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RAS

Rajasthan Administrative Services

Rajasthan Public Service Commission

Volume - 9

General Science and Technology

SIERRA INNOVATIONS

RAS

General Science and Technology

6 CHAPTER

BIO TECHNOLOGY

PREVIOUS YEAR QUESTIONS

Q1. The basis of DNA finger printing is (2018)

(1) The double helix
(2) Errors in base sequence
(3) DNA replication
(4) DNA Polymorphism

Q2. Which of the facts about Dolly (sheep), the first mammal cloned from an adult somatic cell is not correct? (2016)

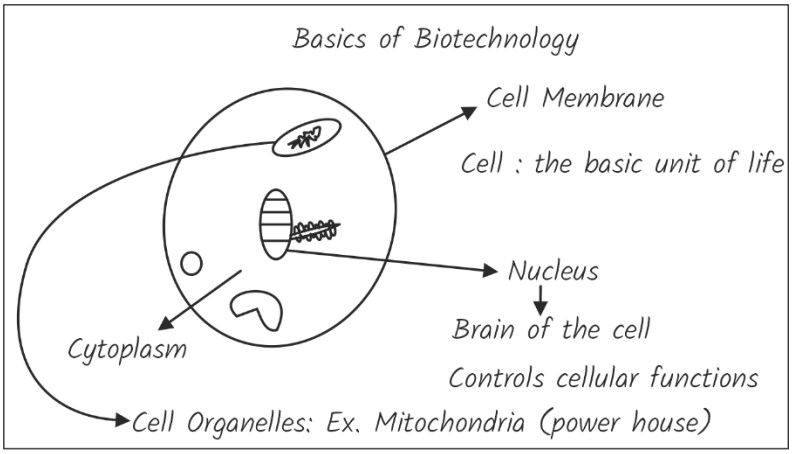
(1) Dolly was born in the year 1998.
(2) Dolly died in the year 2003.
(3) Dolly was born at Scotland.
(4) Dolly died due to lung disease.

Analysis- Generally one question is asked every year in the RAS pre exam,Nature of the question is direct and application based in daily life. Students need to update with current developments in the field of biotechnology to maximize score so keeping that in mind we have holistically covered these aspects very well.

Biotechnology is the use of living organisms, cells, and biological systems to develop products and technologies for various applications. It involves manipulating biological processes for industrial, agricultural, medical, and environmental purposes, such as creating genetically modified organisms, producing biofuels, and developing new medical therapies.

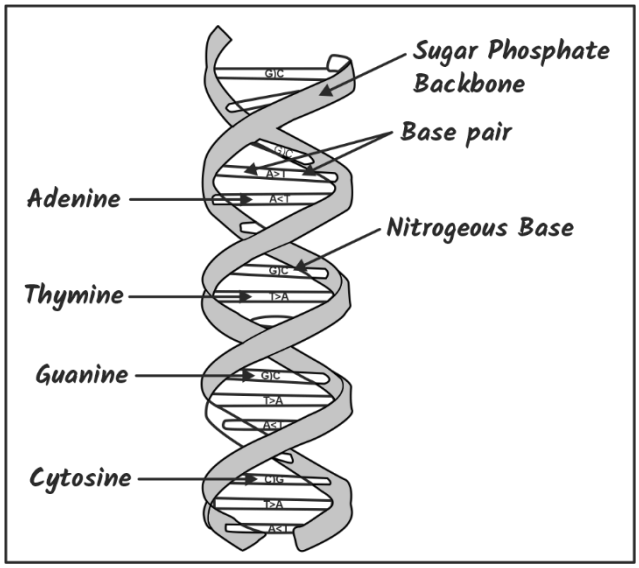
Basics of Biotechnology

Cell: A cell is the basic structural and functional unit of all living organisms, capable of carrying out life processes independently. // draw this diagram



Nucleus: The nucleus, a crucial organelle within the cell, contains genetic material (DNA) essential for cellular regulation and is fundamental to biotechnology.

