Human Reproduction

SHORT NOTES

Reproduction

- The male and female reproductive organs work together to produce offspring.
- **Primary sex organs**: Formation of the gametes (sperm and egg) and hormones.
- **Secondary Sex Organs:** Do not produce gametes, but provide passage for the gametes.

Male Reproductive System

- Male reproductive system consists of **primary sex organ** which are testes (one pair) and secondary sex organs which are male accessory ducts which include rete testis, vasa efferentia, epididymis, vas deferens, urethra.
- Male accessory glands include seminal vesicles, prostate gland, Cowper's glands/Bulbourethral gland and male external genitalia is penis.
- Testes are situated outside the abdominal cavity within a pouch called **scrotum,** for the process of spermatogenesis, which provide **2-2.5°C less temperature** than normal body temperature.
- Each testis has **250 compartments** called testicular **lobules**. Each lobule contains one to three seminiferous tubules. Each seminiferous tubules contain 2 types of cells- Sertoli cells (nurse cells) and Leydig cells (interstitial cells) which synthesise and secrete testosterone.
- Sertoli cells function as an endocrine gland, i.e., secrete biochemicals:
  (*i*) Antimullerian hormone
  (*ii*) Inhibin hormone
  (*iii*) Androgen binding protein (ABP)
- The **pathway of sperm** through the male body is:

Seminiferous tubule → Rete testis → Vasa efferentia → Epididymis → Vas deferens → Ejaculatory duct →Urethra

Female Reproductive System

- It consists of primary sex organ which are pair of ovaries and secondary sex organs which are oviducts (fallopian tubes), uterus, vagina, female external genitalia and mammary glands.
- **Oviduct:** Infundibulum (funnel-shaped), Ampulla (fertilization takes place) and Isthmus (last part).
- **Uterus:** Corpus/Body, Fundus and Cervix.
- The wall of the uterus has three layers of tissue: perimetrium, myometrium, endometrium.
- Birth canal = Cervical canal + Vagina.
- Female external genitalia consists of mons pubis, labia majora, labia minora, hymen, clitoris.
- Accessory glands in female is of 2 types- greater vestibular or bartholin's gland and lesser vestibular glands or paraurethral or skene's glands.
- Pathway of milk ejection

Glandular tissue → Mammary lobes → Mammary alveoli → Mammary tubules → Mammary duct → Mammary ampulla → Lactiferous duct

Gametogenesis

- The process of formation of gametes. It is divided in to three phases:

1. Multiplication phase
2. Growth phase
3. Maturation phase

Spermatogenesis

- The process of formation of sperms.
- Spermatogonia, Primary spermatocytes, Secondary spermatocytes, Spermatids, Spermatozoa
- **Spermiogenesis:** Transformation of the spermatid into a mature sperm cell, or spermatozoon.
- **Spermiation:** The process of release of sperm from the sperm head into the lumen of seminiferous tubule.
- Sperm is divided in 3 parts- Head, Middle piece (production of energy due to presence of mitochondria) and Tail (help in movement).
- Human male ejaculates about 200–300 million sperms during a coitus of which for normal fertility, at least **60 percent** sperms must have normal **shape and size**, at least **40 percent** of sperm among them must show **vigorous motility**.

Oogenesis

- Process of formation of a mature female gamete. It is initiated during the embryonic development stage with million of gamete mother cells called oogonia. **Tertiary follicle** shows the presence of fluid filled cavity known as **antrum**.
- LH acts on corpus luteum to secrete four hormones- progesterone, oestrogen, relaxin, and inhibin.
- FSH causes the development of ovarian follicles.

Menstrual Cycle (28/29 days)

- The cycle of non-pregnant females in their ovaries and uterus, which involves the periodic shedding of the endometrium. It is divided in 3 phases:

1. Menstrual Phase/Bleeding phase (3-5 days).
2. Preovulatory phase/Proliferative phase/Follicular phase (6 to 13 days).
3. Postovulatory phase/Secretory phase/Luteal phase (**14 days fixed**).

Fertilisation

- The process of fusion of a sperm with an ovum to form a diploid cell is called fertilisation. It involves:

1. Acrosomal reaction
2. Fast block to polyspermy
3. Slow block to polyspermy

Cleavage

- The fertilized egg, undergoes repeated cell divisions which occur rapidly to produce a multicellular structure without changing its size. It involves following stages:

