HUMAN REPRODUCTION

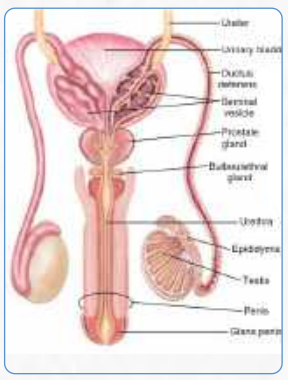
1 INTRODUCTION

- Humans are sexually reproducing viviparous organisms
- Reproductive system is composed of
  - **Primary sex organs** - Site for gamete formation
  - **External genitalia** - Involved In copulation
- Accessory ducts, Accessory glands → Facilitate transport of gametes

2 THE MALE REPRODUCTIVE SYSTEM

- Location: Pelvic region
- **Seminal plasma** from these contains fructose, calcium, enzymes
- Its secretions **lubricate the penis**
- Vas deferens receives a duct from seminal vesicle and opens into the urethra as the **Ejaculatory duct**

Accessory glands
- Seminal vesicle (1 pair)
- Prostate gland (one)
- Bulbourethral gland (1 pair)



External genitalia of Male/Penis

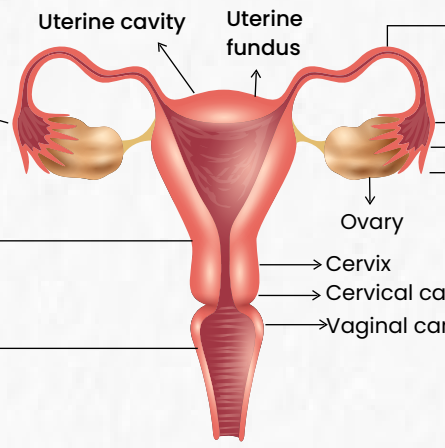
Parts	Features
Urethra	Originates from the urinary bladder and extends through the penis
Special tissues	Help in erection of penis to facilitate insemination
Glans penis	Enlarged end of penis covered by loose fold of skin called foreskin

3 FEMALE REPRODUCTIVE SYSTEM

- **Location:** Pelvic region

Accessory ducts

- **Oviduct/fallopian tube**
  - 10-12 cm (length)
  - Extend from ovary to uterus
- **Uterus/womb**
  - Inverted pear shaped
  - Attached to pelvic wall by ligaments
- **Vagina**



Parts of Fallopian tube
- Isthmus → Last part of oviduct, Narrow lumen, Joins the uterus
- Ampulla - Wider part
- Infundibulum - Funnel shaped
- Fimbriae - Finger like projections that collect ovum after ovulation

Birth canal = Cervical canal + Vaginal canal

- Uterine wall consists of three layers:
- **Endometrium** - Lines lumen, glandular a undergoes cyclic changes during menstruation
- **Myometrium** - Thick layer of smooth muscles that show strong contractions during delivery
- **Perimetrium** - External thin membrane

Parts	Features
Mons pubis	Cushion of fatty tissue covered by skin and pubic hair
Labia majora	Fleshy folds of tissue that extend down mons pubis and surround the vaginal opening
Labia minora	Paired folds of tissue under the labia majora
Clitoris	Tiny finger like structure which lies at the upper junction of labia minora above the urethral opening
Hymen	• Membrane that partially covers the opening of vagina • Can be torn while-sudden jolt/fall, horse riding, cycling, insertion of vaginal tampon • May or may not be torn during the first coitus so its presence or absence is not reliable indicator of virginity or sexual experience.

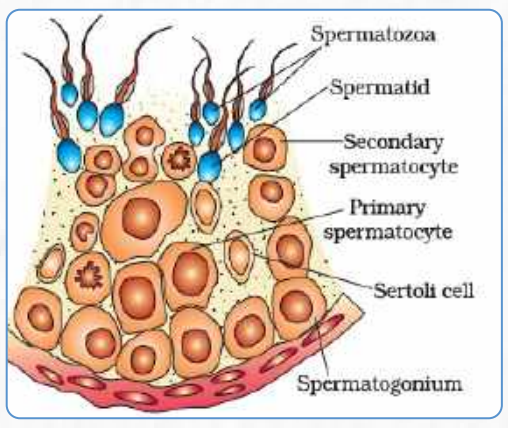
4 PRIMARY SEX ORGANS

Parameters	Male	Female
Organ	Testis	Ovary
Number	2	2
Shape	Oval	Almond shaped
Location	Outside abdominal cavity in s pouch called **scrotum**	Lower abdomen one on on each side
Dimensions	Length 4-5 cm, Width 2-3 cm	Length 2 to 4 cm
Covering	Dense connective tissue (outermost)	Thin epithelium (outermost)
Functions	Sperm formation; synthesise steroidal **testicular** hormones like androgens	Ova formation; synthesise steroidal **ovarian** hormones like estrogen and progesterone
Compartments	250 testicular lobules; 1-3 coiled seminiferous tubules/lobule	Peripheral cortex and Inner medulla zones in ovarian stroma have follicles in various developing stages

• **Cells lining the seminiferous tubules**	**Functions**
1. Male germ cells/spermatogonia	Sperm formation
2. Sertoli cells	Provide nutrition to the germ cells

- Scrotum helps in maintaining the temperature 2 to 2.5°C lower than body temperature, necessary for **spermatogenesis.**
- Interstitial spaces outside the seminiferous tubules contain **immunocompetent** cells and **Leydig cells**
- Ovary is connected to pelvic wall and uterus by **ligaments.**

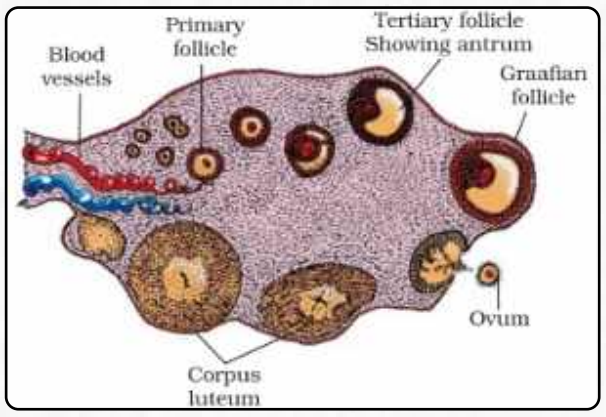
5 GAMETOGENESIS



- **Process of gamete formation**

Parameters	Male	Female
• **Term**	Spermatogenesis	Oogenesis
• **Process begins**	At puberty	During embryonic development
• **Ploidy & number of chromosomes**		
2n = 46	Male germ cells/ Spermatogonia	Female mother cells/ Oogonia
	Mitosis & differentiation	**In** Fetus (arrested at prophase I)
2n = 46	Primary spermatocytes	Primary oocytes
	Periodic Meiosis I	Birth / Puberty
n = 23	Secondary spermatocytes	1st polar body / Secondary oocyte
	Meiosis II	
n = 23	Spermatids	2nd polar body / Ovum
	Spermiogenesis	
n = 23	Sperms	
	Spermiation	
	Released from seminiferous tubules	

Follicies
Primary
(Single layer of granulosa cells)
↓
Secondary
(More layers of granulosa cells and a new theca layer)
↓
Tertiary
(Fluid filled cavity **antrum** and theca layers are organised into external and internal layers Secondary oocyte forms **acellular zona pellucida** a round it)
↓
Graafian mature
(No more oogonia are formed and added after birth)
↓
Ovulation



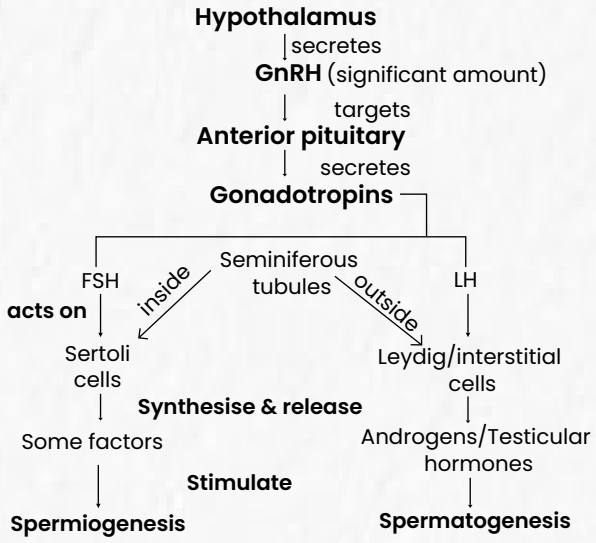
- No more oogonia are formed and added after birth
- A large number of follicles degenerate from birth to puberty so only **60,000-80,000 primary follicles are left in each ovary at puberty.**
- **Meiosis** in oogenesis results in unequal sized **cells** and the secondary oocyte retains bulk of the nutrient rich cytoplasm of the primary oocyte,
- Fate of polar body is not certain
- During the embryonic development, a couple of million gamete mother cells (oogonia) are formed within each fetal ovary

Spermiogenesis is transformation of spermatids to sperms and sperms head embedded in Sertoli cells

6 SEMEN

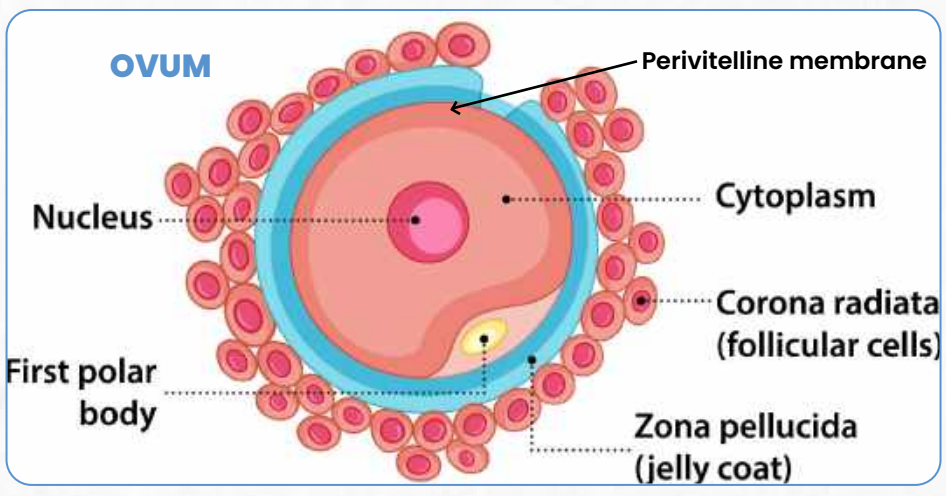
- Secretions of epididymis and vas deferens are essential for maturation and motility of sperms
- **Male ejaculates** about 200-300 million sperms during a coitus.
- **For normal fertility:**
  - 60% sperms must have normal shape and size
  - **40% of 60% sperms must show vigorous motility**

7 HORMONAL REGULATION IN MALES

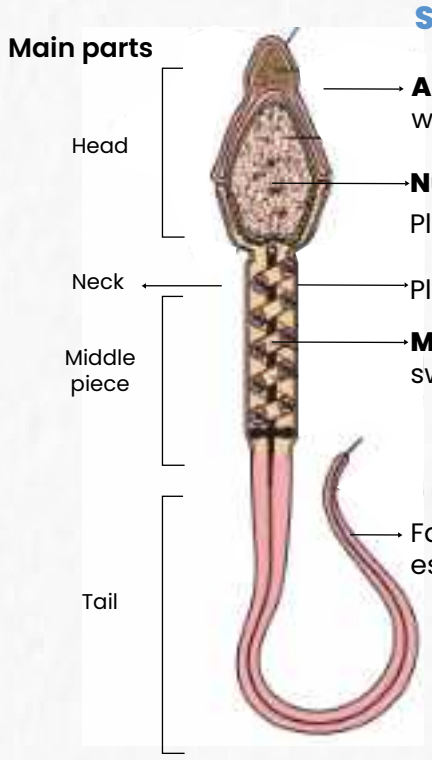


The function of male sex accessory ducts and glands are maintained by the testicular hormones (androgens)

8 STRUCTURE OF GAMETES



SPERM



9 HORMONAL REGULATION IN FEMALES AND MENSTRUAL CYCLE

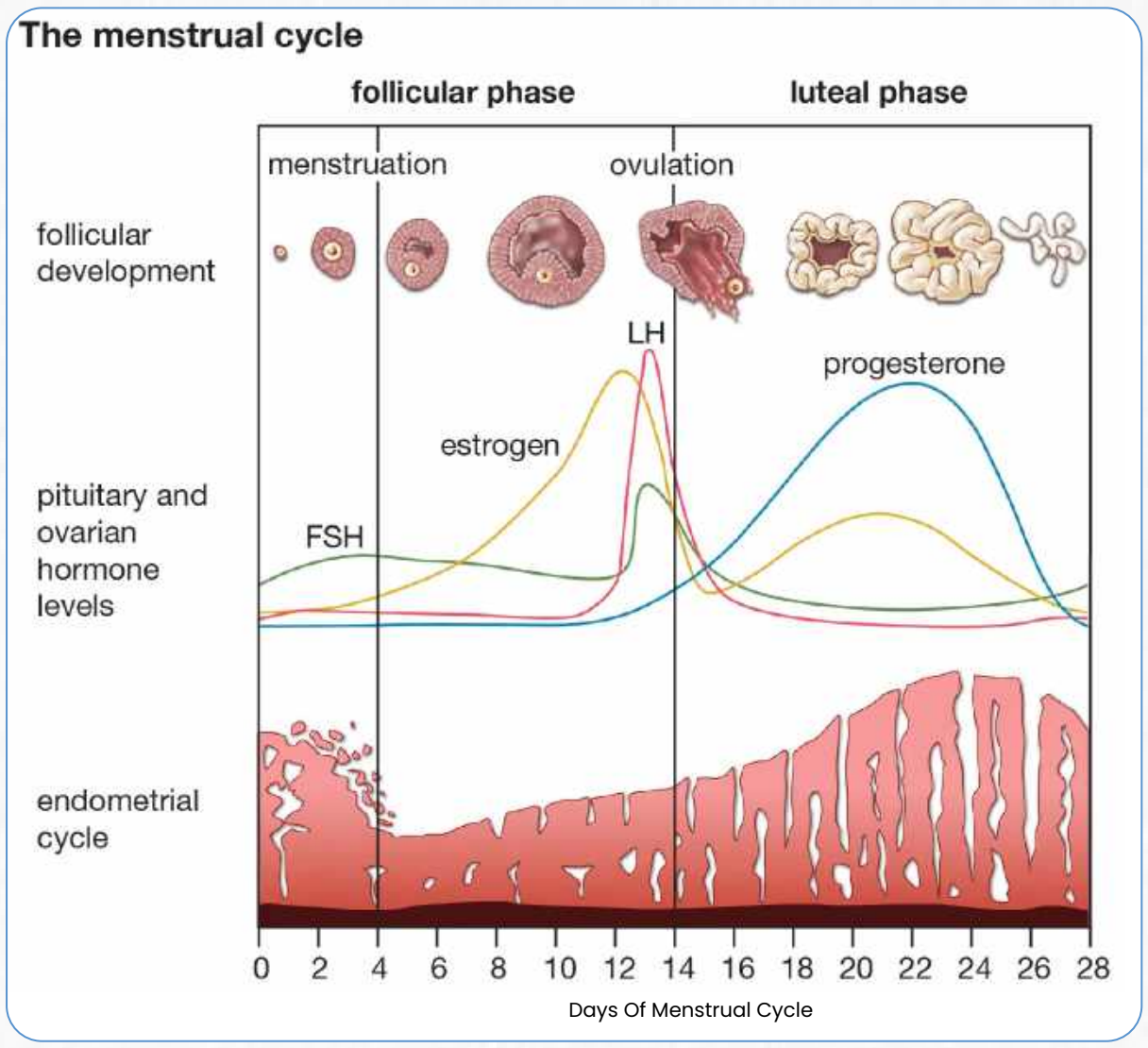
- The cycle of events starting from one menstruation till the next one is termed **menstrual cycle**
- **Characteristic** of female primates
  - Monkeys
  - Apes
  - Humans
- **Begins** at puberty - **menarche**
- **Ceases** at 50 years - **menopause**