DNA or Deoxyribonucleic Acid



- A hereditary material in humans and almost all other organisms.
- It was discovered by Friedrich Miescher in 1869. Whatson and Crick proposed the double helix structure of DNA.
- DNA is a polymer of nucleotides; nucleotide is made up of nucleoside and phosphate; nucleoside is made up of deoxyribose sugar and nitrogen base.
- Nitrogen base:- adenine, guanine (purine); thymine cytosine (pyrimidine)
- Structure of DNA :- double helix (Watson and crick).
- Most DNA is located in the cell nucleus (where it is called nuclear DNA), but a small amount of DNA can also be found in the mitochondria (where it is called mitochondrial DNA or mtDNA).
- Information in DNA is stored as a code made up of four chemical bases: adenine (A), guanine (G), cytosine (C), and thymine (T).
- Human DNA consists of about 3 billion bases, and more than 99 percent of those bases are the same in all people.
- DNA contains our unique genetic code, it holds the instruction for making all the proteins in our bodies (Proteins are the building blocks of our body).
- The Central Dogma: The central dogma of molecular biology describes the flow of genetic information within a biological system, where DNA is transcribed into RNA, which is then translated into proteins. This process is fundamental to understanding how genetic information directs cellular functions and development.

$$\text{DNA} \xrightarrow{\text{Transcription}} \text{RNA} \xrightarrow{\text{Translation}} \text{Proteins(enzymes)}$$

RNA or Ribonucleic Acid

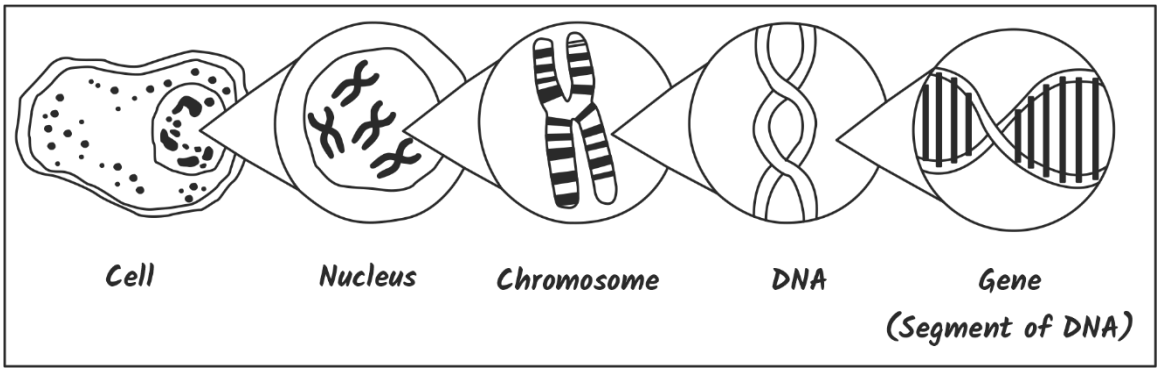
- RNA acts as a messenger (mRNA) carrying genetic information from DNA to ribosomes for protein synthesis, essential in biotechnology.
- Certain RNA molecules (ribozymes) have catalytic properties, facilitating biochemical reactions important for biotechnological applications.

- RNA molecules like microRNAs (miRNAs) and small interfering RNAs (siRNAs) regulate gene expression, crucial for gene therapy and genetic engineering.

Comparison of DNA and RNA

DNA	RNA
Deoxyribonucleic acid	Ribonucleic acid
Animals- nucleus of cell & cell organelles Plants - mitochondria and plant cell.	Found in cytoplasm but very little is found inside the nucleus.
Double-stranded molecule consisting of a long chain of nucleotides.	Single-strand helix having shorter chains of nucleotides.
Stores genetic information.	Translates genetic information into Protein (DNA first gets converted to RNA through process of Transcription and then RNA is used to produce Protein through the process of Translation)
Nitrogen base pairs - Adenine linked to Thymine (A-T) and Cytosine linked to Guanine (C-G)	Nitrogen base pairs - Adenine linked to Uracil (A-U) and Cytosine linked to Guanine (C-G).
Self-replicating	Synthesised from DNA when needed.
Damaged by exposure of ultraviolet rays.	More resistant to ultraviolet rays.
Occurs in chromosomes/ chromatin fibres.	Occurs in ribosomes.
Life of DNA is long.	Its life is short.