1. Morula 2. Blastula 3. Gastrula

Extra Embryonic Membranes

- There are 4 types of extra embryonic membranes

1. Chorion 2. Yolk sac 3. Allantois 4. Amnion

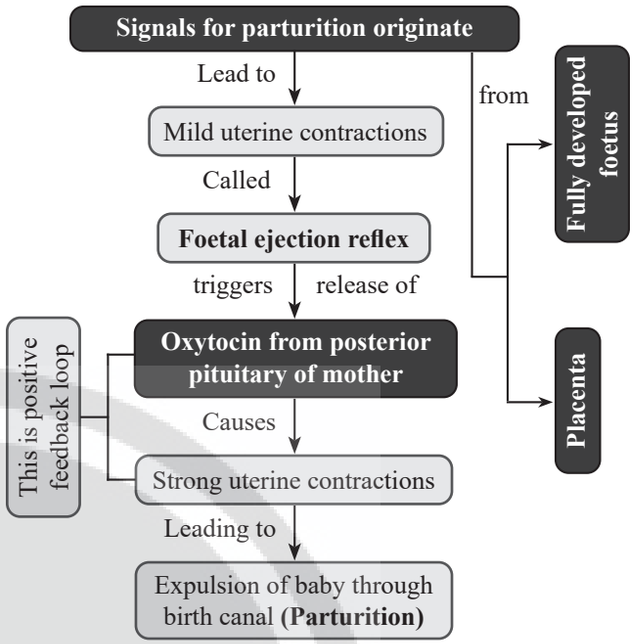
Organogenesis

Major Events During Gestation Period in Humans:

Trimester	Month	Week	Event
1st	I	4	Heart is formed, sign of growing foetus noticed by listening to the heart sounds through stethoscope.
	II (end)	8	Foetus develops limbs and digits.
	III (end)	12	Most of major organ systems are formed including external genital organs, limbs.
2nd	V	20	First movement of foetus, appearance of hair on head.
	VI (end)	24	Body is covered with fine hair, eyelids separate, eyelashes are formed.
3rd	IX (end)	36	Foetus is fully developed and is ready for delivery.

Parturition

- The process of childbirth which is induced by a complex neuroendocrine mechanism.



Lactation

- The process of formation of milk at the end of pregnancy for bringing up a healthy baby.
- **Prolactin** helps in production of milk.
- **Oxytocin** causes ejection of milk.
- In initial few days of lactation **colostrum** (contains IgA) is released by lactating mother.

Reproductive Health

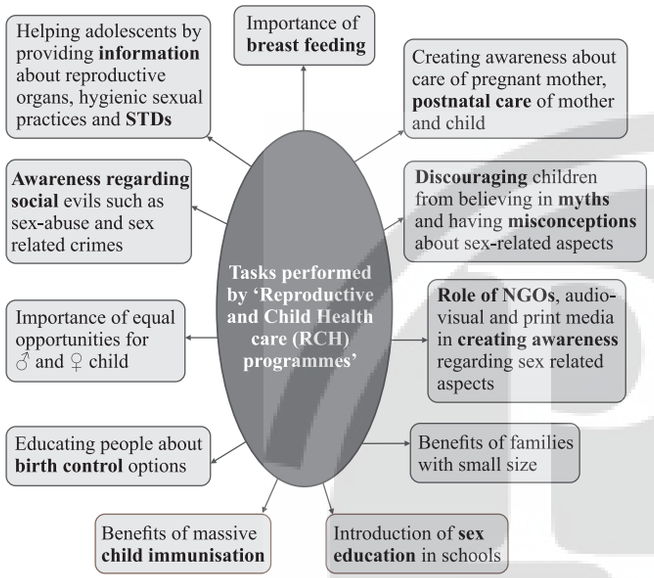
SHORT NOTES

Introduction

- According to WHO, reproductive health means a total well being in all aspects of reproduction i.e., physical, emotional, social and behavioural.

Problems and Strategies

- India was amongst first country in the world to initiate action plans at a national level to attain total reproductive health such as family planning programmes in 1951.



Amniocentesis

- Analyse foetal cells and dissolved substances from amniotic fluids.
- Technique used to check for genetic disorders such as Down's syndrome, sickle-cell anemia, etc.
- Statutory ban on this technique in India to prevent female foeticide.

Population Stabilisation

- According to 2001 census, our population growth rate was around 1.7 per cent, i.e., 17/1000/year.

Year / POPn	1900	1947	2000	2011
World	2 billion		6 billion	7.2 billion
India		approx: 350 million	close to 1 billion	crossed 1.2 billion

Reasons for Increase in Population Size:

- Decline in death rate
- Rapid decline in maternal mortality rate (MMR)
- Decrease in infant mortality rate (IMR)
- Increase in number of people in reproducible age
- Increase in health facilities

Measures Taken by Government to Check Population

- Motivate smaller families for using various contraceptive methods and by slogans "Hum do Hamare do", in advertisements and posters.
- Urban couples adopting: **"One child norm"**.
- Statutory raising of marriageable age:
  - Female- 18 years
  - Male- 21 years
- Incentives given to couples with small families.

Birth Control/Contraception

Features of an Ideal Contraceptive:

- User-friendly
- Easily available
- Effective
- Reversible
- No/least side-effects
- No interference with libido or act of coitus

Two Principle Methods of Birth Control:

- Natural methods
- Artificial methods

Natural/Traditional Methods

- Periodic abstinence
- Withdrawal method/Coitus interruptus
- Lactational amenorrhea
- Based on the principle of avoiding physical meeting of the egg and sperms
- Chances of failure are high

Artificial Methods

- Barrier methods
- Spermicidal jellies
- IUDs
- Oral pills
- Injections and implants
- Emergency contraceptives
- Surgical methods

Medical Termination of Pregnancy (MTP)/Induced Abortion

- **MTP:** Intentional or voluntary termination of pregnancy before full term.
- MTP was legalized in India in 1971.
- 40–50 million MTPs are performed every year.
- $1/5^{th}$ of the total number of conceived pregnancies.

