father, a suspect or a person

involved in an immigration problem.

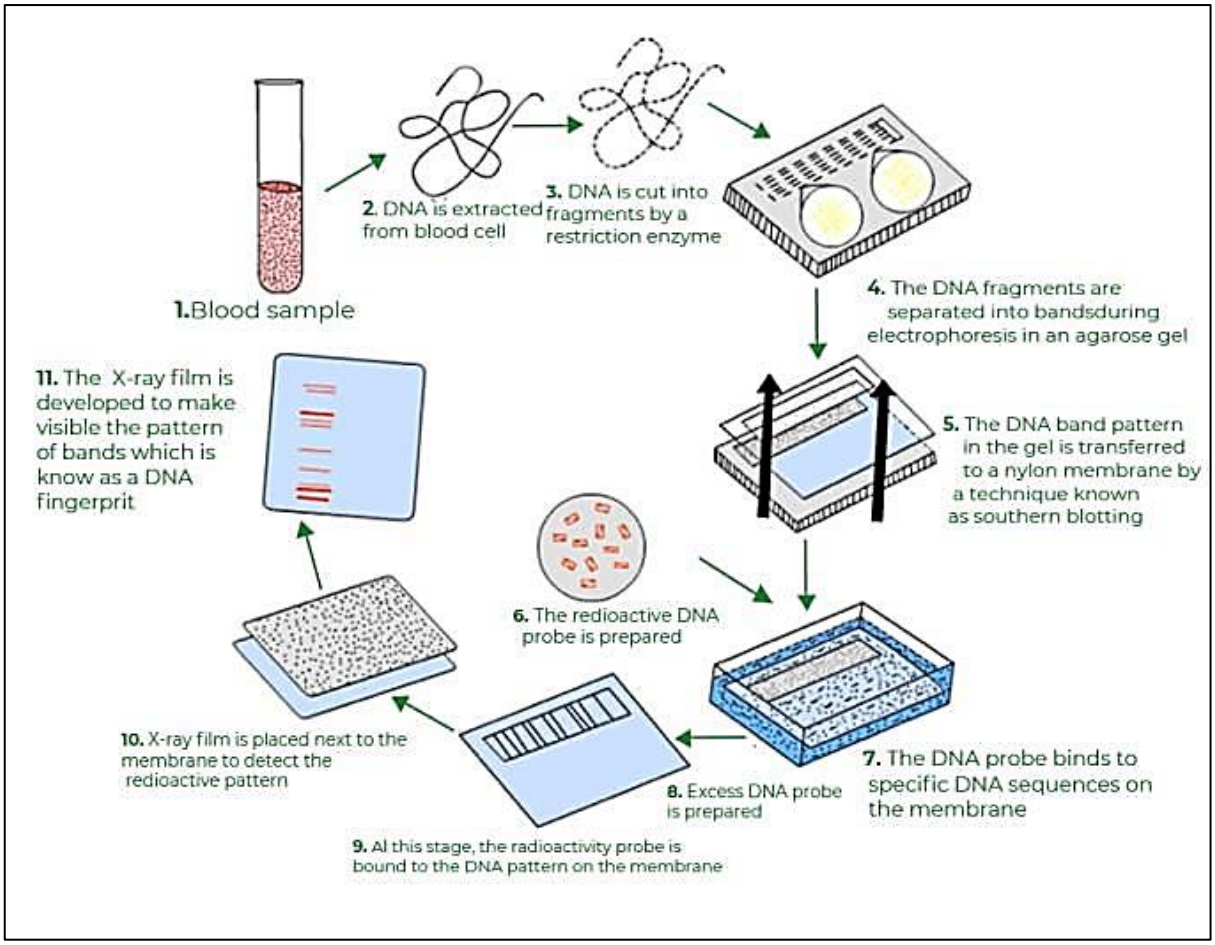
1. The process of DNA fingerprinting starts with isolating DNA from any part of the body such as blood, semen, vaginal fluids, hair roots, teeth, bones, etc.
2. Polymerase chain reaction (PCR) is the next step in the process. In many situations, there is only a small amount of DNA available for DNA fingerprinting. Because of this, in a test tube, DNA replication is must occur to make more DNA. The DNA and the cells will undergo DNA replication in order to make more DNA to be tested.
3. After the DNA is isolated and more copies of the DNA have been made, the DNA will be tested. The scientist will treat DNA with restriction enzymes (an enzymes that cuts DNA near specific recognition nucleotide sequences known as restriction sites).

   -This will produce different sized fragments which are known as restriction fragment length polymorphisms (**RFLP**s).

   -These fragments can then be observed doing an experiment called gel electrophoresis which separates DNA based on fragment sizes.
4. Gel electrophoresis is the next step in this process of DNA fingerprinting. During gel electrophoresis, an electrical current is applied to a gel mixture, which includes the samples of the DNA.

   -The electric current causes the DNA strands to move through the gel. This separates the molecules of different sizes.

   -The fragments of separated DNA are sieved out of the gel using a nylon membrane (treated with chemicals that allow for it to break the hydrogen bonds of DNA so there are sing strands).
5. The DNA (single stranded) is cross-linked against the nylon using heat or a UV light.
6. The probe shows up on photographic film because the strands of DNA decay and give off light.
7. In the end it leaves dark spots on the film which are also known as the DNA bands of a person.
8. What make up the fingerprint are the unique patterns of bands. The pattern of bands are different because we are all different and unique (other than identical twins).
9. Once the filter is exposed to the x-ray film, the radioactive DNA sequences are shown and can be seen with the naked eye. This creates a banding pattern or what we know as DNA fingerprints. This technique is called southern blotting.