Reproductive phase

- **Cycle occurs if ovum remains unfertilized**
- **Lack of cycle** may be an indication of Pregnancy, stress, poor health etc
- Average duration in humans = 28/29 days

Menstrual Hygiene

- Maintenance of hygiene and sanitation during menstruation isvery important
- Take bath and clean yourself regularly use sanitary napkins/home made pads
- Change sanitary pads after every 4-5 hrs.
- Dispose of used sanitary napkins properly by wrapping it in used paper.
- After handling the napkin wash hands with soap



• **If ovum gets fertilized, endometrium is maintained by progesterone** necessary for implantation and other events of pregnancy.
• During pregnancy all events of menstrual cycle stop

Menstrual Cycle

• Changes in the ovary and the uterus are induced by changes in the levels of pituitary and ovarian hormones

Phase	Duration	Hormones & their effects	Events in ovary	Events in uterus
Menstrual	3-5 days	Drastic decline in progesterone	Corpus luteum degenerates	• Breakdown of endometrial lining and its blood vessels which forms liquid that comes out through vagina constituting menstrual flow
Follicular or Proliferative phase	Variable	Gradual increase in FSH & LH that stimulate secretion of estrogen from follicles	Primary follicle gradually matures to Graafian follicle	• Endometrium regenerates through proliferation
Ovulation	14 day (Middle of cycle)	FSH and LH at peak, (LH surge)	Rupture of Graafian. follicle and release of only one ovum/ cycle	• Proliferation of endometrium continues
Luteal or Secretory	Fixed (14 days)	Secretion of progesterone and estrogen	Remnants of the Graafian follicle transforms into corpus luteum	• Endometrium is maintained • If ovum remains unfertilized, endometrium is sloughed off, marking a new cycle

10 SEQUENCE OF REPRODUCTIVE EVENTS OCURING IN HUMANS INCLUDE:

Gametogenesis → Insemination → Fertilization → Implantation → Gestation → Parturition/Birth

11 PATH FOLLOWED BY GAMETES IN FEMALE REPRODUCTIVE TRACT

- During coitus, semen is released from male reproductive tract by the penis into the female reproductive tract i.e., the vagina by process termed Insemination
- **Sperms** → Released in vagina → swim through → Cervix → enters → Uterus
- **Oocyte** → Released in body cavity → Captured by → Fimbriae → Infundibulum → Reach towards Ampulla
- **Ampulla (site of fertilisation)** → Fusion of gametes/**syngamy/Fertilization** (vital event of sexual reproduction)
- Fertilization can only occur if the ovum and sperms are transported simultaneously to the ampullary region. This is the reason why not all copulations leads to fertilization and pregnancy

12 CHANGES IN GAMETES DURING FERTILIZATION

- Secretions of **Acrosome part of sperm**

  ↓ allow

  Entry of sperm into cytoplasm of oocyte through zona pellucida and plasma membrane
- Changes in zona pellucida prevent entry of additional sperms and **ensures that only one sperm can fertilise an ovum** Inducing completion of Meiosis II of secondary oocyte

Form → 2^{nd} Polar body (n) (1) → (1) (Released in Perivitelline space)

Form → Cytoplasmically unequal 2nd meiotic division → egg/ovum

Ovum/ootid (n) (1) → Nuclei fuse ← Sperm (n)

Nuclei fuse → **Zygote** (2n)

- **Characteristics :**
  - Vital link that ensures continuity of species between organism of one generation and the next.
  - Sex of a child is decided at this stage

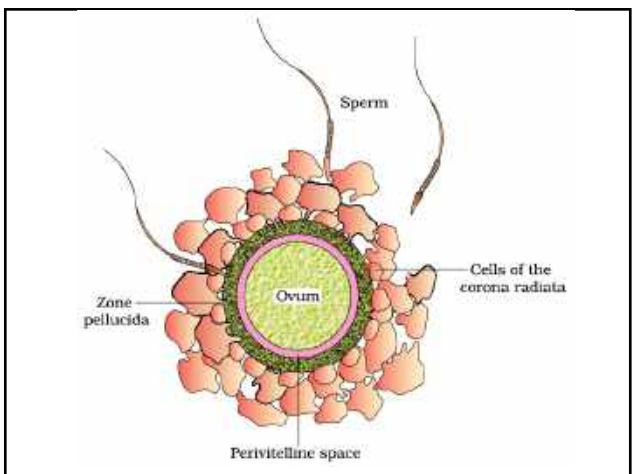
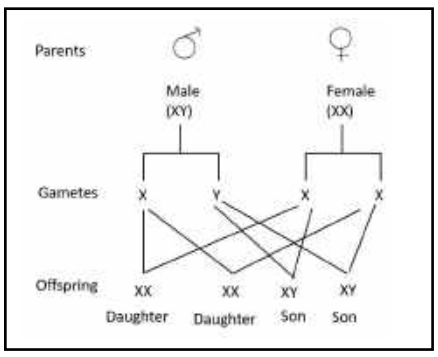


Fig. : Ovum surrounded by few sperms

13 SEX OF A BABY IS DETERMINED BY THE FATHER

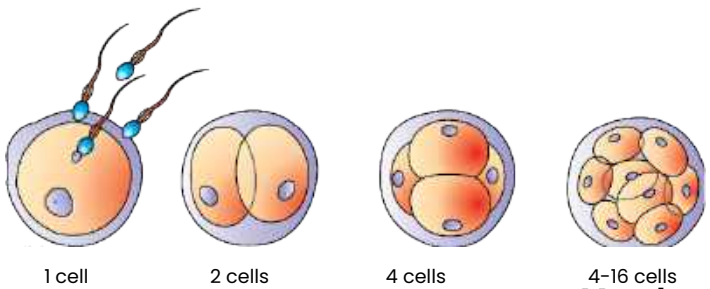
Parameters : Chromosome pattern Gametes formed Fusion of gametes

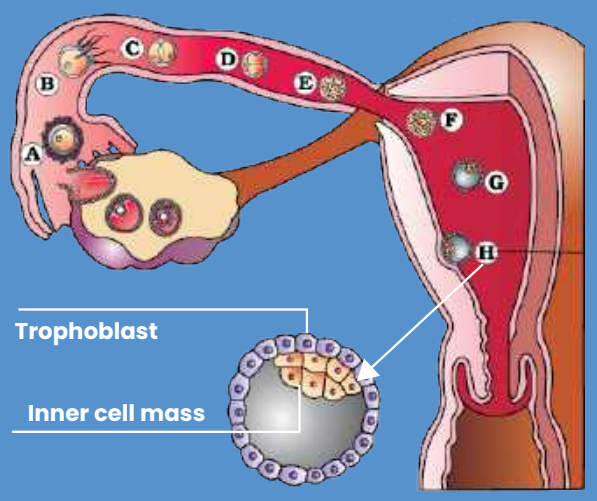


- 50% male gametes carry X chromosome and 50% carry Y chromosome
- Zygote would carry either XX or XY depending on whether the sperm carrying X or Y fertilizes the ovum

14 DEVELOPMENT OF THE ZYGOTE

- Every sexually reproducing organism, including human beings begin life as a single cell i.e., the zygote.
- During embryogenesis, zygote undergo cell divisions and cell differentiation
- The process of development of embryo from zygote is called embryogenesis.
- Cleavage starts as zygote moves through isthmus to the uterus
- Daughters formed after cleavage are called Blastomeres





Trophoblast – Outer layer of blastomeres attaches to endometrium

Inner cell mass – Inner group of cells attached to trophoblast ⟶ Differentiates into embryo with three germ layers

Blastocyst Implants in uterus
The inner cell mass contains certain cells called stem cells which have the potency to give rise to all the tissues and organs.

After attachment, uterine cells divide rapidly and cover the blastocyst. Embedding of blastocyst in endometrium is called

Implantation Leads to ⟶ **Pregnancy**

Germ layers
- Outer – Ectoderm
- Middle – Mesoderm
- Inner – Endoderm

These together give rise to all tissues/organ in adults.

15 CHANGES AFTER IMPLANTATION

Embryo/Foetus
Finger like projections appear on the trophoblast called chorionic villi

Mother
Uterine tissue

Interdigitate to form

Placenta **(Structural and functional unit between embryo/foetus and mother)**

- **Functions:**
  - Supply of O_2 and nutrients to the embryo
  - Removal of CO_2 and excretory/waste materials produced by the embryo
- **Acts as endocrine tissue :**
  - hCG/human chorionic gonadotropin
  - hPL/human placental lactogen

  → **Produced only during pregnancy**
  - Relaxin (Also secreted by ovary in later phase of pregnancy)
  - Estrogens
  - Progestogens
  - Cortisol
  - Prolactin
  - Thyroxine

Increase several folds during pregnancy, essential for supporting:
- Foetal growth
- Metabolic changes in mother
- Maintenance of pregnancy

•Placenta is connected to the embryo through an umbilical cord which helps in the transport of substances to and from the embryo

16 GESTATION PERIOD

- Average duration of pregnancy in
- Dog ~ 63 days
- Cat ~ 63 days
- Elephant ~ 18-22 months
- Human ~ 9 months

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	8	Foetus develops limbs and digits
	III (end)	12	Most of major organ systems are formed including external genital organs
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

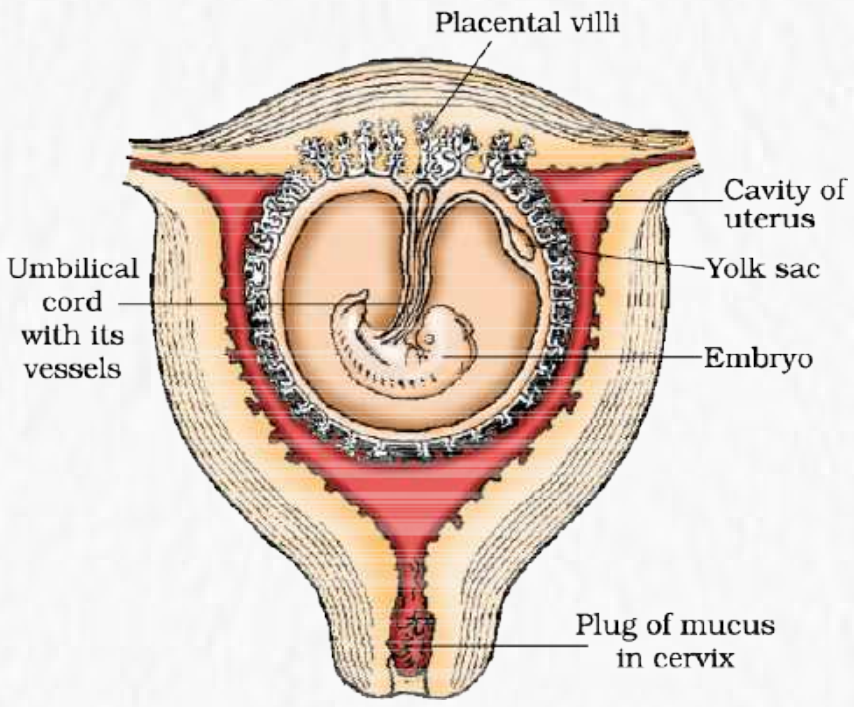
17 PARTURITION

- Defined as delivery of the foetus (Child birth)
- Signals for parturition originate from → Fully developed foetus, Placenta
  - Lead to ↓
  - Mild uterine contractions
  - called ↓
  - **Foetal ejection reflex**
  - triggers | release of ↓
  - **Oxytocin from posterior pituitary of mother** → **This is positive feedback loop**
  - Causes ↓
  - Strong uterine contractions
  - leading to ↓
  - Expulsion of baby through birth canal **(Parturition)**
  - followed by ↓
  - Expulsion of placenta

- Oxytocin is synthesized by its **source gland hypothalamus** but released from posterior pituitary.

- Child birth is induced by a complex neuroendocrine mechanism involving cortisol, estrogens and oxytocin.

Fully developed foetus
Placenta



The human foetus within the uterus

18 MAMMARY GLANDS AND LACTATION

- Functional mammary gland is characteristic of all female mammals
- Paired structures (Breasts) that contain variable amount of fat.

Glandular tissue ↓
(15-20) Mammary lobes
Contain ↓
Mammary alveolus
cluster of cells (secrete and store milk) in their lumen
opens into ↓
Mammary tubules
join to form ↓
Mammary duct
Many join to form ↓
Mammary ampulla
connected to ↓
Lactiferous duct
(Through which milk is sucked out)

Fig.: Sectional view of Mammary gland

- **Mammary glands**
  - Undergo differentiation during pregnancy
  - Secrete milk after child birth that helps mother in feeding new born by process called lactation.

- Milk produced during initial few days of lactation is called colostrum which contains several antibodies, absolutely essential to develop resistance for the newt born babies.
- Breast feeding during the initial period of infant growth is recommended by doctors for bringing up a healthy baby

REPRODUCTIVE HEALTH

INTRODUCTION

1) DEFINITION

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

2) REPRODUCTIVE HEALTH : PROBLEMS AND STRATEGIES

India was amongst first countries in the world to initiate action plans to attain reproductive health such as Family Planning Programmes (FPP) in 1951.

3) POPULATION STABILISATION

• According to 2011 census, our population growth rate was less than 2 % i.e. 20/1000/year.

Year	World Population	Indian Population
1900	2 billion	350 million
2000	6 billion	1 billion
2011	7.2 billion	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 Growth Rate :**
  - Motivate smaller families by using various contraceptive methods with slogans "Hum do Hamare do", advertisements and posters.
  - Urban couples adopting : "One child norm".
- **Statutory raising of marriageable age:**
  - Female to 18 years.
  - Male to 21 years.
  - Incentives given to couples with small families.

4) 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.

- **There are two principle methods of birth control:**
  - Natural methods.
  - Artificial methods.

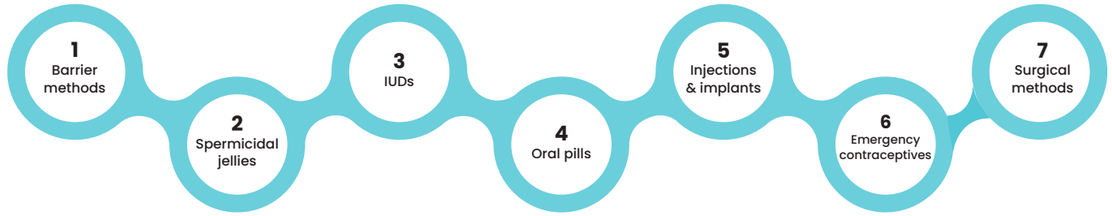
5) NATURALTRADITIONAL METHODS

- **Principle of avoiding physical meeting of the egg and sperms.**
- **Chances of failure are high.**

Method	Mode of Action (MoA)
Periodic abstinence :	Couples abstain from coitus from day 10 to 17 of the menstrual cycle i.e. fertile period.
Withdrawal method :	Insemination is avoided as the male partner, Coitus interruptus, withdraws his penis from the vagina just prior to ejaculation.
Lactational amenorrhea :	Absence of menstruation upto 6 months during period of intense lactation following parturition.

6) ARTIFICIAL METHODS

The various methods of contraception are enlisted below:



7) ARTIFICIAL METHODS

(I) Barrier methods :

- Prevent ovum and sperm from physically meeting.
- Self inserted and offer privacy to user.