- Intention behind MTP amendment act 2017, (Government of India)
  - Reducing the incidence of illegal abortion.
  - Decrease consequent maternal mortality and morbidity.
  - MTPs are safe upto 12 weeks but riskier in 2nd trimester yet both are legal.
  - Amniocentesis and MTPs have been misused in context of female foeticide.

Sexually Transmitted Diseases (STDs)

- **Alternately named:** Venereal diseases (VD) or reproductive tract infections (RTIs).
- **High vulnerability/risk group:** 15-24 years.
- Bacterial and protozoan diseases are completely curable if detected early and treated properly.
- **Mode of Transmission:**
  - Sexual intercourse.
  - Sharing of injection needles, surgical instruments with infected persons.
  - Transfusion of blood.
  - From infected mother to foetus.
- **Preventive measures to avoid STDs:**
  - Avoid sex with unknown partners/multiple partners
  - Always try to use condoms during coitus

Infertility

- Unable to produce children inspite of unprotected sexual co-habitation.
- Infertility as a problem could be with either the male or female partner.
- In India, female is blamed often than male for the couple being childless.

Assisted Reproductive Technologies (ART)

In-vitro fertilization	*In-vivo* fertilization
Outside the body in simulated conditions in laboratory	In the female reproductive tract
Yes	No
ZIFT, IUT, ICSI	GIFT, AI, IUI

Evolution

SHORT NOTES

Evolution

- **Evolutionary biology** is the study of history of life forms on earth.
- **Stellar distances** are measured in light years.
- **Big bang explosion** (Singular huge explosion).

↓Resulted in

Parameters	Origin of Universe —Leading to→ Origin of Earth	
Time scale	20 billion years ago.	4.5 billion years ago.
Feature	Comprises cluster of galaxies (stars, clouds of gases, dust).	Occurred in solar system of Milkyway galaxy.

Events after expansion of universe:

- Temperature declined
- H_2 & He formed
- Gases condensed
- Galaxies formed

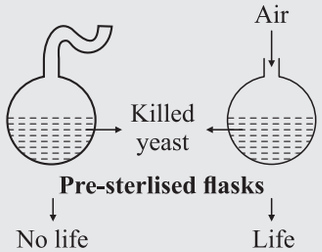
Events after expansion of universe:

- No atmosphere existed on early earth.
- Water vapour, methane, carbon dioxide and ammonia released form molten mass covered the surface.
- $H_2O \xrightarrow{\text{UV rays}} H_2 + O_2$
- NH_3, CH_4 $+ O_2 \rightarrow CO_2 + H_2O$ + other contents

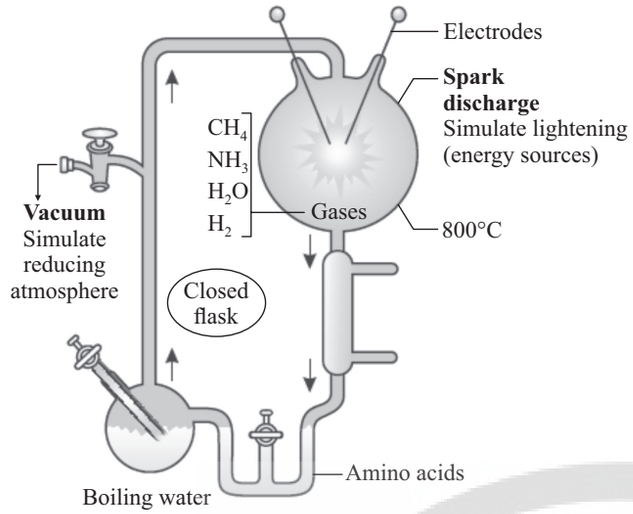
Origin of Life

- Life appeared 500 million years after the formation of earth, i.e., almost 4 billion years ago.
- **First non-cellular forms of life**
  - Probably originated **3 billion years ago**
  - Would have possibly originated from giant molecules (RNA, protein, polysaccharides, etc.)
  - These capsules **reproduced** their molecules
- **First cellular forms of life**
  - Possibly originated **2 million years ago**
  - Arose in aquatic environment
- This version of Biogenesis i.e., the first form of life arose slowly through evolutionary forces from non-living molecules is accepted by majority.

Table: Theories for Origin of Life

Theory	Proponents	Significance
Special creation	Conventional religious literature	❖ All living organisms that we see today were created. ❖ Diversity was always the same since creation and will be the same in future also. ❖ Earth is 4000 years old.
Cosmozoic Panspermia	Early Greek thinkers, Astronomers	❖ Life came from outerspace. ❖ Units of life called **spores** were transferred to different planets including earth.
Spontaneous generation	–	❖ Life came out from decaying and rotting matter like straw, mud etc. ❖ Disapproved by Louis Pasteur.
Theory of Biogenesis	Louis Pasteur 	❖ **Life comes only from pre-existing life**. ❖ He showed that in pre-sterlised flasks, life did not come from **"killed yeast"**.