Applications of DNA Fingerprinting

DNA fingerprinting is a way to identify using DNA. Some applications of DNA fingerprinting include:

- identifying a microbe causing an infection (diagnostic test)
- identifying microbes for scientific research
- paternity testing
- forensic DNA analysis to match DNA to criminal suspects
- a wide variety of genetic research

DNA fingerprinting involves multiple biotechnologies, including PCR but here this laboratory focuses on creating DNA fingerprints using restriction enzymes and visualizing the DNA fingerprints using gel electrophoresis.

B. Karyotyping

Karyotyping is a test to examine chromosomes in a sample of cells. This test can help identify genetic problems as the cause of a disorder or disease.

- **Procedure**

Karyotyping can be done on any sample from which nucleated cells (which can be cell cultured – that is, grown in a laboratory) can be obtained, most commonly:

a. blood;
b. skin;
c. prenatal samples (such as chorionic villus or amniotic fluid); and
d. bone marrow.

Cell culturing is required for karyotyping. This can take anything from three days (blood and bone marrow) up to 7 to 14 days (skin and prenatal samples). The process is outlined below.

- Cells are grown in an environment that includes stimulants for cell division (this may not be required for cancer cells, which divide rapidly themselves).
- Cell growth is arrested at metaphase and harvested for analysis.
- Once harvested, the cells are mounted on slides, treated with enzymes and Giemsa stain to produce G-banding patterns, then viewed under a light microscope (x1,000).
- The banding patterns for each different chromosome are characteristic and reproducible, resembling a barcode.
- The cytogeneticist identifies and counts the chromosomes, 'cuts and pastes' the image to pair the homologues, and compares the banding pattern in each pair to identify any rearrangements, deletions or duplications.

Many attempts have been made to develop computer software to replace this labour-intensive work, but it has not been possible to replace the pattern-recognition skills of a highly trained cytogeneticist.

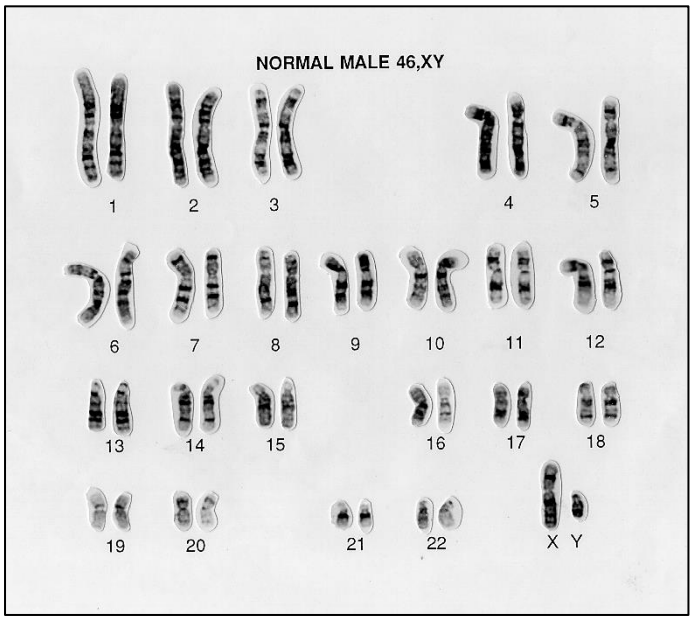


Figure 2: A normal male karyotype (note the X and Y sex chromosomes)

- **Advantages**

Advantages of Karyotyping are

i. screens the whole genome;

ii. detects balanced and unbalanced rearrangements;

iii. provides positional information;

iv. can detect mosaicism; and

v. can identify structural rearrangements that may be missed by arrays; for example, ring chromosome 20 in epilepsy.

vi. Clinical applications:

A karyotype provides a visual, genome-wide screen for chromosomal variants such as deletions, duplications and structural rearrangements. It has a limited resolution of 5–10mb, which means that it cannot detect variants that are smaller than this.

In most contexts, karyotyping has been superseded by the use of microarray, which can also detect chromosomal variants but at much higher resolution (50–200Kb). Nevertheless, karyotyping is still in use in certain clinical situations.

One such situation is in the investigation of infertility or recurrent miscarriage. Here, karyotyping has an advantage over microarray because it is able to detect balanced rearrangements, which can lead to infertility. Karyotyping can do this because it provides an image of the structure of whole chromosomes, unlike microarray, which shows the amount of chromosomal material present but not its position.

Another common application of karyotyping is the detection of structural chromosome rearrangements resulting in gene fusions that drive cancer. An example is the Philadelphia chromosome in chronic myeloid leukaemia (a translocation of chromosomes 9 and 22).

- **Limitations**

Karyotyping:

i. is labour intensive;
ii. has a slow turnaround time;
iii. is unable to detect uniparental disomy;
iv. requires dividing cells (and cell culture);
v. has much lower resolution than arrays (only changes over 5Mb can be detected);
vi. carries a risk of cell culture artefacts (abnormal chromosomes arising during cell culture, but not actually present in the patient); and
vii. carries a (rare) risk that some variants are not detected in cultured cells because they are lost during cell culture (for example, the 12p isochromosome causing Pallister-Killian syndrome is usually lost when blood lymphocytes are cultured).

Probable Questions:

1. Define DNA finger printing?
2. Mention the steps of DNA finger printing.
3. Define karyotyping?
4. Mention the advantages and limitation of karyotyping.

Suggested Readings:

1. Principles of Genetics. Snustad and Simmons.
2. Genetics . Verma and Agarwal.
3. Principles of Genetics by Tamarin.
4. Biotechnology by V. Kumaresan
5. Embryology by N. Kumarsen

DISCLAIMER: This Self Learning Material (SLM) has been compiled from various authentic books, Journals articles, e-journals and other web sources.