(a) Condoms & its types

Made up of rubber and thin latex

Parameter	Male	Female
• Region covered • Provides production from STIs	• Penis • Yes	• Vagina and cervix • Yes

Male condom (Nirodh) | Female condom

(b) Diaphragms, cervical caps, vaults :

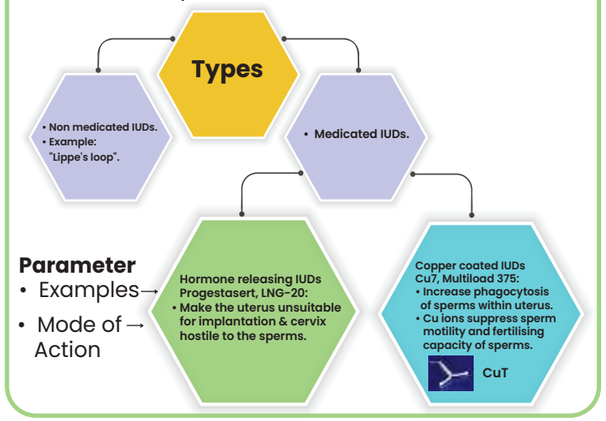
- Rubber barriers that cover the cervix during coitus.
- Reusable.
- Do not protect from STIs.
- Used by females only.

(II) Spermicidal jellies, foams and creams :

- Kill the sperms by creating acidic pH.
- Used along with barrier methods to increase their efficiency.

(III) Intra-uterine devices (IUDs) :

- Inserted by doctors or expert nurses in uterus.
- IUDs are one of the most widely accepted method of contraception in India.



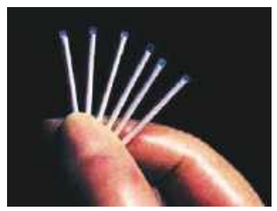
(IV) Oral Contraceptive Pills (OCP$_s$) or tablets :

Parameter	Non-steroidal	Steroidal
Example or composition.	Saheli.	Progestogens (Prg) alone or combination of Prg and Estrogens (Est).
Mode of action.	Interferes with implantation.	Inhibit ovulation & implantation; also alter the quality of cervical mucus to retard entry of sperms.
Dosage.	'Once a week' pill.	Pills have to be taken daily for a period of 21 days starting preferably within first 5 days of menstrual cycle.
Effectiveness.	High contraceptive value with very few side effects.	Pills are very effective with lesser side effects & well accepted by females.

Saheli was developed at CDRI, Lucknow, Uttar Pradesh.

(V) Implants :

- Placed under skin.
- Effective periods are much longer.
- Composition: Progestagens alone/Combination of Progestagens and Estrogens.
- **Mode of Action (MoA).**
  - Inhibit ovulation and implantation.
  - Alter the quality of cervical mucus to retard entry of sperms.
  - Injections usually share similar MoA and composition as implants.



(VI) Emergency contraceptives :

Types

- Progestogens.
- Combination of Prg + Est.
- IUDs.

Charateristics

- Effective within 72 hrs of coitus.
- Used to prevent contraception resulting from rape or unprotected intercourse.

(VII) Surgical/Sterilisation methods :

- Poor reversibility but highly effective.
- **Mode of action**
  Blocks gamete transport.

Types

Tubectomy

- In females:
- Cut and tie fallopian tubes.
- Incision in abdomen or through vagina.

Vasectomy

- In males:
- Cut and tie vas deferens.
- Small incision on the scrotum.

8) MEDICAL TERMINATION OF PREGNANCY (MTP)/INDUCED ABORTION :

- **MTP** : Intentional or voluntary termination of pregnancy before full term.
- MTP was legalized in India in **1971 .**
- **When can MTP be performed?**
- Unwanted pregnancy due to rape, failure of contraception, casual unprotected intercourse.
- If continuation of pregnancy could harm the mother of foetus or both.

- **Permission of how many medical practitioners is needed for MTP depending on duration of gestation?**

01 less than/upto 12 weeks.

02 More than 12 but less than 24 weeks.

- **Intention behind MTP amendment act 2017 :**
- Reduction in 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.

9) AMNIOCENTESIS :

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

10) SEXUALLY TRANSMITTED INFECTIONS (STIS) :

- **Alternately named:** Venereal diseases (VD) or reproductive tract infections (RTIs).
- **High vulnerability/risk group:** 15-24 years.
- **Mode of transmission (MoT):** Sexual intercouse.

Category		Disease
1	Bacterial	Gonorrhea, Syphilis, Chlamydiasis.
2	Protozoan	Trichomoniasis.
3	Viral	Genital herpes, Hepatitis-B, Genital warts, AIDS.

- Bacterial and protozoan diseases are completely curable if detected early and treated properly.
- **Other MoT for hepatitis-B virus and HIV infection include:**
- Sharing of injection needles, surgical instruments with infected persons.
- Transfusion of blood.
- From infected mother to foetus.

- **Symptoms and complications of STIs :**

1 Early detection

Symptoms

Itching, fluid discharge, slight pain, swellings in the genital region.

2 Late detection

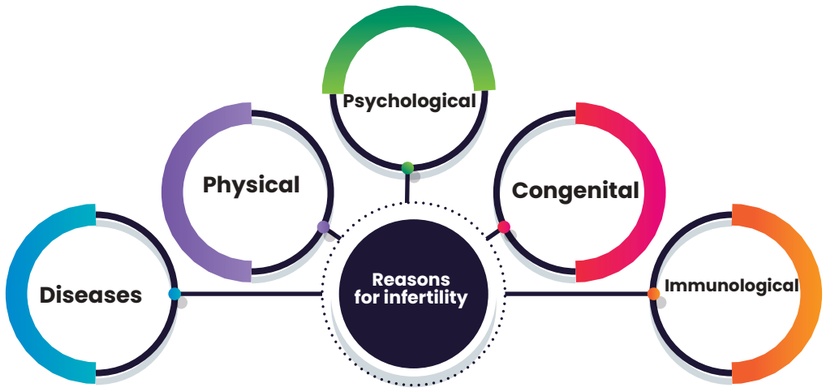
Complications

Pelvic inflammatory diseases (PIDs), abortions, still births, ectopic pregnancies, infertility, cancer of reproductive tract.

- **Preventive measures to avoid STIs:**
- Avoid sex with unknown partners/multiple partners.
- Always try to use condoms during coitus.

11) INFERTILITY

- **Infertile couple :** Unable to produce children inspite of 2 years of unprotected sexual co-habitation.



Help for infertile couples comes in the form of ASSISTED REPRODUCTIVE TECHNOLOGIES (ART).

Parameter :
(i) Site of fertilisation
(ii) Can female produce ova ?
(iii) Embryo transfer.
(iv) Example of techniques.

In-vitro fertilisation :
- Outside the body in simulated conditions in laboratory
- Yes
- Yes
- ZIFT, IUT, ICSI

in-vivo fertilisation:
- In the female reproductive tract
- No
- No
- GIFT, AI, IUI

Site of Embryo Transfer (ET) based on number of blastomeres :

Parameter :
- Location.
- Technique.

Upto 8 blastomeres :
In fallopian tube.
- ZIFT: zygote Intra fallopian transfer.

More than 8 blastomeres:
In uterus.
- IUT: Intra uterine transfer fallopian transfer.

- **Other details of ART involved :**

(i) ICSI : Intra cytoplasmic sperm injection :
- Sperm injected directly into the egg.

(ii) Artificial Insemination (AI):
- Semen introduced in vagina or uterus.
- Low sperm count or inability of male to inseminate female.
- **IUI :** Intra uterine insemination.

(iii) GIFT : Gamete intra fallopian transfer:
- Female can provide conditions for fertilisation and further development.

EVOLUTION

1 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 gas, dust)		• Occurred in solar system of Milkyway **galaxy**

Events after expansion of universe :

- Temperature declined
- H_2 + He formed
- Gases condensed
- Galaxies of present day formed

Hypothesis for Origin of life on early earth :

- No atmosphere existed on early earth.
- Water vapours, methane, carbon dioxide and ammonia release from molten mass covered the surface.

$$H_2O \xrightarrow{U.V\ rays} H_2 + O_2$$

$$\left.\begin{matrix} NH_3 \\ CH_4 \end{matrix}\right] + O_2 \rightarrow CO_2 + H_2O + \text{other contents}$$

- H_2O vapour falls as rain to fill all the depression and form oceans.
- Ozone layer was formed

2 ORIGIN OF LIFE

Life appeared 500 million years after the formation of earth, i.e, almost **4 billion years ago.**

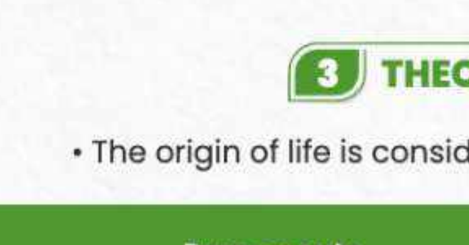
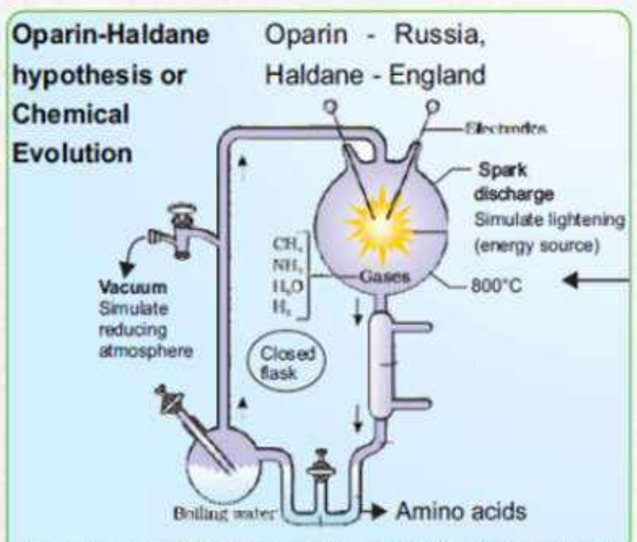
First non-cellular forms of life
- **Probably originated 3 billion years ago**
- Would have possibly originated from giant molecules (RNA, protein, polysaccharide, etc.)
- These capsules **reproduced** their molecules perhaps.

First cellular forms of life
- **Probably originated 2 billion years ago**
- Arose in water environment (aquatic)

This version of Biogenesis i.e., the first form of life arose slowly through evolutionary forces from non-living molecules is accepted by majority.

3 THEORIES FOR ORIGIN OF LIFE

- The origin of life is considered a unique event in the history of universe

Theory	Proponents	Connotations
Special creation	Conventional religious literature	• All living organisms that we see today were created as such. • 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		• **Life comes only from pre-existing life** • He showed that in pre-sterlised flasks, life did not come from **"killed yeast".**
Oparin-Haldane hypothesis or Chemical Evolution	Oparin - Russia, Haldane - England  **Fig.: Diagrammatic representation of Miller's experiment**	• Formation of life was preceded by chemical evolution i.e., formation of diverse organic molecules from inorganic constituents • First form 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 others observed, formation of sugars, nitrogen bases, pigments and fats. • **Analysis of meteorite content also revealed similar compounds indicating that similar processes are occuring elsewhere in space**

4 EVIDENCES OF EVOLUTION

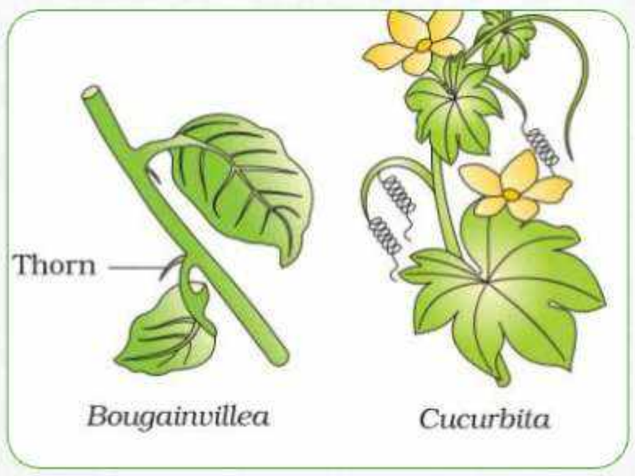
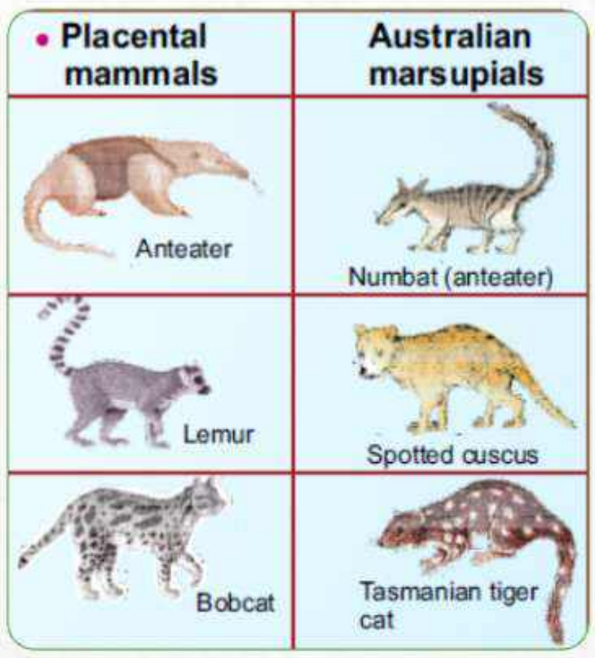
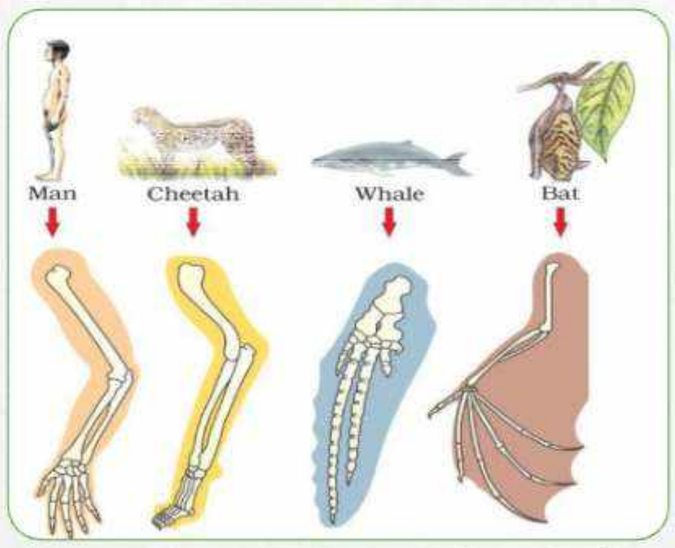
1. Palaeontological evidences (Evidences from fossils)

- **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)**
- Rocks form sediments and a cross-section of Earth's crust indicates the arrangement of sediments one over the other during the long history of earth.
- Different-aged rock sediments contain fossils of different life-forms who probably died during the formation of the particular sediment. Some of them appear similar to modern organisms. They **represent extinct organisms (e.g., Dinosaurs).**

2. Embryological evidences

- Proposed by **Ernst Heckel**
- 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.
- **Molecular homology**
  Similarities in proteins and genes performing a given function among diverse organisms indicates common ancestry. These biochemical similarities point to the same shared ancestry as structural similarities among diverse organisms.

3. Morphological and anatomical evidences

Parameters	Homologous organs	Analogous organs
Common ancestory	✓	✗
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		
fore limbs of vertebrates		• Wings of butterfly and birds • Eyes of octopus and mammals • Flippers of Penguins and Dolphins • Sweet potato (root modification) and potato (stem modification
Vertebrate hearts, Vertebrate brains		

5 ADAPTIVE RADIATION

The process of evolution of different species in a given geographical area starting from a point and literally radiating to other areas of geography (habitats)

Examples

Darwin's finches

- Small black birds
- Evolved fromseed eating birds inGalapagos Island
- Altered beaks arose, enabling them to become insectivorous and vegetarian finches.