S.L Millers experimental setup



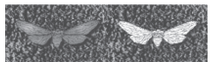
- Formation of life was preceded by chemical evolution i.e., formation of diverse organic molecules from inorganic constituents.
- First from of life could have come from pre-existing non-living organic molecules (e.g. RNA, proteins, etc.).
- This hypothesis was proved by Miller's experiment, **1953, S.L. Miller (American scientist)**.
- In similar experiments, other observed formation of sugars, nitrogen bases, pigments and fats.

Evidences of Evolution

1. **Palaeontological evidences** (Evidences from fossils):
   - Fossils are remains of hard parts of life forms found in rocks.
   - Age of fossils is determined by radioactive-dating method.
   - Fossils of different life forms in different sedimentary layers indicates the geological period in which they existed (epochs, periods, eras).
2. **Embryological evidences:**
   - Proposed by **Ernst Haeckel**.
   - Based upon the observations of certain features during embryonic stage common to all vertebrates that are absent in adults e.g., embryos of all vertebrates develop a row of vestigial gill slits functional only fish and not found in another adult vertebrates.
   - It was disproved by **Karl Ernst Von Baer**. He noted that embryos never pass through the adult stages of other animals.
3. **Morphological and anatomical evidences**

Parameters	Homologous organs	Analogous organs
Common ancestory	Yes	No
Anatomical structures	Similar but developed along different directions due to adaptations to different needs	Not similar but resulted in selection of similar adaptive features in different groups of organisms, thus, evolving for the same function.
Function performed	Different	Similar
Type of evolution	Divergent	Convergent

Examples of Evolution by Anthropogenic Action

Parameters	Before industrialization (1850s)	After industrialization (1920s)
Figure		
Tree trunks	White, covered by **lichens**	Became dark due to deposition of soot and smoke
White moths	More	Less
Melanised moths	Less	More
Predators feed on	Melanised moths	White winged moths

- Lichens do not grow in polluted area **(pollution indicator)**.
- **Agent of natural selection:** Predator/birds.
- Moths that were able to camouflage themselves (i.e., hide in the background) survived **but no variant is completely wiped out**.

Hardy–Weinberg Principle

This principle was given by G.H Hardy & W. Weinbeng in 1908.

- The principle states that **allele frequencies in a population are stable and constant from generation to generation.**

Sum total of all the allelic frequencies is 1.
$(p + q)^2 = p^2 + 2pq + q^2 = 1$.

A Brief Account of Evolution

Form of life	Appeared around
1st non cellular form	3 bya
1st cellular forms	2000 mya
Invertebrates	350 mya
Sea weeds and few plants	320 mya

- Land reptiles were dinosaurs which disappeared 65 mya
  - Reason → Climatic change
  - Reason → Evolved in birds
- Some land reptiles went back to water to evolve fish like reptiles e.g., *Ichthyosaurus* (200 mya)
- Modern day descendants of reptiles e.g., Turtles, Tortoises, Crocodiles

Lobbed fin fish (Coelocanth) → (Possibly evolved into) Amphibians → (Possibly evolved into) Reptiles → Mammals (Arose from ancestral extinct reptiles)

- Lobbed fin fish (Coelocanth)
  - ❖ Primarily aquatic
  - ❖ 1938, fish caught in South Africa was earlier thought to be extinct
- Amphibians Frogs, salamanders
  - ❖ Aquatic and land
- Reptiles
  - ❖ Lay thick shelled eggs which do not dry up in sun
- Mammals (Arose from ancestral extinct reptiles)
  - ❖ Viviparous i.e., protect young ones inside mothers body
  - ❖ More intelligent in sensing and avoiding danger

❖ Restricted Distribution of Pouched Mammals in Australia due to continental drift.

Origin and Evolution of Man

Human ancestors	Years back	Cranial capacity	Specific features
Dryopithecus / ***Ramapithecus***	15 mya	100 cc / 150-300 cc	More ape-like → Hair and walked like Gorillas and chimpanzees / More man-like → Hair and walked like Gorillas and chimpanzees
Australopithecus	2 mya	500 cc	❖ Few fossils of man-like bones have been discovered in Ethiopia and Tanzania. ❖ 3-4 mya, man-like primates walked in East African grasslands. ❖ They were probably **not taller than 4 feet but walked upright** ❖ Evidence shows they hunted with stone weapons but essentially ate fruit.
Homo habilis	–	650 cc – 800 cc	❖ **First human-like being, the homind.** ❖ Probably did not eat meat.
Homo erectus	1.5 mya	900 cc	❖ Fossils discovered in **Java in 1891**. ❖ Probably ate meat.
Neanderthal man	1,00,000-40,000 years back	1400 cc	❖ Lived in near East and Central Asia. ❖ **Used hides to protect their body and buried their dead**.
Homo Sapiens	75,000-10,000 years ago (ice age)	–	❖ Arose in Africa and moved across continents and developed into distinct races.

Human Health and Disease

SHORT NOTES

HEALTH

- Health is not simply 'absence of disease' or 'physical fitness'.

Factors Affecting Health:

Mental state, genetic disorders, infections and life style (habits, rest and exercise)

Condition of health → Increase → Productivity, longevity; Bring economic prosperity
Condition of health → Decrease → Infant and maternal mortality

DISEASE

- It is state of the body when functioning of one or more organ systems is adversely affected, characterized by various signs and symptoms.