Gene – Gene is a sequence of nucleotides in DNA which contains genetic information and transfers it from one generation to another, it is also responsible for protein synthesis in body.



TYPES OF BIOTECHNOLOGY

1. **Genetic Biotechnology** – A field of biotechnology that involves modifying or interacting with the gene of organisms to create new or improved organisms. This alteration and modification in genes are also called the genetic engineering.

Example :- DNA Recombinant technique, gene therapy, gene editing

2. **Nongenetic Biotechnology** - Use of living organisms, cells, and biomolecules to create products and technologies without genetically modifying them. In this Technology natural properties of organisms or microorganisms are used to obtain desired products.

Example :- Bioinformatics, biosensors, biosignature

Techniques of Genetic Engineering

1. DNA Recombinant Technique

Recombinant DNA technology is a process that uses enzymes and other techniques to create new DNA molecules by combining DNA fragments from different sources. The resulting DNA is called recombinant DNA.

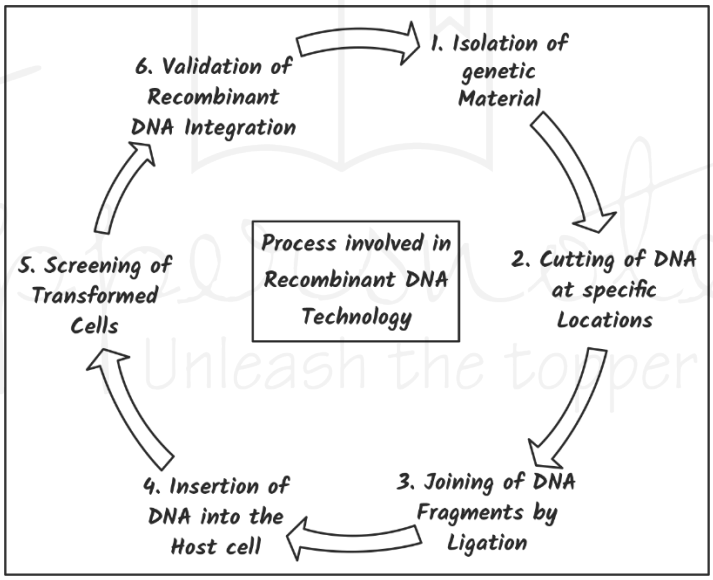
Tools of Recombinant DNA Technology

Tools	Utility	Examples
Vector	They are used as carriers to introduce foreign DNA into a host cell	- Plasmids, - Viruses (Phages), etc.
Restriction Enzymes	It recognises specific DNA sequences and cleaves the DNA at the precise location	- CRISPR Cas9 (used primarily nowadays) - Zinc-Finger Nuclease (ZFN)
DNA Ligase	It joins together DNA fragments	- T4 DNA Ligase
Selectable markers	To distinguish transformed cells from non-transformed ones	- Antibiotic resistance genes, herbicide resistance genes, etc

Process-

- **Isolation of Genetic Material**: The extraction of genetic material is performed from the source organism's DNA, such as bacteria, plants, or animals.
- **Selection of a Suitable Cloning Vector:** Vectors are carrier molecules used to introduce rDNA into a host organism.
  - ✓ Plasmids, which are small, circular DNA molecules, are commonly used vectors. They can replicate independently within a host cell, allowing for the propagation of the foreign DNA.

- **Cutting of DNA at Specific Locations**: It uses the specialized enzymes known as restriction endonucleases (RE) or restriction enzymes, which recognize specific DNA sequences (recognition sites) and cleave the DNA at those precise locations. Nuclease enzyme is known as molecular scissors.
- **Joining of DNA Fragments by Ligation**:
  - ✓ The isolated DNA fragments are combined with a vector, which is typically a plasmid or a viral genome modified to accept foreign DNA.
  - ✓ DNA ligase is used
- **Gene Transfer**: There are several methods which are employed in the process of Gene Transfer-
  - ✓ Physical methods: Gene gun or Biolistics, Electroporation, Microinjection, etc., which make the direct entry of the rDNA into the host's cell.
  - ✓ Chemical methods: Using Lipofection, calcium phosphate, etc. make it easier for the rDNA to enter into the host's cell.
  - ✓ Biological methods: This is an indirect method of gene transfer, using vectors (for example, bacteria) as a means.