Fig.: Variety of beaks of finches that Darwin found in Galapagos Island

Fig.: Adaptive radiation of marsupials of Australia

When more than one adaptive radiation appeared to have occurred in an isolated geographical area (representing different habitats), one can call this **convergent evolution.**

Examples	
Placental mammals	**Australian marsupials**
Mole	Marsupial mole
Anteater	Numbat (banded anteater)
Mouse	Marsupial mouse
Lemur	Spotted cuscus
Flying squirrel	Flying phalanger (Sugar glider)
Bobcat	Tasmanian tiger cat
Wolf	Tasmanian wolf

6 BIOLOGICAL EVOLUTION

- Metabolic capabilities of different cellular forms of life under the influence of natural selection contributed to biological evolution.

- Rate of appearance of new forms is linked to the life cycle or the life span. e.g., Microbes have the ability to multiply and become millions within hours whereas fish or fowl would take millions of years as life span of these animals are in years.

Theories For The Evolution Of Life Forms

Lamarck's theory of evolution

- **Given by French naturalist Lamarch**

Main postulates :
- Use and disuse of organs
- Inheritance of acquired charcters

Darwin's theory of natural selection

- Darwin visited Galapagos island, Ship - **H.M.S Beagle**
- **Observations by Charles Darwin:**

(i) Existing living forms share similarities to varying degrees not only among themselves but also with life forms that existed millions of years ago.

(ii) The geological history of earth correlates with the biological history of earth.

Influence of the work of Thomas Malthus on Darwinism:

(i) Population can grow exponentially

(ii) Limited resources

(iii) Nature keeps control/check on population size which are, hence, nearly stable

Darwin's postulates:

- According to Darwin, variations are **small** and **directional** and evolution for Darwin was **gradual** process.
- Darwin asserted that in a population exist variations, which are heritable and which make resource utilisation better for few will enable only those to reproduce and leave more progeny.

- Those characteristics which enable some to survive better in natural conditions (climate,food, physical factors etc.) would outbreed others that are less-endowed to survive under such natural conditions.
- The fitness, according to Darwin, refers **ultimately and only to reproductive fitness.**
- Adaptive ability is inherited and has a genetic basis
- **Fitness is the end result of the ability to adapt and get selected by nature.**
- Branching descent and natural selection are the two key concepts of Darwinian theory of evolution.
- **Habitat fragmentation and genetic drift may accentuate variations leading to speciation.**

Weaknesses of Darwinism :

(i) Unable to explain the origin of variations

(ii) Could not explain speciation

(iii) Ignored work done by Mendel.

Mutation theory

- Given by Hugo de Vries in first decade of 20th century
- Worked on evening primrose
- New species originate as a result of mutations which are single step (saltation) large, random and directionless variations arising suddenly in a population

1

- Alfred Wallace, a naturalist who worked in Malay Archipelago had also come to similar conclusions as Charles Darwin around the same time

2

- Evolution is not a directed process in the sense of determinism. It is a stochastic process based on chance events in nature and chance mutations in the organisms

3

- **When we describe story of this world we describe evolution as a process. When we describe the story of life on earth, we treat evolution as a consequence of a process called natural selection.**

4

- Artificial selection : By intensive breeding programme, man has created breeds that differ from other breeds (e.g., dogs)

7 EXAMPLES OF EVOLUTION BY ANTHROPOGENIC ACTION

1. **Antibiotic resistant microbes**
2. **Herbicides resistant varieties**
3. **Pesticides resistant varieties**
4. **The case of Industrial melanism :** Classical example of natural selection among variants in moth population observed in England.

Parameters	Before industrialisation (1850s)	After industrialisation (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**

8 A BRIEF ACCOUNT OF EVOLUTION

Form Of Life	Appeared Around
1st non cellular form	3 bya
1st cellular form	2000 mya
invertebrates	350 mya
sea weeds and few plants	320 mya

- **Lobed fin fish** (Coelocanth) → Possibly evolved into → **Amphibians** → Possibly evolved into → Reptiles → **Mammals** (Arose from ancestral extinct reptiles)
  - Primarily aquatic
  - **1938,** fish caught in **South Africa** was earlier thought to be extinct
  - Frog salamanders
  - Aquatic and land
  - **Lay thick shelled eggs which do not dry up in sun**
  - **Viviparous** i.e., protect young ones inside mothers body
  - More intelligent in sensing and avoiding danger.
- Land reptiles were dinosaurs which disappeared 65 mya
  - **Probable reason** → Climatic change / Evolved in birds
- Some land reptiles went back water to evolve fish like reptiles eg. Ichthyosaurus (200mya)
- Modern day descendants of reptiles e.g., Turtles, Tortoised, Crocodiles.

• A Likely Reason for Restricted Distribution of Pouched Mammals in Australia

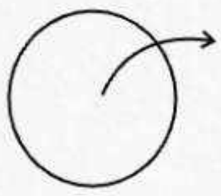
• Due to continental drift, when South America joined North America, these animals were overridden by North American fauna. Due to the same continental drift pouched mammals of Australia survived because of lack of competition from any other mammal.
• The First Mammal were Like Shrews.
• whales, dolphins seals and sea cows are aquatic mammals.

9 HARDY-WEINBERG PRINCIPLE

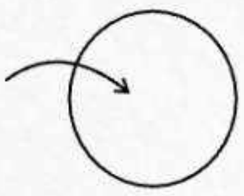
- Allele frequencies in a population are stable and is constant from generation to generation. This is called **genetic equilibrium.**
- Sum total of all the allelic frequencies is 1 and is represented as :
  p + q = 1 [p = Frequency of recessive allele (a); q = Frequency of dominant allele (A)]
  $p^2 + q^2 + 2pq$ = 1 [p^2 = Frequency of homozygous dominant (AA);q^2 = Frequency of homozygous recessive (aa); 2pq = Frequency of heterozygotes (Aa)]
- The **gene pool** (total genes and their alleles in a population) remains a constant.
- When frequency measured, differs from expected values, the difference **indicates the extent of evolutionary change or disturbance in genetic equilibrium.**

• Factors affecting Hardy-Weinberg equilibrium:

1.Gene migration : When migration of a section of population to another place or population occurs, gene frequencies change in the original as well as in the new population.



• Emigration (–)
• Some genes/alleles lost



• Immigration (+)
• New genes/alleles added

- When gene migration occurs multiple times, it is called **gene flow.**

2. Genetic recombination: Variations due to recombination during gametogenesis

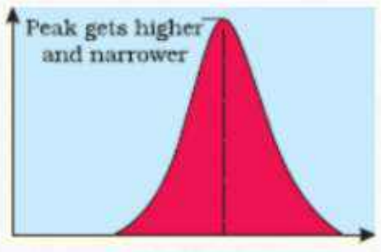
3. Mutation: Microbial experiments show that pre-existing advantageous mutations when selected will result in observation of new phenotypes. Over few generations, this would result in speciation.

4. Genetic drift: Change in gene frequencies in a small population by chance. Sometimes the change in allele frequency is so different in the new sample of population that they become a different species. The original drifted population becomes founders and effect is called founders effect.

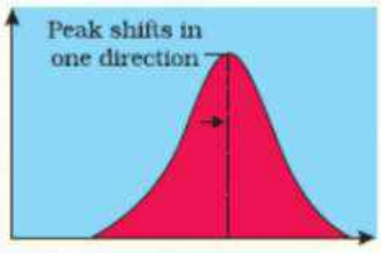
5. Natural selection : A process is which heritable variations enabling better survival are enabled to reproduce and leave greater number of progeny.

Types

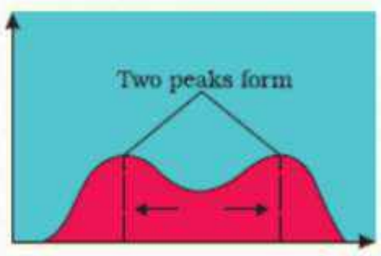
Stabilising selection
- More individuals acquire mean character value



Directional selection
- More individuals acquire value other than the mean character value



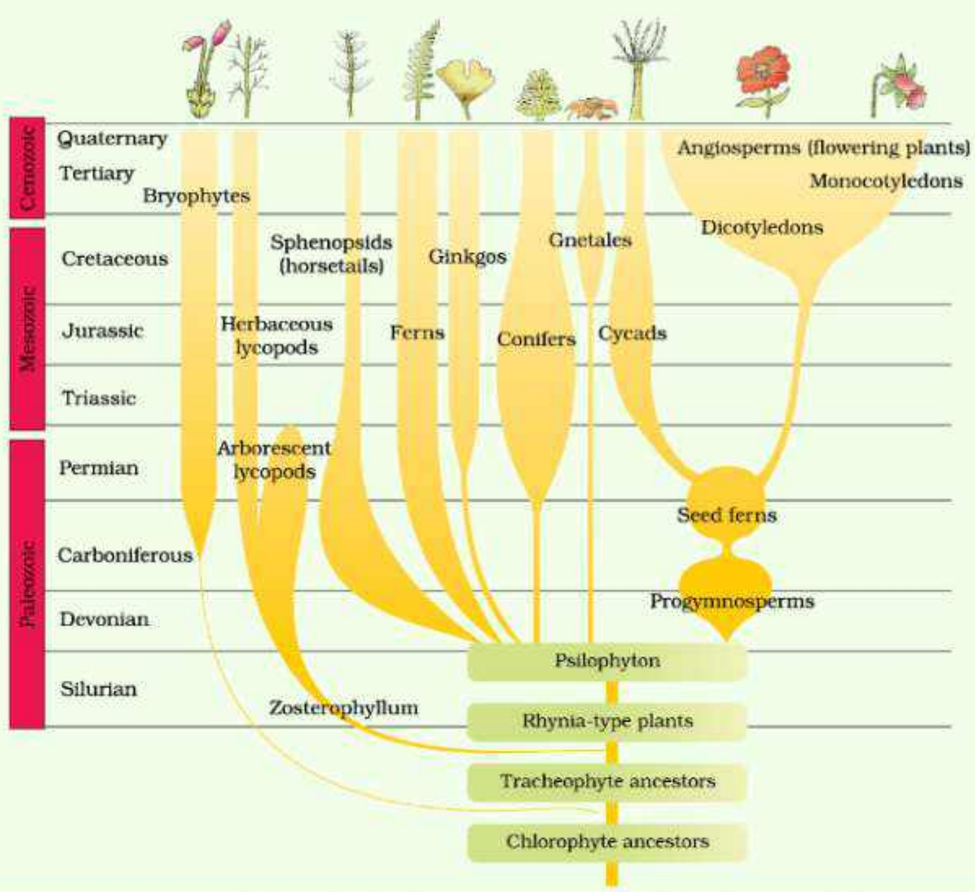
Disruptive selection
- More individuals acquire peripheral character value to both ends at the distribution curve



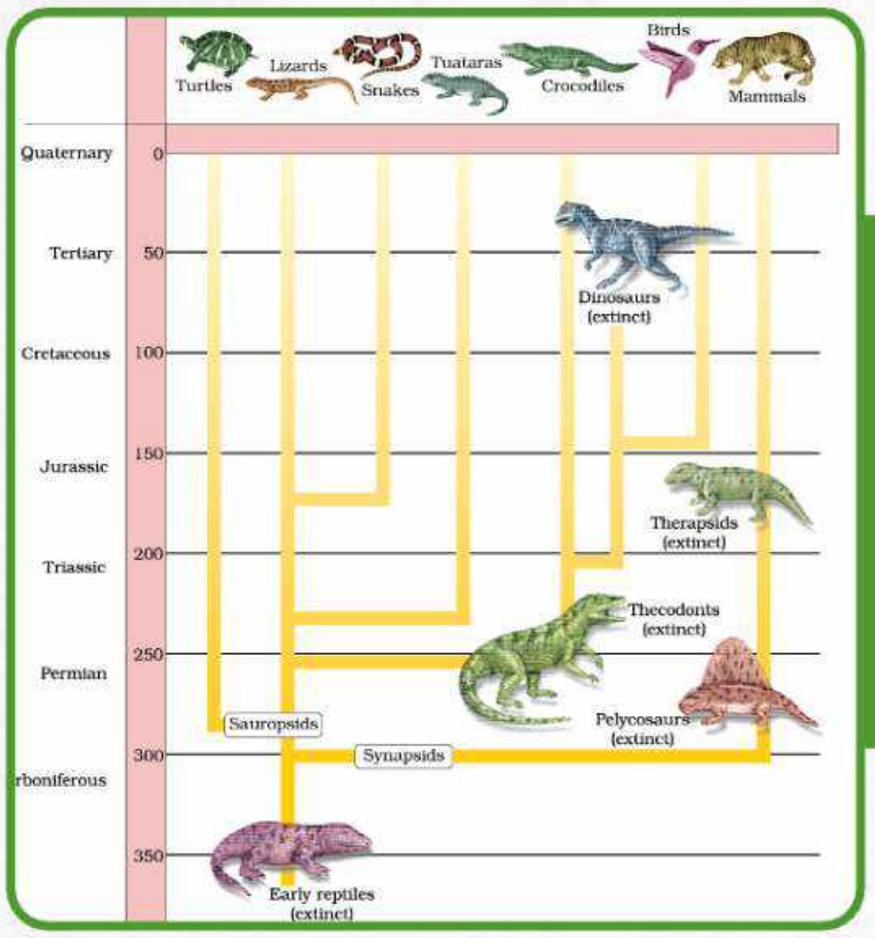
10 A BRIEF ACCOUNT OF EVOLUTION OF PLANTS

Derivatives

- Different types of plants evolved in different periods of time: Palaeozoic, Mesozoic and Coenozoic
- Chlorophyte ancestors: Bryophytes : Tracheophyte ancestors : Lycopods
- *Psilophytons* were common ancestors for Progymnosperms, Ferns, Ginkgos, Conifers, Gnetales, Sphenopsids
- Origin of monocotyledons is more recent than that of dioctyledons
- Giant ferns (pteridophytes) were present (200 mya) but they all fell to form coal deposits slowly.