Parameters	Types of Diseases	
	Non-infectious	Infectious
Transmission from one person to another	No	Yes
Example	Cancer	AIDS

- **Pathogens:** Disease causing organisms:
  - **Most parasites are pathogens** living in (or on) the host, multiply and interfere with normal vital activities resulting in morphological and functional damage.
  - Gut pathogens can survive harsh pH & digestive enzymes.
- **Vector:** Transmits disease from one organism to another e.g., female *Aedes* mosquito is the vector for dengue and chikungunya while, *Anopheles* spreads malaria.

Table: Classification of diseases on the basis of transmission

Mode of Transmission	Bacterial	Viral	Protozoan	Helminthic
Air (droplet/aerosol) or **object borne** (pens, knobs etc.)	Pneumonia, diphtheria	Common cold, Smallpox	–	–
Direct contact	Tetanus	Smallpox	–	–
Contaminated food and water	Typhoid, dysentery	Polio	Amoebiasis	Ascariasis
Insect vector/vector borne	Plague	Chikungunya, Dengue	Malaria	Filariasis
Body fluids	Syphilis	AIDS	Trichomoniasis	–

Bacterial Diseases

Disease	Pathogen	Organ Affected	Common Symptoms
Typhoid	*Salmonella typhi* **Diagnostic test: Widal test**	Small intestine and other organs by migrating through blood	Sustained high fever (39–40°C) ❖ Stomach pain ❖ Weakness ❖ Constipation ❖ Headache ❖ Loss of appetite **In severe cases, intestinal perforation and death** may occur.
Pneumonia	*Streptococcus pneumoniae, Haemophilus influenzae*	Alveoli of lungs	❖ Problem in respiration due to fluid filled alveoli ❖ Fever, chills, cough, headache **In severe cases, lips and finger nails turn grey to bluish**

Viral Diseases

Disease	Pathogen	Organ Affected	Symptoms
Common cold	Rhino virus	❖ Nose and respiratory passage ❖ Common cold does not infect lungs	❖ Nasal congestion and discharge ❖ Sore throat ❖ Hoarseness, cough ❖ Headache, tiredness ❖ Symptoms usually lasts for 3-7 days

Helminthic Diseases

Disease	Pathogen	Organ/Structure Affected	Symptoms
Ascariasis	*Ascaris* **(Roundworm)**	Intestine	Internal bleeding, fever, muscular pain, anemia, **blockage of intestinal passage**
Elephantiasis/ Filariasis	*Wuchereria bancrofti* *W. malayi* **(Filarial worm)**	Lymphatic vessels	**Chronic inflammation** of organs in which they live for many years resulting in gross deformities e.g., limbs, genital organs etc.

Fungal Diseases

Disease	Pathogen	Body Parts Affected	Symptoms
Ringworm	*Microsporum*, *Trichophyton*, *Epidermophyton*	Skin, nails, scalp	Dry, scaly lesions Intense itching

Protozoan Diseases

Disease	Pathogen	Area Affected	Symptoms
Amoebiasis/Amoebic dysentery	*Entamoeba histolytica*	Large intestine	❖ Constipation ❖ Abdominal pain ❖ Cramps ❖ Stool with excess mucous and blood clots
Malaria	*Plasmodium* ❖ *P. vivax* ❖ *P. malariae* ❖ *P. falciparum* ❖ *P. ovale*	RBCs	❖ Chills ❖ High fever recurring 3-4 days ❖ If not treated, can prove to be fatal

AIDS (ACQUIRED IMMUNO DEFICIENCY SYNDROME)

- ❖ It is the deficiency of immune system, acquired during the lifetime of an individual.
- ❖ It is non congenital and fatal infectious disease. It is caused by HIV (Human Immunodeficiency Virus).

Table: Enveloped virus enclosing 2 single stranded RNA genome

Life Cycle	Mode of Transmission	High Risk Individuals
Entry of virus in body ↓	Sexual contact	Multiple sexual partners
	Placenta	HIV infected mother to foetus
	Blood transfusion	Repeated blood transfusion
	Infected needles	Drug addicts **(intravenous)**
Entry into body cells **(Macrophages, helper T-cells)**		

- ❖ There is progressive decrease in number of helper T-cells.
- ❖ **Initial symptoms:** Fever, diarrhoea, weight loss.
- ❖ **Later the immune-deficient patient** is prone to infections especially those due to *Mycobacterium,* viruses, fungi, *Toxoplasma*, etc.
- ❖ There is always a time-lag between infection and appearance of AIDS symptoms. This may vary from a few months to many years (usually 5-10 years).

Diagnostic Test: ELISA (Enzyme Linked Immuno-Sorbent Assay)

Treatment: Anti-retroviral drugs, can only prolong life but cannot prevent death.

CANCER

A dreaded non-infectious disease; major cause of death all across the globe.