- **Gene Cloning**: Once inside the host, the rDNA replicates itself independently (due to self-replicating plasmid). This is called Gene Cloning. It can also be done using the PCR method for amplifying a gene of interest.
- **Polymerase Chain Reaction (PCR)**: PCR is a tool that allows for the amplification of the target DNA sequences outside the cell. It needs much less time than the traditional cloning methods.
- **Selection and Screening of Transformed Cells**: This step involves identifying and isolating cells that have successfully taken up the recombinant DNA.
  - ✓ Selectable markers, such as antibiotic resistance genes carried by the vector, are often used to distinguish transformed cells from non-transformed ones.
- **Validation of Recombinant DNA Integration**: To ensure that the recombinant DNA has integrated into the host genome as intended, various techniques may be employed.

- ✓ For example, nucleic acid hybridization, blue-white screening, etc.

Examples –

- ✓ Golden Rice - Vit-A beta carotene
- ✓ Super Potato - protein rich
- ✓ Super Banana - protein rich
- ✓ BT Cotton - Insect resistant (Bacillus Thuringiensis)
- ✓ BT Brinjal - Insect resistant (Bacillus Thuringiensis)

Genetically Modified Organisms (GMO)-Genetically modified organisms (GMOs) are organisms whose genetic material has been altered using genetic engineering techniques to introduce new traits or characteristics.

Example: Bt cotton, which has been genetically modified to express a bacterial protein that acts as an insecticide, protecting the plant from pests.

GM CROPS- Genetically Modified (GM) crops are plants whose genetic material has been altered through genetic engineering techniques to introduce desirable traits such as resistance to pests, tolerance to herbicides, or enhanced nutritional content. These modifications typically involve inserting specific genes from bacteria, viruses, or other plants to improve the crop's performance or sustainability.

Important GM Crops

Crop	Features	Genes introduced	Bacteria Used
Bt Cotton	Insect resistance	Cry I Ac, Cry 2ab	Bacillus thuringiensis
Bt Corn	Insect resistance	Cry I ab, Cry 2ab	Bacillus thuringiensis
Golden rice	Enhanced vitamin A Production	Psy (Phytoene synthase,) crtl (carotene desaturase)	Erwinia uredovora
Round Ready Soybean	Herbicide tolerance	CP4 EPSPS	Agrobacterium tumefaciens
Flavr Savr Tomato	Delayed ripening	Antisense polygalacturonase	-
Rainbow Papaya	Virus resistance	Coat protein gene	Papaya ringspot virus (PRSV)
GM Canola	Herbicide tolerance	CP4 EPSPS	Agrobacterium tumefaciens
Bt Brinjal	Insect resistance	Cry IAc	Bacillus Thuringiensis

DMH II (Dhara Mustard Hybrid)-GM hybrid variety of mustard

NOTE: - Only GM crop approved in India (For commercial cultivation)- Bt cotton.