11 A BRIEF ACCOUNT OF EVOLUTION HISTORY OF VERTEBRATES



Derivatives

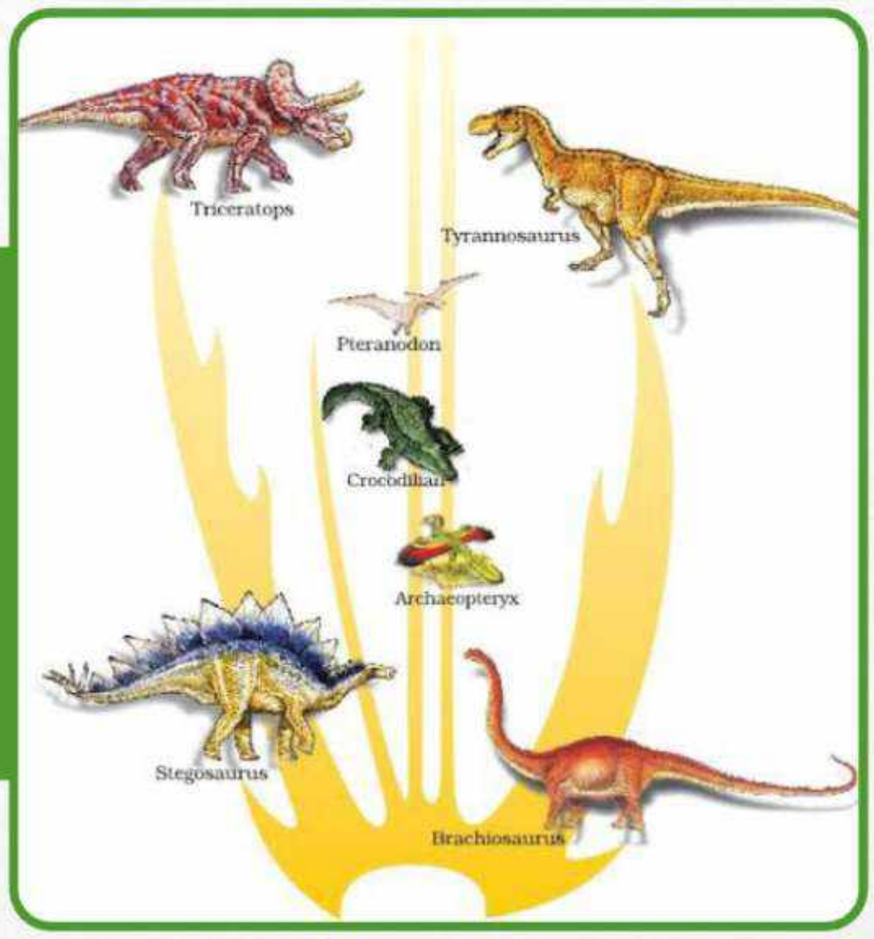
- Turtles, Lizards, Snakes and Tuatara arose from common ancestor sauropsids
- Thecodont ancestors gave way to existing crocodiles and birds and extinct dinosaurs.
- Story of origin of mammals:
  Extinct reptiles → Synapsids → Pelycosaurs → Therapsids → Mammals

12 A FAMILY TREE OF DINOSAURS

- *Triceratops* – three horned dinosaur with bony frill around back of its head.

Stegosaurus - Large triangular bony plates along the back and spiked tail	*Tyrannosaurus rex* - 20 feet in height, had huge fearsome dagger-like teeth	*Brachiosaurus* - Long giraffe like neck, long forelimbs

- *Pteranodon* were possibly flying reptiles
- *Archaeoptervx* is a transitional fossil between non avian dinosaurs and birds
- *Brachiosaurus* and *Tyrannosaurus* arose separately, mostly likely, from a common ancestor.



13 ORIGIN AND EVOLUTION OF MAN

• Among the stories of evolution of individual species, the story of evolution of modern man is most interesting and appears to parallel evolution of human brain and language.

Human ancestors	Years back	Cranial capacity	Specific features
Dryopithecus ***Ramapithecus***	15 mya	–	more ape-like, more man-like: Hairy and walked like Gorillas and Chimpanzees
Australopithecus	2 mya	–	• 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 hominid • 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

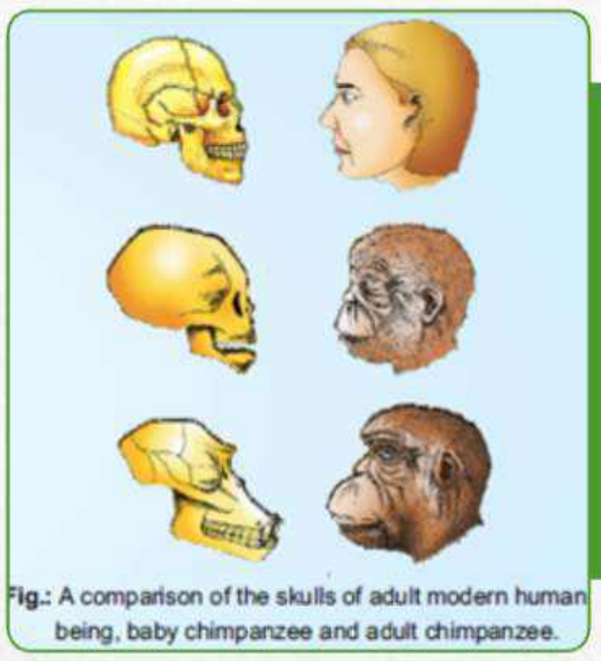


Fig.: A comparison of the skulls of adult modern human being, baby chimpanzee and adult chimpanzee.

• The skull of baby chimpanzee is more like adult human skull than adult chimpanzee skull

• **Pre historic cave art**-18,000 years ago. One such cave paintings by pre-historic humans can be seen at **Bhimbetka rock shelter** in Raisen district of Madhya Pradesh.

• Agriculture came around **10,000 years back** and human settlements started.

HUMAN HEALTH AND DISEASE

1 HEALTH

1. • As per '**Good humor' hypothesis** arrived at by reflective thought and asserted by **Hippocrates** along with **Indian Ayurveda System.**
   Health is a state of body and mind where there was a balance of certain 'humors' e.g., persons with black bile belonged to hot personality and had fevers.

2. **William Harvey** (discovered blood circulation experimentally) disproved this 'good humor' hypothesis of health by demonstrating normal body temperature in persons with black bile using thermometer.**View of biologists in later years:**

3. Mind influences our immune system through neural and endocrine systems, and that our immune system maintains our health i.e., state of complete physical, mental and social and psychological well being.

4. 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)

• **Healthy conditions** → Increase → Productivity, longevity; Bring economic prosperity
• **Healthy conditions** → Decrease → Infant and maternal mortality

2 DISEASE

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

Types of diseases

Parameters	Non-infectious	Infectious
• **Transmission from one person to another**	×	✓
• **Example**	Cancer	AIDS

• **Pathogens:** are disease causing organisms

• **Most parasites arepathogens** living in (or on) the host multiplyand interfere with normalvital activities resulting in morphological and functional damage.

• Gut pathogens can survive harsh pH & digestive enzymes.

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	–

• **Vector:** Transmits disease from one organism to another e.g. female Aedes mosquito is the vector for dengue and chikungunya, while, Anopheles spreads malaria.

4 MEASURES FOR PREVENTING SPREAD OF INFECTIOUS DISEASES

Parameters	Measures
Personal Hygiene	• Keeping the body clean • Consumption of clean drinking water, food, vegetables, fruits etc.
Public Hygiene	• Proper disposal of waste and excreta • Periodic cleaning and disinfection of water reservoirs, pools, cesspools and tanks. • Decontamination of drinking water
Avoid close contact	• Contact with infected persons and belongings should be avoided.
Control vectors and their breeding places	• Avoid stagnation of water in and around residential areas. • Regular cleaning of house old coolers • Use of mosquito nets • Introducing larvicidal fishes like Gambusia in ponds that feed on mosquito larvae • Spraying of insecticides in ditches, drainage areas and swamps • Doors and windows should be provided with wire mesh.

• Balanced diet, yoga and regular exercise, personal hygiene, awareness about diseases and vaccination are very important to **maintain good health.**

• Use of vaccines and immunisation programmes have enabled us tocompletely eradicate a deadly diseaselike **smallpox.** Large number of infectious diseases like polio, diphtheria, pneumoniaand tetanus have been controlled to a large extent by the use of vaccines.

• Biotechnology is at the verge of making available newer and safer vaccines.

• Discovery of antibiotics and various drugs have enabled us to effectively treat infection

5 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 gray to bluish**

Typhoid Mary (Mary Mallon), a cook by profession was a typhoid carrier who spread typhoid through the food she prepared.

VIRAL DISEASES

Disease	Pathogen	Organ affected	Symptoms
• Common cold	Rhino virus	Nose and respiratory passage	• Nasal congestion and discharge • Sore throat • Hoarseness, cough • Headache, tiredness

Common cold does not infect lungs and its 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 DISEASE

Disease	Pathogen	Body parts affected	Symptoms	
• Ringworm	*Microsporum, Trichophyton, Epidermo- phyton*	Skin, nails, scalp	• Dry, scaly lesions • Intense itching	

- **Heat and moisture** makes the fungi thrive in **skin folds** such as in groin and between toes
- Acquired from soil or belongings of infected individuals such as towels, combs, clothes etc.

6 PROTOZOAN DISEASES

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

- House flies act as **mechanical carrier for amoebiasis**
- *P. falciparum* causes **malignant malaria (Most serious form)**

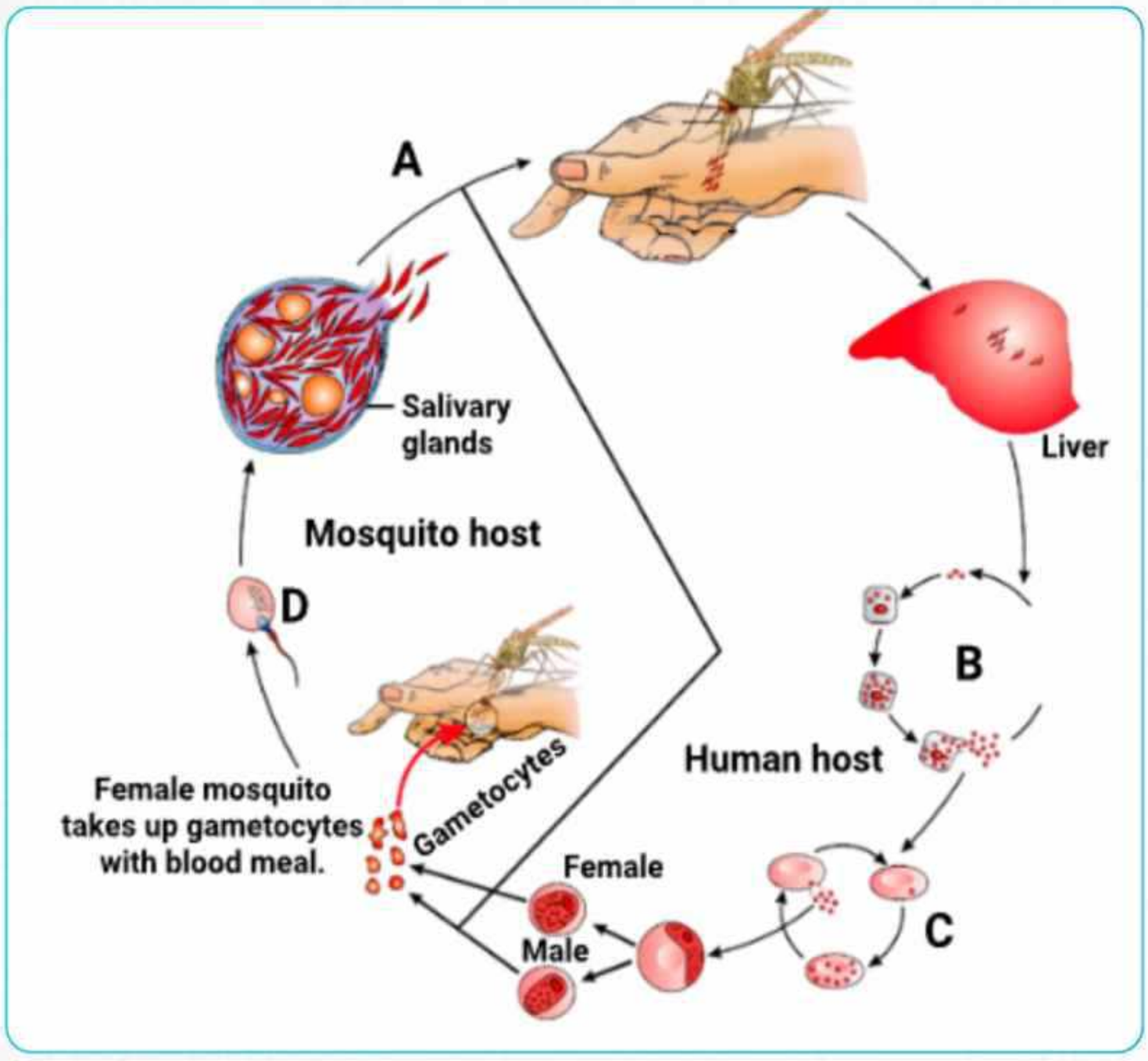


Fig: Stages in the life cycle of Plasmodium

Rupturing Of RBCs releases the toxic substance, **haemozoin** responsible for symptoms of disease

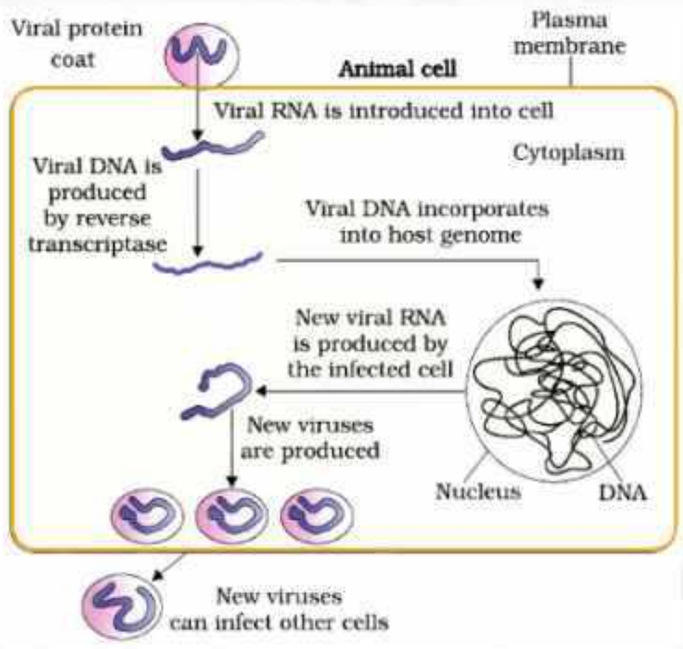
7 AIDS/ACQUIRED IMMUNO DEFICIENCY SYNDROME

- 1st reported - 1981, Killed – Approximately 25 million people in last 25 years
- It is deficiency of immune system, acquired during the lifetime of an individual
- **Syndrome** means 'group of symptoms'
- **Non congenital, fatal infectious disease**

- **Causative agent** – HIV / Human Immuno deficiency virus
  - **Enveloped virus enclosing RNA genome**

• **Life cycle**	**Mode of Transmission**	**High Risk Individuals**
Entry of virus in body	Sexual contact	Multiple sexual partners
	Placenta	Mother to foetus
	Blood transfusion	Repeated blood transfusion,
	Infected needles	Drug addicts **(intra venous)**

Entry into body cells **(Macrophages, helper T-cells)**



Sequence of events:

01

Inflected cells, **(Macrophages)** can survive while viruses are being replicated and released **hence called HIV factory**

02

HIV enters into macrophages and T-helper cells (T_H) simultaneously

03

There is progressive decrease in number of helper T-cells

04

Initial symptoms: Bouts of fever, diarrhoea, weight loss

05

Later the immuno-deficient patient is prone to infections especially *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

8 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
Cell growth and differentiation	Confined to original place	Grow rapidly and spread to other parts.
Contact inhibition	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).**

Cause: **Normal cell**
Proto/cellular oncogene (c-onc) → (Neoplastic transformation; causative agents called **carcinogens**) → **Cancerous cell** Oncogene

01 • **Ionising radiations** -X-rays, γ- rays

02 **Non**-ionising radiations-UV rays ·

03 **Physical agents**

04 **Non**-ionising radiations-UV rays ·

05 Chemical agents-in tobacco smoke

06 • **Biological agents**- Oncogenic viruses **(carry viral oncogenes)**

07 **Diagnosis/ Detection:** Early detection allows the disease to be treated successfully in many cases.

Technique	Basis	Detect
Biopsy	Histopathological studies	Changes in tissue
Blood and bone marrow test	Cell counts	Leukemias
Radiography	X-rays	Internal organ cancers
Computed tomography (CT)	X-rays	Internal organ cancers (3D image)
Magnetic resonance Imaging (MRI)	Strong magnetic fields and non-ionising radiations	Accurately detect pathological and physiological changes in living tissue
Molecular techniques	Identification of genes responsible for susceptibility to certain cancers	
Antibodies based	Against cancer specific antigens	Certain cancers

• 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.