Parameters	Normal Cells	Cancerous Cells/ Neoplastic Cells
Cell growth and differentiation	Highly controlled and regulated	Uncontrolled & non-regulated
Contact inhibition	**Present**, virtue of which contact with other cells inhibits their growth	**Lost**, so these cells keep on dividing and form mass of cells called **Tumor/ Neoplasm**

Types of Tumor

Parameters	Benign	Malignant Tumor/Cancer
Location	Confined to original place	Grow rapidly and spread to other parts
Damage	Little damage	Invade and damage other cells starving normal cells by competing for vital nutrients.
Metastasis	**No**	**Yes**, cells sloughed from such tumors reach distant sites through blood and start new tumor called **metastasis (most feared property).**

- **Tumor cells have ability to avoid detection and destruction by immune system.**
- **Approaches for Treatment:**
  - Surgery
  - **Radiotherapy:** Tumor cells irradiated lethally
  - **Chemotherapy:** Side effects like hair loss, anemia
  - **Immunotherapy:** **α-interferons** (Biological response modifiers) activate immune system and helps in destroying the tumor.

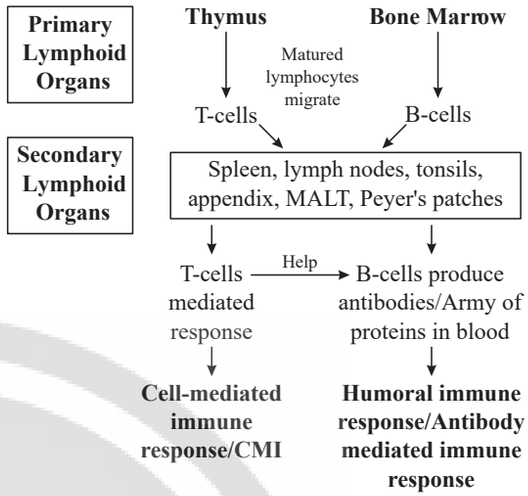
IMMUNITY

- The ability of the host to fight the disease causing organisms, conferred by the immune system is called **immunity**.

Parameters	Types	
	Innate	Acquired
Observed	Time of birth	After birth
Exposure to infection	Not required	Required
Defense	Non specific	Specific
Memory record	No	Yes

LYMPHOID ORGANS

- The human immune system consists of lymphoid organs, tissues, cells and soluble molecules like antibodies. This response is carried out by two special types of lymphocytes present in our blood i.e., **B** and **T-lymphocytes**.



	Immune Response	
Exposure to pathogens	First time	Subsequent times
Intensity	Low ← Based on memory of first encounter → High	

- **Responses are carried out by B and T lymphocytes.**
- **Each antibody has 4 peptide chains (H_2L_2):** 2 long heavy chains and 2 short light chains. They are called immunoglobulins (Ig). The different types of immunoglobulins are:
  IgA, IgM, IgE, IgG, IgD
- T-lymphocytes are responsible for graft rejection.

VACCINATION AND IMMUNISATION

	Types of Immunity	
Antibodies	**Active** Produced within the host body	**Passive** Ready-made/preformed antibodies are directly given
Time taken for full/ effective response	Longer	Shorter
Memory cells	Yes	No
Examples	Natural infection / **Vaccination** → Antibody production in host Deliberate injection of living/dead microbes/ proteins	Mother $\xrightarrow[(IgG)]{\text{Placenta}}$ Foetus Mother $\xrightarrow[(IgA)]{\text{Colostrum}}$ Infant

- Recombinant **DNA technology** has allowed the large scale production of antigenic polypeptides of pathogen in **bacteria/yeast**. Hence, greater availability for immunization, e.g., **hepatitis B vaccine produced from yeast**.,

ALLERGIES

- **Exaggerated response** of immune system to certain antigens present in the environment.

Allergens	Substances to which exaggerated immune response is produced e.g., pollens, mites in dust, animal dander, etc.
	lgE type
Symptoms	Sneezing, watery eyes, running nose, difficulty in breathing
	Histamine and **serotonin** from mast cells
Diagnosis	Patient is exposed to or injected with very small doses of possible allergens and reactions studied.
	Anti-histamine, adrenaline and steroids quickly reduce the symptoms of allergy

DRUG ABUSE

- Chemical when taken for a purpose other than medicinal use or in amounts/frequency that impairs one's physical, physiological or psychological functions and constitutes **drug abuse**.
- **Source:** Majorly from flowering plants and some from fungi.

Drug	Receptors	Source	Intake	Examples	Action and Anything Specific
Opioids	CNS, GIT	Latex of poppy plant, *Papaver somniferum*	Snorting, injection	❖ Morphine ❖ Heroin/Smack (Diacetylmorphine)	❖ Effective sedative and pain killer ❖ Useful in patients undergone surgery ❖ Depressant and slows down body functions ❖ Odourless, white, bitter crystalline compound
Cannabinoids	Principally in brain	Inflorescence, flower tops, leaves and resin of cannabis plant	Inhalation, oral ingestion	❖ Charas ❖ Hashish ❖ Ganja ❖ Marijuana	❖ Effects on cardiovascular system of the body ❖ Also being abused by some sportspersons
Stimulants	CNS	Coca plant *Erythroxylum coca* (Native of South America)	Snorting	❖ Cocaine/coka alkaloid ❖ Commonly called (coke/crack)	❖ Interferes with transport of neurotransmitter dopamine ❖ Potent stimulating action on CNS, producing **sense of euphoria** and increased energy ❖ **Excessive dosage causes hallucinations**
Hallucinogens		*Atropa belladona, Datura*	–	–	❖ Have been used for hundreds of years in folk medicine, religious ceremonies and rituals all over the globe
Other drugs		Synthetic		Barbiturates, Benzodiazepines, Amphetamines	❖ Help patients cope with mental illnesses like **depression** and **insomnia**

- Chronic use of drugs and alcohol damages nervous system and liver (Cirrhosis)

CHAPTER

6 Biotechnology: Principles and Processes

INTRODUCTION

- **Biotechnology** deals with techniques of using live organisms or enzymes from organisms to produce products and processes useful to humans.