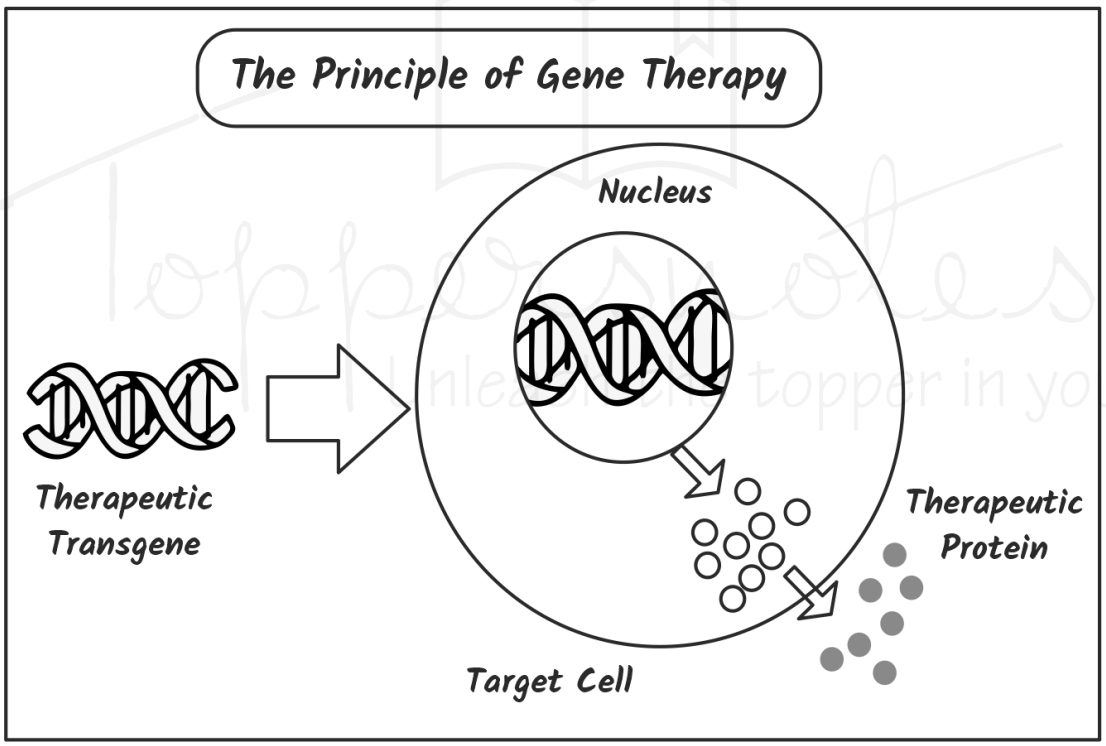
Approval is given by Genetic Engineering Appraisal Committee (GEAC) under Ministry of Environment Forest and climate change.

2. DNA FINGERPRINTING / DNA PROFILING

- Technique developed by British scientist Alec Jeffreys.
- The technique of determining an individual's identity by uniqueness of its DNA (found on chromosome) is called DNA fingerprinting or DNA profiling. Determine the nucleotide sequence at certain part of the DNA that is unique in all human beings.
- Lal ji Singh is known as father of DNA fingerprinting in India.

3. Gene Therapy

Gene therapy is a collection of methods that allows correction of genetic defects to treat or prevent disease.



Methods of Gene Therapy

(1) **Gene Replacement:-** In this technique a faulty/unhealthy gene is replaced by a healthy gene with same functioning.

(2) **Gene Augmentation:-** In this technique a healthy gene with the same functioning is adjusted in DNA while faulty gene also exists there.

(3) **Gene Correction:-** In this technique a faulty gene is repaired.

Gene/ Genome Editing Techniques

1. **CRISPR-Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats):**
   - ✓ Widely used for its precision and ease of use.
   - ✓ Can introduce, delete, or replace specific DNA sequences.
2. **TALENs (Transcription Activator-Like Effector Nucleases):**
   - ✓ Customized proteins that can bind to specific DNA sequences and create double-strand breaks.
   - ✓ Used for targeted gene modification.
3. **ZFN (Zinc Finger Nucleases):**
   - ✓ Engineered DNA-binding proteins that create targeted double-strand breaks in DNA.
   - ✓ Used for precise gene editing.
4. **Meganucleases:**
   - ✓ Naturally occurring enzymes with long recognition sequences.
   - ✓ Used for highly specific gene editing tasks.
5. **RNA Interference (RNAi):**
   - ✓ A process where RNA molecules inhibit gene expression or translation.
   - ✓ Used for gene silencing rather than editing DNA.
6. **Base Editing:**
   - ✓ Allows precise conversion of one DNA base into another without causing double-strand breaks.
   - ✓ Used for correcting point mutations.
7. **Prime Editing:**
   - ✓ A newer, more versatile form of gene editing that can insert, delete, and replace DNA sequences.
   - ✓ Combines aspects of CRISPR and reverse transcriptase.