9 IMMUNITY

• The ability of the host to fight the disease causing organisms, conferred by the immune system is called Immunity

Parameters	Innate	Acquired
Observed from	Time of birth	After birth
Exposure to infection	Not required	Required
Defence	Non specific	Specific
Memory record	×	✓

Types

Memory based immunity evolved in higher vertebrates

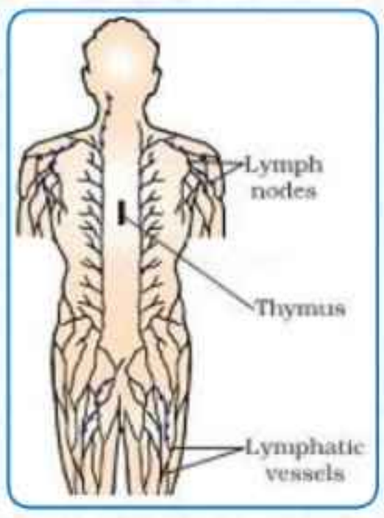
10 INNATE IMMUNITY

• This immunity is accomplished by providing different types of barriers to the entry of the foreign agents.

Types of Barrier	Structures involved/Barrier	Basic function
Physical	• Skin • Mucus coating of the epithelium lining the respiratory, gastrointestinal and urogenital tracts	• Prevent entry of microbes. • Trap microbes entering our body.
Physiological	• Saliva in the mouth •Acid in stomach • Tears from eyes	• Prevent microbial growth.
Cellular	• Neutrophils/PMNL •Monocytes • Macrophages •Natural killer cells (type of lymphocytes)	• Phagocytose microbes. • Destroy microbes.
Cytokine	• Interferons	• Produced by virus infected cells that protect **non-infected cells** from further infection.

11 ACQUIRED IMMUNITY

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.**



Primary lymphoid organs

Secondary lymphoid organs

Thymus → T-cells

Bone marrow → B-cells

Matured lymphocytes migrate

Spleen, lymph nodes, tonsils, appendix, MALT, Peyer's patches

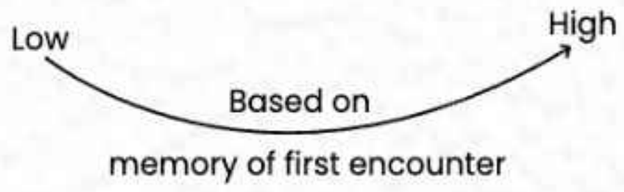
T-cells mediated response → **• Cell-mediated immune response/CMI**

B-cells produce antibodies/Army of proteins in blood → **• Humoral immune response/Antibody mediated immune response**

- Origin and/or maturation of lymphocytes
- **lymphocytes become antigen sensitive**
- Provide micro-environments for development and maturation of lymphocytes
- Provide sites for interaction of lymphocytes with antigen which proliferate to become **effector cells.**

- **Type**
- **Exposure to pathogens**

- **Intensity**



These responses are carried out by B and T-lymphocytes.

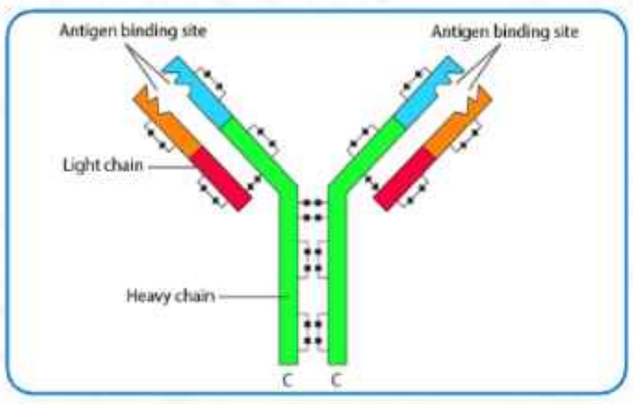


Fig: Structure Of An Antibody Molecule

- Each antibody has 4 peptide chains (H_2L_2)
  - 2 long heavy chains
  - 2 short light chains
- Called immunoglobulins (Ig)
- **Types – IgA, IgM, IgE, IgG**

- T-lymphocytes are responsible for graft rejection. Tissue and blood group matching are essential before undertaking any graft/transplant and even after this patient has to take immunosuppressants throughout life.
- If the pathogens succeed in gaining entry to our body, specific antibodies and T-cells serve to kill these pathogens.

12 LYMPHOID STRUCTURES/ORGANS

Structure	Typical
Bone marrow	• **Main lymphoid organ** where all bold cells are produced including lymphocytes.
Thymus	• **Lobed** organ located **near the heart** and **beneath the breastbone.** Quite large at the time of birth, **keeps reducing in size** with age and by the time puberty is attained it is reduced to a very small size.
Spleen	• Large **bean shaped organ,** mainly contains lymphocytes and phagocytes • Acts as a **filter of the blood** by trapping blood borne micro-organisms • **Large reservoir of erythrocytes.**
Lymph nodes	• Small solid structures located at different points along the lymphatic system • Serve to trap the microbes/antigens which happen to get into the lymph and tissue fluid. Antigens trapped in the lymph nodes are responsible for the **activation of lymphocytes** present there and cause the immune response.
MALT	• Mucosa-associated lymphoid tissue is located within the lining of major tracts like respiratory, digestive and urinogenital tracts • Constitutes about **50%** of lymphoid tissue in human body.

13 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	Longer	Shorter
Memory cells	✓	✗
Examples	•Natural infection → Antibody production in host • **Vaccination** Deliberate injection of living/dead microbes/proteins	• Mother —Placenta→ Foetus • Mother —Colostrum (IgA)→ Infant

Immunisation (Principle : Property of 'Memory' of immune system)
Active

1

Active
preparation of antigenic proteins of pathogen/inactivated /weakened pathogen introduced into body

2

Active
Direct preformed antibodies/antitoxin is injected such as antitoxin against snake bites

01

• The antibodies produced in the host body against antigens would neutralize the pathogenic agents during actual infection.

02

• The vaccines also generate **memory B-cells and memory T-cells** that recognise the pathogen quickly on subsequent exposure and overwhelm the invaders with a massive production of antibodies.

03

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

14 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.

Antibodies – IgE type

Symptoms – Sneezing, watery eyes, running nose, difficulty in breathing.

Chemical released – Histamine and **serotonin** from mast cells.

Diagnosis – Patient is exposed to or injected with very small doses of possible allergens, and reactions studied.

Treatment – Anti-histamine antihistamine, adrenaline and steroids quickly reduce the symptoms of allergy.

Effects of modern-day life style

- More and more children in metro cities of India suffer from allergies and asthma due to more sensitivity to the environment.
- Protected environment provided early in life has resulted in lowering of immunity and person is more sensitive to allergens.

15 AUTOIMMUNITY

- Memory based acquired immunity evolved in higher vertebrates can **distinguish foreign** molecules as well as foreign organisms (pathogens) **from self-cells.**

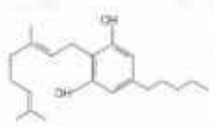
01 **Results –** Self destruction /body attack self cells

02 **Reason –** Genetic /unknown

03 **Example – Rheumatoid arthritis**

16 DRUG ABUSE

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

Drug	Receptors	Source	Intake	Examples	Action and anything specific
Opioids	CNS, GIT	Latex of poppy plant, *Papaver somniferum*  Opium poppy	Snorting, injection  Chemical structure of Morphine	• 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, *Cannabis sativa*  Leaves of *Cannabis sativa*	Inhalation, oral ingestion  Skeletal structure of cannabinoid molecule	• Charas • Hashish • Ganja • Marijuana	• Produced by virus infected cells that protect **non-infected cells** from further infection • Effects on cardiovascular system of the body • These days cannabinoids are formating 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  *Flowering branch of 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 illness like **depression insomnia.**

17 DRUGS AND SPORTSPERSON

Why to use?

- Increase muscle strength & bulk.
- Promote aggressiveness.
- Enhance athletic performance.

Commonly abused drugs

- Narcotic analgesics.
- Diuretics.
- Anabolic steroids.
- Certain hormones.

Common side effects

- Increased aggressiveness.
- Mood swings.
- Depression.
- Stunted growth because of premature closure of growth centres of long bones.
- Severe facial and body acne.

Typical side effects

Male

- **Breast enlargement**
- Decreased sperm production
- Reduction in size of testicles
- Acne, premature baldness, enlargement of prostate gland
- Potential for liver and kidney dysfunction

Female

- **Masculinisation** (features like males)
- Abnormal menstrual cycles
- Enlargement of clitoris
- Excessive hair growth on face & body
- Deepening of voice

• These side effects may be permanent with prolonged use.

18 TABACCO/SMOKING-PAVES THE WAY TO HARD DRUGS

01

- **Intake**
- Smoked
- Chewed
- Snuff

02

- **Chemical substance**
- Nicotine, an alkaloid.

03

- **Action of nicotine**
- Stimulates adrenal gland to release adrenaline and non-adrenaline into blood circulation.

04

Effects

- **Respiratory system**
- Increases carbon monoxide (CO) in blood and reduces concentration of haembound oxygen, causes oxygen deficiency in the body.

05

- **Circulatory system**
- Increase heart rate and blood pressure.

06

- **Common diseases**
- Bronchitis.
- Emphysema.
- Coronary heart disease.
- Gastric ulcer.

07

- **Risk of cancers**
- Oral cavity.
- Throat.
- Lungs.
- Urinary bladder.

- Tobacco has been used by humans for more than 400 years
- Packets of cigarettes, warns against smoking and says how it is injurious to health.

19 ADOLESCENCE AND DRUG/ALCOHOL ABUSE

1

- **Adolescence** means both "a period" and process" during "a which a child mature in terms of his/her attitudes and beliefs for effective participation in society.

2

- **Adolescence** is a **bridge linking childhood and adulthood.**

3

- It's a period between **12-18 years of age, a vulnerable phase of mental and psychological development** of an individual.

4

- it is accompanied by several biological and behavioural changes.

5

- Curiosity, need for adventure and excitement, and experimentation, motivate youngsters towards drug and alcohol use

6

- First use may be out of curiosity but later used to escape from stress, pressures to excel in academics, perception that it is cool.

7

- Television, movies, newspapers, internet, promote this perception.

8

- Unstable or unsupportive family structures and peer pressure also promote drug and alcohol abuse.

use of drugs even once be **"fore-runner to addiction"** and pull the user into a vicious circle leading to their regular use/abuse

20 ADDICTION AND DEPENDENCE

Addiction

• Because of perceived benefits, drugs are frequently used repeatedly that leads to **psychological attachment to certain effects** like euphoria and temporary feeling of well being

Dependence

It is the tendency of the body to manifest a characteristic and unpleasant "withdrawal syndrome" if regular dose of drugs/alcohol is abruptly discontinues. Addiction drive people to take drug even when its use become self-destructive

- **With repeated** use of drug, tolerance **level of receptors increases**
- Receptors respond only to higher doses of drugs leading to greater intake.

Effects of drug/alcohol abuse

• Reckless behavior. • Vandalism • Violence • Depression • Fatigue • Drop in academic performance

Warning signs

- Unexplained absence from school/college
- Aggressive and rebellious behaviour
- Change in sleeping and eating habits
- Deteriorating relationships with family and friends
- Poor personal hygiene, withdrawal, isolation
- Loss of interest in hobbies
- Fluctuations in weight and appetite
- High doses lead to coma and death due to respiratory failure, heart failure or cerebral hemorrhage
- Chronic use of drugs/alcohol damage nervous system and liver (cirrhosis)
- Use of drugs during pregnancy adversely affect foetus. **Some far-reaching implications**
- Abuser may turn to stealing
- Addict becomes the cause of mental and financial distress to entire family and friends

Withdrawl syndrome

If drug is abruptly discontinued, symptoms include:

• Anxiety • Nausea • Shakiness • Sweating

• In severe cases, can be life threatening, person needs a medical supervision.

Prevention and control "Prevention is better than cure"

- **Avoid undue peer pressure** on child related to sudies, sports or other activities
- **Education and counselling:** Channelise energy of child into healthy pursuits like sports, yoga, reading, music, etc.
- Sort out problems by **seeking help from parents and peers.**
- **Looking for danger signs :** Alert parents, teachers and close friends need to look for and identify the danger signs of substance (drug/alcohol) abuse and appropriate measures would then be required to diagnose the malady and underlying cause.
- Proper remedial steps or treatment should be taken by **seeking professional and medical help** in the form of highly qualified psychologists, psychiatrists and de-addiction and rehabilitation programmes. This will totally relieve the individual from these evils.

Strategies for Enhancement in Food Production

1 ANIMAL HUSBANDRY

01 • Agricultural practice of breeding and raising livestock useful to humans.

02 • Some of the products and the animals involved are

Useful products	Source
Milk	Cows, buffaloes, Goats
Eggs	Poultry birds; Chicken, Ducks, Turkey, Geese
Meat	Cattle, Sheep, Pigs
Wool	Sheep
Silk	Silk worms
Honey	Bees

• More than 70% population of livestock is in India and China but contribution to world's farm produce is 25% that result in

Problem ↓

• Productivity per unit is low
• Insufficient food supply due to ever increasing population size.

Solution ↓

• Improvement in conventional practices of animal breeding by applying biological principles.
• Application of newer technologies to Animal Husbandry

Extension of Animal husbandry → **Poultry farming**, **Fisheries**

2 FISHERIES

• Industry devoted **to** → Rearing, Catching, Processing & Selling **of** → Fish, Molluscs (shell-fish), Crustaceans (prawns, crabs), Aquatic animals

• **Aquaculture:**
Enhancement in aquatic yield including plants and animals

• **Pisciculture:**
Increase in total fish yield

Products from fishery
Food : lobster, prawn, fish, edible oyster

- **Fresh water (FW)**: Catla, Rohu, Common carp
- **Marine (M)**: Hilsa, Sardines, Mackerel, Pomfrets

To meet the increasing demand on fisheries, different techniques have been employed that led to Blue revolution

3 MANAGEMENT OF FARM AND FARM ANIMALS

• A professional approach to boost our food production. Some of the management procedures employed in animal farm

Management : system are { Dairy farm, Poultry farm }

1. Dairy farm management/Dairying: Management of animals for milk and its products for human consumption
e.g., cows, buffaloes

Aimed at
↓
Increasing yield
↓
Depends on
↓
Quality of breed

- High yielding potential under given climatic conditions
- Resistance to diseases

led to

Improving quality of milk

- **Practices involved**
  - Effective housing
  - Adequate water
  - Maintain disease free conditions
  - cattle diet fodder
    - Quality↑(oil cake rich)
    - Quality balanced
  - Stringent cleanliness of
    - Cattle
    - Handlers
  - Regular inspection by veterinary doctor

2. Poultry farm management

- Involves use of birds for food → Eggs, Meat
- Birds : Fowl, chicken, ducks, turkey, geese.

- **Practices involved**
  - Selection of disease free and suitable breeds.
  - Proper and safe farm conditions.
  - Proper feed and water.
  - Maintaining proper health care and hygiene.