Principles of Biotechnology/Core Techniques Involved in Modern Biotechnology

Parameters	Genetic engineering	Bioprocess engineering
Definition	Techniques to alter the chemistry of genetic material to introduce these into host organisms and thus change the phenotype of host organism	Maintenance of sterile ambience in **chemical engineering processes** to enable growth of only the desired microbe/ eukaryotic cell in large quantities
Include	Creation of rDNA Gene cloning Gene transfer	Manufacture of biotechnological products like antibiotics, vaccines, enzymes, etc.
The ability to multiply copies of antibiotic resistance gene in *E. coli* was called **cloning** of antibiotic resistance gene in *E. coli*.		

Three Basic Steps in Genetically Modifying Organisms (GMO)

- Identification of DNA with desirable genes;
- Introduction of the identified DNA into the host;
- Maintenance of introduced DNA in the host and transfer of the DNA to its progeny.

KEY TOOLS OF RECOMBINANT DNA TECHNOLOGY

- **(1) Enzymes**
- **(2) Vectors**
- **(3) Competent host cells**

ENZYMES

Restriction Endonuclease

- More than 900 restriction enzymes have been isolated from over 230 strains of bacteria each of which recognise different recognition sequences.

First restriction endonuclease-***Hin*dII:** Isolated and characterised **in 1968** later, recognises sequence of **6** bp.

The first recombinant DNA was constructed by **Stanley Cohen** and **Herbert Boyer**, **1972**.

Functions:

- Cuts the two strands of dsDNA at specific points in their sugar-phosphate backbones and leaves single stranded portions at the ends.

Ligase

- When source DNA and vector DNA are cut by the same restriction enzyme, the resultant DNA fragments have the same kind of sticky-ends .
- Sticky ends are named so because they form hydrogen bonds with their complementary cut counterparts.
- Stickiness facilitates the action of the enzyme DNA ligase.

CLONING VECTORS

Vectors are vehicles for delivering foreign DNA into recipient cells.

Features of cloning vectors:

- **Origin of Replication (*ori*)**
- **Selectable Marker**
- **Cloning Sites/Restriction Sites**

Transformation: Procedure through which piece of foreign DNA is introduced in a host bacterium.

- **Insertional inactivation:** Insertion of gene of interest within antibiotic resistance gene/selectable marker results in inactivation.

All transformants are not recombinants but all recombinants are transformants.

- **Non-Transformants:** Hosts that do not take up the vector DNA (Non-recombinant).
- **Transformants:** Hosts that take up the vector DNA (Recombinant or Non-recombinant).
- **Recombinants:** Transformant hosts that take up the recombinant DNA (Vector DNA with desired DNA).
- **Non-Recombinants:** Transformant hosts that take up the nonrecombinant DNA (Vector DNA without desired DNA)
- rop → Codes for the proteins involved in the **replication** of the plasmid.

Plasmids as vectors:

- Extra-chromosomal, circular, double-stranded DNA.
- Replicate independent of the control of chromosomal DNA (autonomously).
- They may have 1 or 2 copies per cell or even 15-100 copies per cell.

OTHER CLONING VECTORS

Ti-plasmid of *Agrobacterium tumefaciens*

- *Agrobacterium tumefaciens*, a pathogen of several **dicot plants** is able to deliver a piece of DNA known as **'T-DNA'** to transform normal plant cells into a tumor and direct the tumor cells to produce the chemicals required by the pathogen.
- **Disarmed tumour inducing (Ti) plasmid** is used which is no more pathogenic to the plants but is still able to use the mechanism to deliver the genes of our interest into varieties of plants.

Bacteriophages

- High copy number than plasmid.

Retroviruses

- Retroviruses in animals have the ability to transform normal cells into cancerous cells.
- Disarmed retroviruses are used to deliver desirable genes into animal cells.

Methods of Transformation

1. **Micro-injection**
   - Recombinant DNA is directly injected into the nucleus of an animal cell.
2. **Biolistic/Gene gun**
   - Plant cells are bombarded with high velocity microparticles of gold or tungsten coated with DNA.
3. **Heat-shock method**
4. **Disarmed pathogen vectors**

Competent Host for Transformation with recombinant DNA

- DNA is hydrophilic, so it can not pass through cell membranes.
- In order to force cell to take up alien DNA/rDNA, it must first be made 'competent' by treating with **ice cold calcium chloride** ($CaCl_2$).
- Entry of rDNA in host cell is due to transient pores created by heat shock (42°C) and not due to Ca^{2+} ions.
- Divalent cations increases the efficiency with which DNA enters the bacterium through pores in its cell wall.