Stem Cell Therapy

Stem cells are those undifferentiated cells that have unique ability to develop into specialized cell type in body. It is also called Master cell. Using Stem Cells for medical purposes is called Stem cell Therapy.

Types of stem cell on the basis of source –

(1) Embryonic Stem Cell- These stem cells are obtained from embryo.

(2) Cord Stem Cell :- obtained from umbilical cord.

(3) Adult Stem Cell :- Found in various tissues of fully developed mammals.

(4) Induced Pluripotent Stem Cell :-These stem cells are obtained from somatic cells with the help of DNA Recombinant technique. These cells are pluripotent.

Types of stem cells on the basis of capacity -

(1) Unipotent - Those stem cells which have ability to convert in only one type of cell. Source - liver, blood vessels, skeleton, spine.

(2) Multipotent Stem Cell - Those stem cells which have ability to convert in more than one type of cells. Source - brain, bone marrow.

(3) Pluripotent Stem Cell - Those stem cells which have ability to convert in any type of cell excluding Placenta. Source- blastula phase of embryo.

(4) Totipotent Stem Cells - Those stem cells which have ability to convert in any type of cell. Source - 3 - 4 days of zygote.

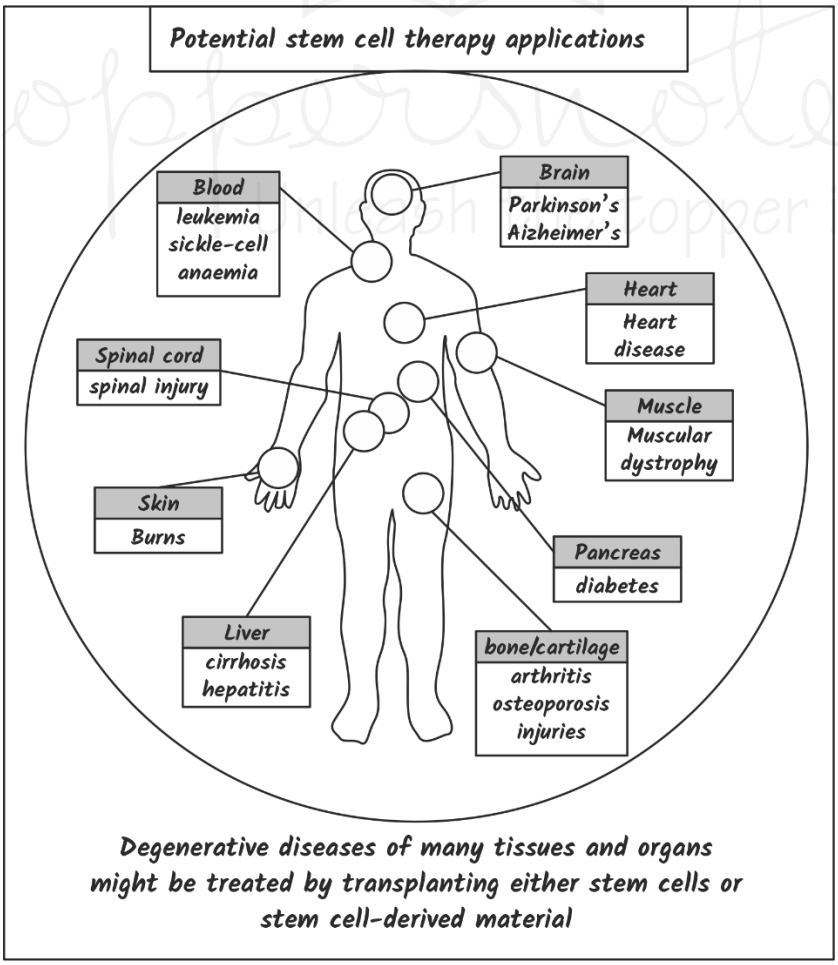
Uses of stem cell -

a. Treatment of serious disorder.

b. Regenerate organ.