Disease	**Bird flu**
Cause Symptoms	H_5N_1 virus
People at risk	Respiratory difficulty, fever, malaise

People at risk
- Poultry farmers exposed to infected birds.
- People who eats under cooked eggs/poultry

Increased mechanisation in dairy farming particularly milking, storage and transport of milk, reduces chances of direct contact of the produce with the handler

4 BEE-KEEPING (Apiculture)

1. • It is the maintenance of hives of honeybees for the production of honey
2. • Age old, high income yield cottage industry and is not labour intensive
3. • Most common bee in India : *Apis indica*

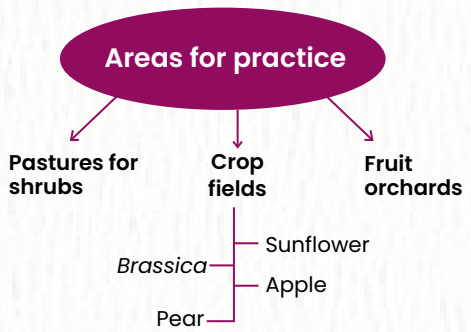
Advantages

a. Increases product yield → **Products obtained from honey bee**

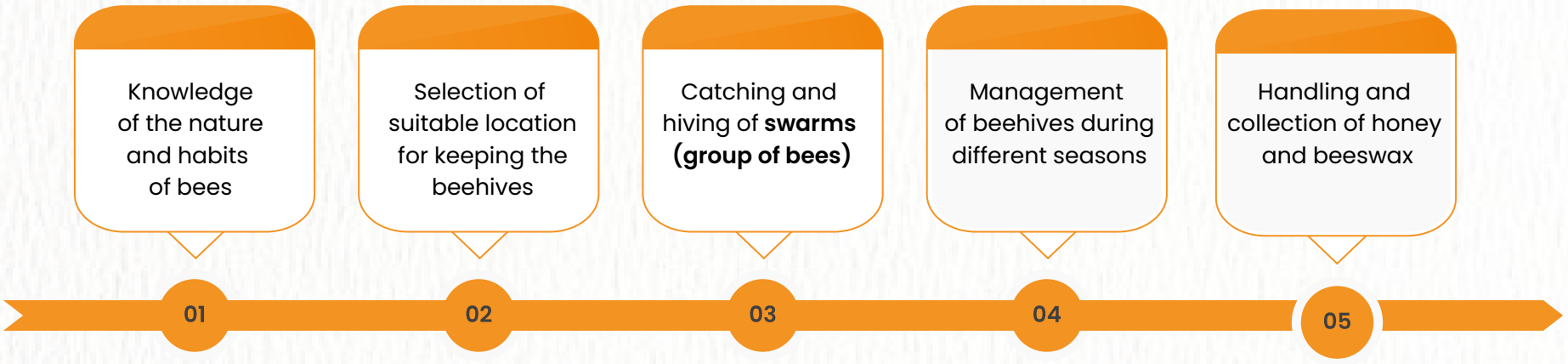
Bees wax
- Preparation of cosmetics and polishes

Honey
- Food: High nutritive value
- Used in Indigenous medicine

B. Enhances crop yield → **Bees are effective pollinators**



Salient points for successful bee keeping



Keeping beehives in crop fields during flowering period increases pollination efficiency and yield.

5 ANIMAL BREEDING

1 • Breed

A group of animals related by descent and similar in most characters like general appearance, features, size, configuration etc.

2 • Aims of animal breeding

- Increase the product yield (Quantity) .
- Improving desirable qualities in produce and cattle.
  - → High yielding variety.
  - → Disease resistant.
  - → High reproductive rate.
  - → Longer productivity span.

6 CONTROLLED BREEDING EXPERIMENTS

Artificial Insemination (AI)

Identify elite bull

↓

Extract semen
Either used immediately or
Cryopreservation (cold storage)

↓

Inject into reproductive tract of superior cow

Multiple Ovulation Embryo Transfer (MOET)

Identify superior cow

↓

Inject with FSH-like hormone

↓

Super ovulation (6-8 ova/cycle)

↓

AI or natural insemination

↓

Non surgical extraction of embryo

↓

Gestation in surrogate cow

Advantages

- Greater chances of fertilisation.
- Economically relevant as all sperms can be used since semen from 1 bull can fertilise more than 1 cow, if needed.
- Helps to overcome several problems of normal mating.

- Increase herd size and quality in less time.
- Genetic mother available for another round of super ovulation.

Disadvantages

- The sucess rate of crossing mature and is fairly low.

- Not applicable to fishes and birds.

- MOET is demonstrated on mammals such as cattle, mares, sheep, rabbitst.
- **Superior cow :** High yield of milk/lactation
- **Superior bull :** Lean meat with less lipid

} Have been bred successfully to increase herd size in a shot time.

7 SELECTIVE BREEDING OR HYBRIDISATION

Types of selective breeding

1 Inbreeding

- Mating of more closely related individuals within the **same breed** for 4-6 generations
- Inbreeding increases **homozygosity** and is useful to evolve

Advantage

- Increases the productivity of population as superior genes are accumulated and harmful recessive genes are eliminated

Disadvantages

- **Inbreeding depression** (ID) due to continued close inbreeding

reduces → Fertility, Productivity

2 Outbreeding

- Mating between male and female of same breed (no recent common ancestor) or different breeds or different species.

1. Outcrossing

Mating of animals within the **same breed** but having **no common ancestors** upto 4-6 generations

Advantage

- A single out cross helps increase productivity of animals below average
  - → increase growth rate in beef cattle
  - → increase milk production

2. Cross breeding

- Mating between superior male of one breed and superior female of another breed
- This method allows combination of superior qualities of two different breeds on a commercial scale.

Advantage

- Stable new breeds superior to existing breeds can be developed
- **Example:** A new breed of sheep

♂/Male Merino ram × ♀/Female Bikaneri ewe

↓

Hisardale

- **Location** : Punjab

3. Interspecific hybridisation

- Male and female of two different related species are mated

Advantage

- Progeny may combine desirable features of both the parentsand may be of considerable economic value
- **Example:**

♂/Male Donkey × ♀/Female Horse (Mare)

↓

Mule (Sterile)

inbreeding depression can be overcome by out crossing i.e.. method to restore fertility and yield

BIOTECHNOLOGY: PRINCIPLES AND PROCESSES

1 INTRODUCTION

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

Parameters	Traditional biotechnology	Modern biotechnology
Organisms involved	• Microbes	• Genetically modified organisms
Production	• Small scale	• Large scale
Examples/Technique include	• Curd, bread or wine making	• In vitro fertilization leading to a 'test -tube' baby • Synthesising a gene and using it • Developing a DNA vaccine • Correcting a defective gene
01	02	03

EFB (European Federation of Biotechnology)

01 • **The integration of natural science and organisms, cells, parts thereof, and molecular analogues for products and services'.**

02 • It encompasses both traditional view and modern molecular biotechnology.

Parameters 01	Genetic engineering 02	Bioprocess engineering 03
• **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	• Maintenance of sterile ambience in chemical engineering processes to enable growth of only the desired microbe/eukaryotic cell in large quantities• Manufacture of biotechnological products like antibiotics, vaccines, enzymes, etc.

The ability to multiply copies of antibiotic resistanse gene in E.coli was called cloning of antibiotic resistance gene in E.coli

3 ADVANTAGES OF BIOTECHNOLOGY OVER OTHER TECHNIQUES

01 Methods	02 Advantage	03 Disadvantage
I. Asexual reproduction	Preserves genetic information.	No variations.
II. Sexual reproduction	Provides opportunities for variations and formulation of unique combinations of genetic setup.	Some of which may be harmful to the organism as well as the population.
III. Traditional hybridisation	Used in plant and animal breeding.	Very often lead to inclusion and multiplication of undesirable genes along with desirable genes.
IV. Genetic engineering	Allows us to isolate and introduce only one or a set of desirable genes without introducing undesirable genes into target organism.	—

4 THREE BASIC STEPS IN GENETICALLY MODIFYING ORGANISMS

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

5 KEY TOOLS OF RECOMBINANT DNA TECHNOLOGY

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

Enzymes - Most commonly used enzymes in genetic engineering are
- **Nucleases**
- **DNA polymerase**
- **Ligases**

Nucleases - Catalyse the cleavage of nucleic acids.

Types

Exonucleases
Remove nucleotides from the ends of the DNA

Endonucleases
Make cuts at specific positions within the DNA i.e. at **recognition/palindromic sequence**

- In the year 1963, the two enzymes responsible for **restricting the growth of bacteriophage** in *Escherichia coli* were isolated

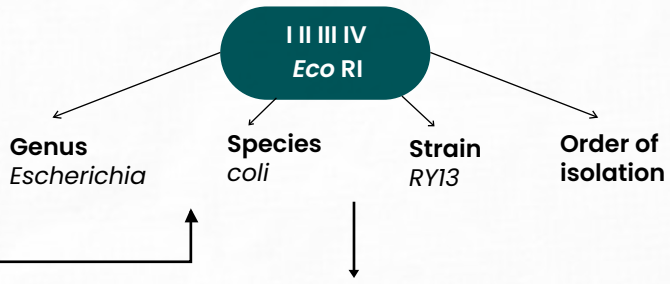
Methylase
Add methyl groups to bacterial DNA

Restriction endonuclease / Molecular scissors
Cut the DNA of bacteriophage

6 ENZYMES

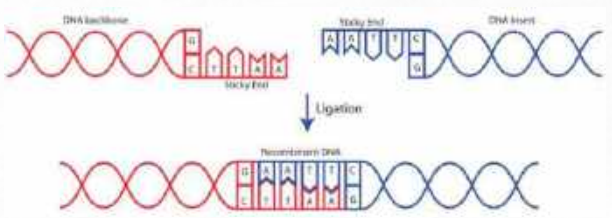
• Restriction endonuclease

More than **900** restriction enzymes have been isolated from over **230** strains of bacteria **(prokaryotic cell)** each of which recognise different recognition sequences.



• **Nomenclature/Naming of enzyme :**

• **Functions by:**
  • **'Inspecting'** the length of DNA sequence
  • **Binds** to the **"specific recognition sequence"**
  • **Cuts** the two strands of **ds DNA** at s pecific points in their **sugar-phosphate backbones** and leaves single stranded portions at the ends.
• These **overhanging stretches** and called **sticky 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 and this stickiness facilitates the action of the enzyme **DNA ligase.**

• First restriction endonuclease - ***Hind* II** : I solated and characterised five years later, recognises sequence of **6 bp.**
• First recombinant DNA was prepared by **Stanley Cohen and Herbert Boyer, 1972 :**
  • Antibiotic resistant gene
  • Plasmid of *Salmonella typhimurium*

→ Recombinant plasmid —(Introduced Into)→ *Escherichia coli*

7 CLONING VECTORS

• **Vectors are vehicles** for delivering foreign DNA into recipient cells.
• Vectors used at present are engineered in such a way that they help **easy linking of foreign** DNA and selection of recombinants from non recombinants

Features of cloning vectors:

01 Origin of Replication (ori):

- Sequence from where replication starts
- Responsible for **controlling copy number** of the linked DNA
- Those vectors are preferred which support high copy number

02 Selectable Marker:

- Helps in selection of transformants
- Normally, the **genes encoding resistance to** antibiotics such as ampicillin, chloramphenicol, tetracycline or kanamycin, etc., are considered useful selectable markers for E coli
- **The normal *E.coli* cells do not carry resistance against any of these antibiotics**

03 Cloning Sites/Restriction Sites

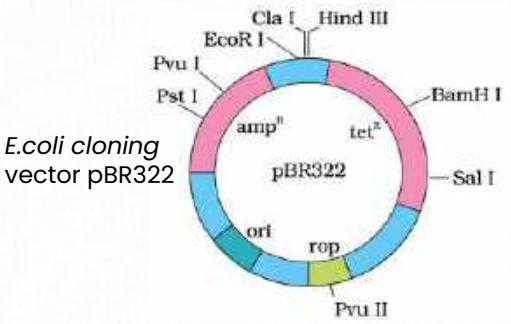
- **Single recognition site** for a restriction enzyme within the vector is a preferable feature.
- Presence of more than one recognition sites within the vector will generate several fragments, which will complicate the gene cloning
- The ligation of alien DNA/**gene of interest (GOI)** is carried out at a restriction site present in one of the antibiotic resistant genes.

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

• **Insertional inactivation:** Insertion of GOI within antibiotic resistance gene/selectable marker results in inactivation/formation of the coded product.
• **Hypothesis:** Insertion of GOI at Bam HI site in tet^R.
• If transformation fails – Non transformants are obtained in antibiotic lacking agar medium but they don't grow on antibiotic rich medium.

• If transformation successful – **Transformants** obtained are of **two types:**

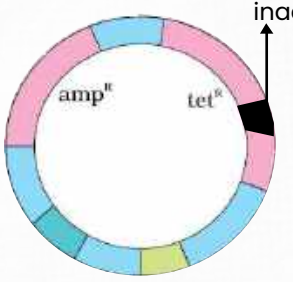
Non Recombinants



E.coli cloning vector pBR322

Gene of interest cloned ✗
Resistance to ampicillin ✓
Resistance to tetracycline ✓

Recombinants



✓
✓
✗
[due to inactivation]

- **All transformants are not recombinants but all recombinants are transformants.**
- One antibiotic resistant gene helps in selecting the transformants whereas the other antibiotic resistant gene helps in selection of recombinants
- *rop* ⟶ codes for the proteins involved in the replication of the plasmid

Plasmids as vectors

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

8 OTHER CLONING VECTORS

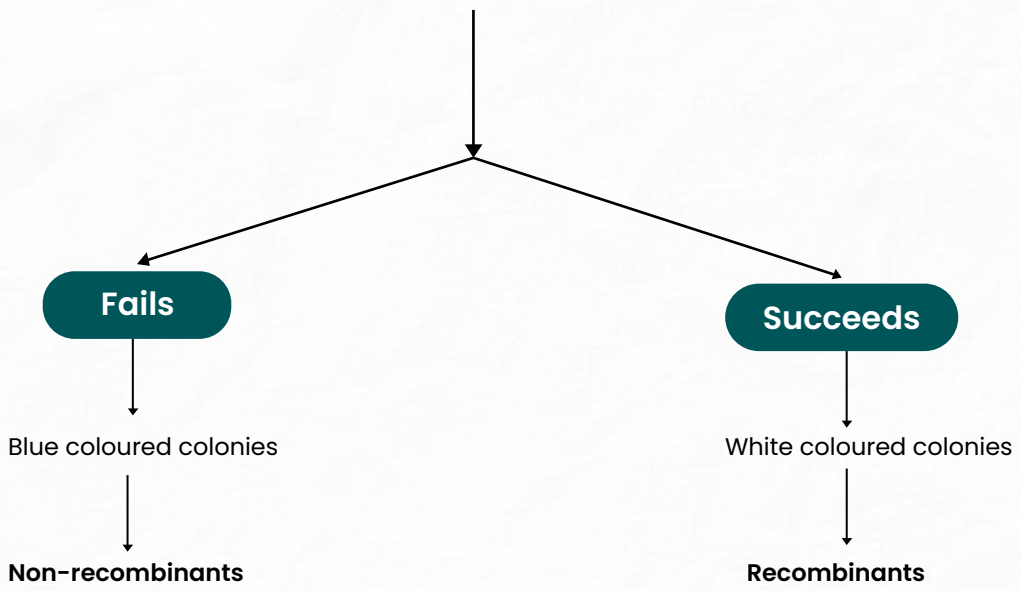
1

- Selection of recombinants due to inactivation of antibiotic resistant gene as in pBR322 is a cumbersome procedure because it requires simultaneous plating of two plates having different antibiotics.

2

To **overcome the disadvantage of pBR322,** alternative selectable markers ***(lac Z)*** acting as **reporter enzyme** have been developed which differentiate recombinants from non-recombinants on the basis of their ability to produce colour in the presence of chromogenic substrate.

- lac Z gene coding for β-galactosidase acts as selectable marker in the plasmid
- **Experiment:** Insert foreign DNA at lac Z gene + transformation in *E.coli*



• 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

• Retro viruses

• Retroviruses in animals have the ability to transform normal cells into cancerous cells

• **Disarmed retroviruses** are used to deliver desirable genes **into animal cells**

9 METHODS OF TRANSFORMATION

I. Micro – injection

• Recombinant DNA is **directly** injected into the nucleus of an **animal cell.**

II. Biolistic/Gene gun

• Plant cells are bombarded with high velocity micro- particles of gold or tungsten coated with DNA.