Process of Recombinant DNA Technology

1. **Isolation of the Genetic Material (DNA)**
2. **Fragmentation by restriction endonucleases**
3. **Separation and isolation of DNA fragments**
   - **Gel electrophoresis:**
     - Separation of negatively charged DNA molecules under an electric field through a medium/matrix.
     - Most commonly used matrix for DNA separation is agarose.
4. **PCR-Polymerase Chain Reaction**
   - *In vitro* **amplification of DNA (gene of interest)**
     - The amplified fragment if desired can now be used to ligate with a vector for further cloning.
5. **Ligation of the DNA fragment into a vector by DNA ligase**
6. **Insertion of recombinant DNA into the host cell**
   - Transformed host cells are selected with the help of selectable marker genes.
7. **Culturing of recombinant host cells (Biosynthetic stage)**
   - The cells harbouring cloned genes of interest may be grown in laboratory/ bioreactors.
   - **Bioreactors:** Vessels in which raw materials are biologically converted into specific products using microbial plant, animal or human cells and provide optimal growth conditions (temperature, pH, substrate, salts, vitamins, oxygen).
8. **Downstream processing**
   - Separation and purification of the desired product/ recombinant protein from heterologous host (non native host).
   - Product has to be formulated with suitable preservatives.
   - Strict quality control testing is done for each product.
   - The downstream processing and quality control testing vary from product to product.
9. **Product is subjected for marketing as a finished product**

In Open Culture System/Continuous Culture System

- Used medium is drained out from one side.
- Fresh medium is added from the other to maintain the cells in their physiologically most active log/exponential phase.
- Larger biomass → Higher yields of desired protein.

Biotechnology and Its Applications

SHORT NOTES

- **Biotechnology:** Deals with industrial scale production of biopharmaceuticals and biologicals using GM microbes, fungi, plants and animals.
- Applications of biotechnology includes:
  - Therapeutics processed food.
  - Diagnostics bioremediation.
  - Genetically modified organisms.
  - Crops for agriculture.
  - Waste treatment.
  - Energy production.

BIOTECHNOLOGICAL APPLICATIONS IN AGRICULTURE

- Made crops more tolerant to abiotic stresses (cold, drought, salt, heat).
- Reduced reliance on chemical pesticides (pest-resistant crops).
- Helped to reduce post harvest losses.
- Increased efficiency of mineral usage by plants (prevents early exhaustion of fertility of soil).
- Enhanced nutritional value of food, e.g., golden rice, i.e., Vitamin 'A' enriched rice.
- Insect resistant plants-Bt Cotton
- Pest resistant plants-Tobacco plant (By RNAi)

BIOTECHNOLOGICAL APPLICATIONS IN MEDICINE

- 30 recombinant therapeutics have been approve for human use the world over. In India, 12 of these are presently being markted.
- Genetically Engineered Human Insulin (humulin) → manufactured by Eli Lilly, an American company in 1983.
- Gene Therapy → First clinical gene therapy was conducted in 1990 in a 4 year old girl to treat adenosine deaminase (ADA) deficiency.

MOLECULAR DIAGNOSIS METHODS

Parameters	Conventional	Modern
Early detection	Not possible	Possible
Examples	Serum and urine analysis	RDT, PCR, ELISA

TRANSGENIC ANIMALS

- Possess manipulated DNA and express foreign gene.
- Transgenic rats, rabbits, pigs, sheep, cows.
- 95% of transgenic animals are mice.

Uses of Transgenic Animals

- Transgenic models exist for study of diseases like cancer, cystic fibrosis, rheumatoid arthritis and alzheimer.
- Biological products:
  - α-1 antitrypsin - Treat emphysema
  - Similar attempts are made for treatment of PKU (Phenylketonuria) and cystic fibrosis.
  - First transgenic cow: Rosie developed in 1997 producing human protein enriched milk (2.4 grams per litre).
- Vaccine Safety:

  Transgenic mice are being used to test the safety of polio vaccine to replace the use of monkey.
- Chemical safety testing:

  Transgenic animals are made more sensitive to toxic substances to obtain results in less time.

ETHICAL ISSUES

- GEAC (Genetic Engineering Approval Committee): Makes decisions regarding the validity of introducing GMO for public services.
- Biopiracy refers to the use of bio-resources by multinational companies and other organisations without proper authorization from the countries and people concerned without compensatory payment.
- The Indian Parliament has recently cleared the second amendment of the Indian Patents Bill.

CONTROVERSIES REGARDING PATENTS AND BIOPIRACY

- Basmatic rice:
  - 2,00,000 varieties of rice in India, 27 documented varieties of Basmati rice in India.
  - In 1997, an American company got patent rights on Basmatic rice through the US Patent and Trademark Office.
- Turmeric and Neem:
  - Though Indian were using turmeric for hundred of years, in 1995, the patent for the use of turmeric in wound healing is given to university of Mississippi medical centre.
  - Several traditionally herbal based medicinal products made up of turmeric and neem were also got patent.