III. Heat shock method

IV. "Disarmed pathogen" vector

10 COMPETENT HOST FOR TRANSFORMATION WITH RECOMBINANT DNA

1

• DNAis **hydrophilic,** so itcan not pass through cell membranes

2

• In order to force cell to take up alien DNA/rDNA, it must first be **made 'competent' by treating** with ice cold calcium **chloride.**

3

• Entry of rDNA in host cell is due to transient pores created by heat shock (42°C) and notdue to Ca^{2+} ions.

4

• Divalent cations **increases the efficiency** with which DNA enters the bacterium through pores in its cell wall.

11 PROCESS OF RECOMBINANT DNA TECHNOLOGY

Isolation of DNA
↓
Fragmentation of DNA by restriction endonucleases
↓
Isolation of desired DNA fragment **(electrophoresis)**
↓
Amplification of gene of interest **(PCR)**
↓
Ligation of the DNA fragment into a vector
↓
Transferring the alien DNA/recombinant DNA into the host
↓
Culturing the host cells in a medium at large scale **(Bioreactors)**
↓
Extraction and **purification** of the desired product

I. Isolation of the Genetic Material (DNA)

- In majority of organisms, DNA is the genetic material
- Since DNA is enclosed within the membranes, we m have to break the cell open to release DNA along with other macromolecules
  - **Bacteria** → **Lysozyme**
  - **Fungi** → **Chitinase**
  - **Plant** cell → **Cellulase**

- In order to get DNA in pure form (free from other macromolecules), it is treated with different enzymes like RNase, protease etc.

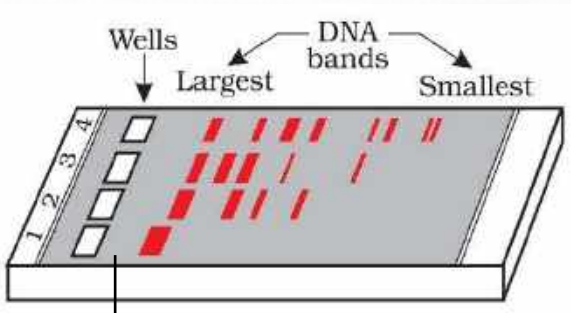
Pure DNA → Centrifuge → **Chilled ethanol to precipitate DNA**

Process: **Spooling**

II. Fragmentation by restriction endonucleases
III. 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
- Natural polymer, obtained from **sea weeds**
- Separate DNA fragments through **seiving effect**



Agarose gel electrophoresis

Lane1: Migration of undigested DNA fragments

Lane 2 to 4: Migration of digested DNA fragments

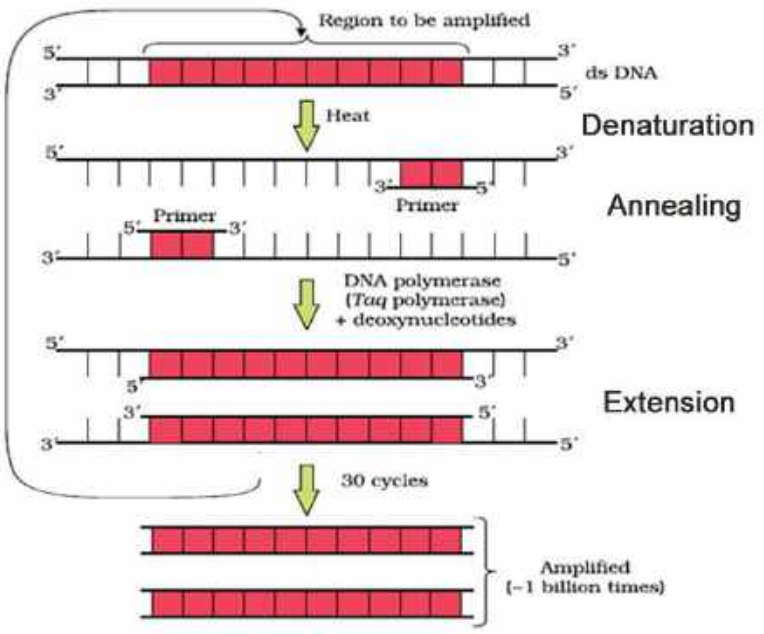
Gel → Stained with → **Ethidium Bromide** → Exposed to → **U.V rays** → Appears → **Bright orange bands** → Removal of DNA fragment from gel → Process → **Elution**

Purified DNA fragments are generally amplified (PCR) before constructing rDNA by joining with cloning vector.

IV. PCR - Polymerase Chain Reaction

- *In vitro* amplification of DNA (gene of interest)

Reaction mixture	Work/Function
Nucleotides	Formation of DNA chain
Primers	2 sets of chemically synthesised oligonucleotides complementary to the regions of DNA
Taq polymerase	**Thermostable** DNA polymerase, isolated from bacterium, ***Thermus aquaticus,*** remains active during high temperature induced denaturation of dsDNA. It extends the primers i.e. meant for chain elongation.
Genome DNA	Template DNA for gene of interest



The amplified fragment if desired can now be used to ligate with a vector for further cloning.

V. Ligation of the DNA fragment into a vector by DNA ligase

↓

VI. Insertion of recombinant DNA into the host cell

• Transformed host cells are selected with the help of selectable marker genes.

VII. Culturing of recombinant host cells (Biosynthetic stage)

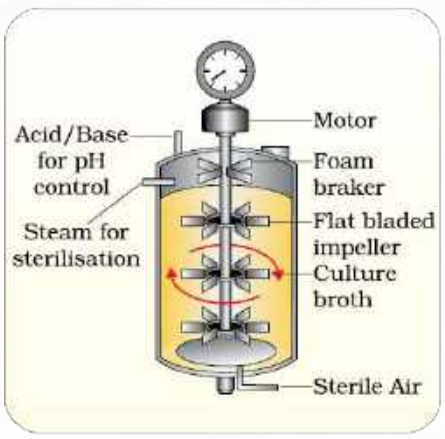
• The cells harbouring cloned genes of interest may be grown in **Laboratory/ Bioreactors**

Parameters	Laboratory	Bioreactors
Culture	Small volume	Large volumes (100 - 1000 lts)
Maintaining optimal conditions	Not possible	✓
Growth rate of cell	Never optimal	Optimum
Production	Small scale	Large scale

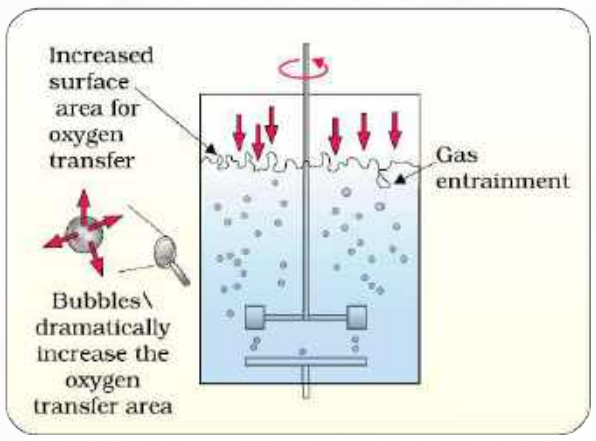
• Commonly used Bioreactors are **stirred type** having
- Cylindrical or with curved base → Facilitate mixing of reactor contents
- Stirrer → **Facilitate even mixing and oxygen availability** throughout the bioreactor
- Agitator system
- Oxygen delivery system
- pH control system
- Foam control system
- Sampling ports → To withdraw small volumes of culture periodically

Types of stirred tanks

Simple stirred tank



Sparged stirred tank



In Open Culture System/Continuous Culture System

• Used medium is drained out from one side while 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.

VIII. 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.

IX. Product is subjected for marketing as a finished product

• **Bioreactors:** Vessels in which raw materials are biologically converted into specific products using microbial plant, animal human cells and provide optimal growth conditions (temperature, pH, substrate, salts, vitamins, oxygen)

Biotechnology and its Applications

1 - INTRODUCTION

Biotechnology: Essentially deals with industrial scale production of biopharmaceuticals and biologicals using GM microbes, fungi, plants and animals.

Applications of biotechnology include:

- Therapeutics
- Processed food
- Diagnostics - Bioremediation
- Genetically modified crops for agriculture
- Waste treatment
- Energy production

Three critical research areas of biotechnology:

- Providing best catalyst in the form of improved microbes or pure enzymes
- Creating optimal conditions through genetic engineering
- Downstream processing technologies for purification

2 - BIOTECHNOLOGICAL APPLICATIONS IN AGRICULTURE

Food production could possibly be increased by three ways:

- Agrochemical based agriculture
- Organic agriculture
- Genetically engineered crop-based agriculture

Green revolution resulted in tripling of food production:

Reasons for success of green revolution:

- Improved crop varieties
- Agrochemicals (fertilisers + pesticides)
- Better management practices

Problem Area and Hinderances:

- Enhancement in food production by green revolution was still not enough to feed growing population
- **Agrochemicals** are often too **expensive** for farmers of **developing world**
- **Increase in yield** with existing varieties is **not possible** using **conventional breeding**

Genetically Modified Organisms

Organisms including plants, bacteria, fungi and animals whose **genes have been altered by manipulation** are called genetically modified organisms **(GMO)**.

Applications of genetic modification:

- 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.

Tailor Made Plants:

Plants have been developed to supply alternative resources to industries in the form of starches, fuels and pharmaceuticals.

3 - INSECT RESISTANT PLANTS

- Provides resistance to insects without the need for insecticides (bio-pesticide)
- Examples of biopesticides are Bt cotton, Bt corn, rice, tomato, potato and soyabean etc.

Bt cotton

Bacillus thuringiensis (A bacterium)

Source of the **cry gene**

Codes for **Bt toxin**

- Produced during a particular phase of their growth
- Inactive protoxins (Protein crystals)
- Endotoxin

Sequence of events

Cry gene from *Bacillus thuringiensis*

↓

Bt cotton (cells express inactive protoxins)

↓

Ingested by cotton bollworms

↓

Alkaline pH in the midgut of insects solubilities protein crystals

↓

Active toxin

↓

Create pores in midgut epithelial cells

↓

Swelling of midgut epithelial cells and thereby lysis

↓

Death of insect

Choice of gene depends on:

(i) Target pest

(ii) Crop

- Cry I AC and cry II Ab
- Cotton bollworm
- Cry I Ab
- Corn borer

Bt toxins are insect group specific :

- **Lepiddoptera:** Tobacco budworm, armyworm, cotton bollworm
- **Coleoptera:** Beetles
- **Diptera:** Flies and mosquitoes

4 - PEST RESISTANT PLANTS

- Method of cellular defense seen in all eukaryotes against pest infestation.
- Technique responsible : RNA interference (RNAi)
- Based on post transcriptional silencing of mRNA
- Translation of mRNA coded from pest specific genes is silenced/prevented due to formation of complementary dsRNA

Source

I.Viruses with RNA genome

II. Mobile genetic elements Transposons replicating via an RNA intermediate

The case of nematode resistant transgenic tobacco:

- Pest causing **roots knot** disease in tobacco plant : ***Meloidogyne incognita*** (Nematode/helminth)
- **Nematode specific gene** is introduced in host plant (tobacco), by using Ti plasmid (vector) of Agrobacterium tumefaciens, in such a manner that it produces both sense and **antisense RNA** in the host cells.
- Sense RNA and antisense RNA being complementary form **dsRNA** that initiates **RNAi**.
- Parasite could not survive in a **transgenic host** expressing **specific interfering RNA**.
- **Host plant – generated dsRNA triggers protection against nematode infestation.**

5 - BIOTECHNOLOGICAL APPLICATIONS IN MEDICINE

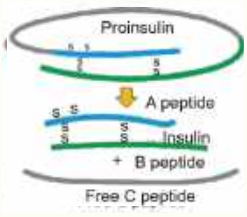
Advantages of Recombinant Therapeutics:

- 30 recombinant therapeutics have been approved for human use the world over. In India, 12 of these are presently being marketed.
- Mass production of safe and effective drugs.
- Do not induce unwanted immunological responses.

Genetically Engineered Human Insulin

- Problem : Insulin extracted from slaughtered cattle and pigs could cause allergy.
- **Solution:** Production of humulin

Proinsulin
A peptide
Insulin
B peptide
Free C peptide



Maturation of proinsulin to insulin

- Recombiant insulin manufactured by Eli Lilly, an American company, in 1983
- The main challenge for production of insulin using rDNA techniques was getting insulin assembled into a mature form

Sequence of events:

Artificially synthesised

DNA sequence for A-chain → Plasmid → E.coli → Chain A

DNA sequence for B-chain → Plasmid → E.coli → Chain B

Chain A + Chain B (Produced separately)

↓

Extracted and combined by creating disulphide bonds

↓

Human insulin

Insulin is a peptide hormone and can be degraded by proteases in our gut

6 - GENE THERAPY

- Insertion of genes into an individual's cells to treat diseases by
  - (i) Replacing a defective mutant allele with a functional one
  - (ii) Gene targeting which involves gene amplification.
- **First clinical gene therapy** was conducted in **1990** in a **4 year old** girl to treat adenosine **deaminase (ADA) deficiency**. ADA enzyme is crucial for immune system to function

Treatment for ADA Deficiency

1. Enzyme replacement therapy
   - Functional ADA is given by injection
2. Bone marrow transplantation in children
3. Gene therapy - Could be a permanent cure if bone marrow transplantion is done at early embryonic stages.

Steps in gene therapy

Lymphocytes of patient + ADA cDNA

↓ Retrovirus mediated transformation

Genetically engineered lymphocytes (not immortal)

↓

Infused in patient

7 - MOLECULAR DIAGNOSIS METHODS

PCR (Polymerase Chain Reaction):

- **Basis:** Nucleic acid amplification
- **Advantage:** Detection of very low concentration of a bacteria or virus
- **Uses:** Detection of HIV infection, mutations in genes in cancer patients, genetic disorders

ELISA (Enzyme Linked Immuno-Sorbent Assay):

- **Basis:** Antigen - antibody interaction
- **Uses:** Detect the presence of antigens or antibodies synthesized against pathogens

Autoradiography

- **Probe:** Radioactive ssDNA or ss**RNA** that hybridises with complementary DNA
- **Probe will not hybridise with mutated gene**, hence mutated gene will not appear on the photographic film due to lack of complementarity.

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

8 - TRANSGENIC ANIMALS

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

Uses of Transgenic Animals

- To study how genes are regulated and how they affect the normal functions of body, e.g. study of insulin – like growth factors
- Transgenic models exist for study of diseases like cancer, cystic fibrosis, rheumatoid arthritis and Alzheimer's

Biological products

- **1 antitrypsin -** To 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)
- The milk contained **alpha-lactalbumin**: More balanced product for human babies than natural cow milk

Vaccine Safety

- **Transgenic mice** are being used to test the safety of **polio vaccine** to replace the use of monkeys.
- **Chemical safety testing**
- Transgenic animals are made **more sensitive to toxic** substances to obtain results in less time.

9 - ETHICAL ISSUES

Genetic modification of organisms (GMO) can have unpredictable results when such organisms are introduced into the ecosystem.

- Genetic manipulation of living organisms by humans has to regulated for moral and biological significance.
- GEAC (Genetic Engineering Approval Committee) : Makes decisions regarding the validity of GM research and the safety of introducing GMO for public services
- Developing in biodiversity countries are rich and traditional knowledge related to bio-resources
- 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.

Controversies regarding patents and biopiracy:

(i) 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.

Basmati rice × Semi-dwarf variety of rice

↓

New variety of Basmati rice

(ii) Turmeric

(iii) Neem

The Indian Parliament has recently cleared the second amendment of the Indian Patents Bill.