c. Fractured tissues can be replaced by stem cells.

d. Artificial organs can be developed

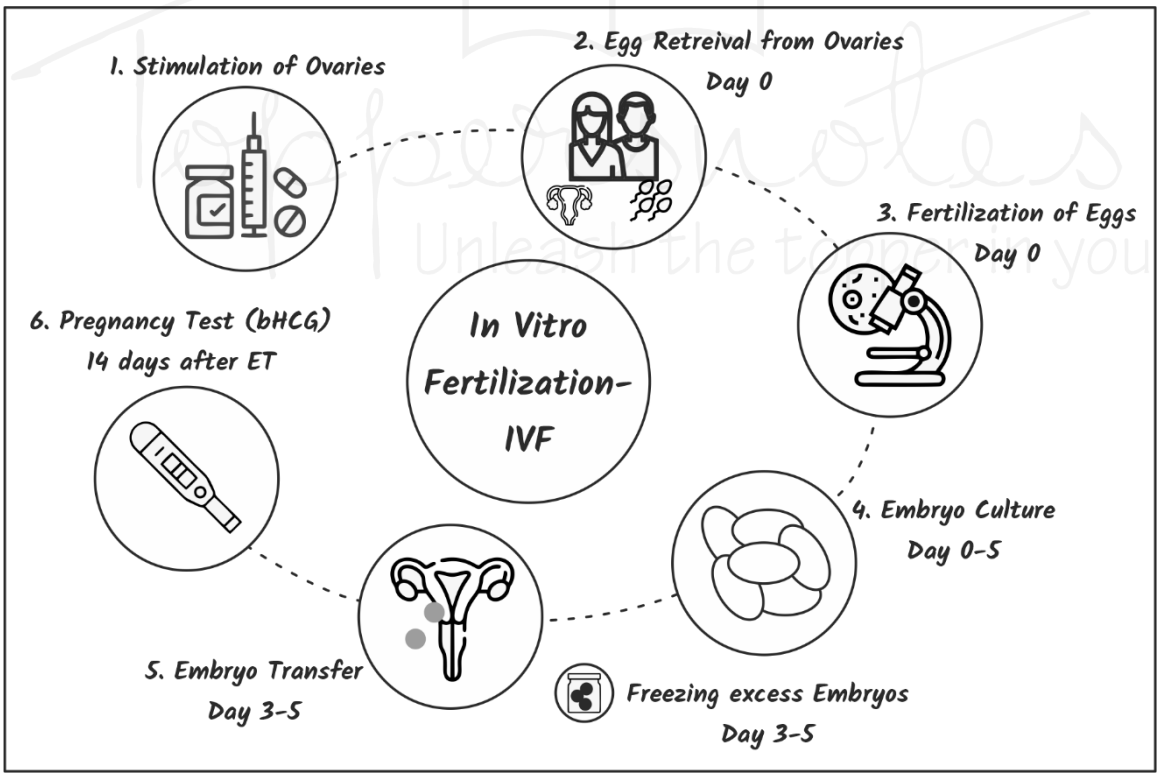


IVF (In Vitro Fertilization / Test Tube Baby)

IVF is a process of fertilization in which an ovum is combined with sperm outside the body in test tube (In lab).

Process

- First step– Ovulation induction-Taking fertility medications for several months to help ovaries produce several eggs that are mature and ready for fertilisation.
- Second step– Egg retrieval– The egg is removed from the woman's body.
- Third step– Semen Analysis– The collected semen sample is sent for testing to verify its motility for fertilisation.
- Fourth step– Fertilisation– In a lab, eggs are mixed with sperm cells from another partner or a donor.
- Fifth step– Embryo Development– The eggs and sperm are stored together in a particular container, and fertilisation happens.
  - ✓ The cells in the fertilised eggs divide and become embryos.
- Sixth step– Embryo transfer– one or more embryos are put into the uterus.



Use : To help infertile parents in conception of a child.

NOTE –

- World's first test tube baby :- Louise brown, 1978, Manchester United Kingdom.
- India's first test tube baby :- Durga (Kanupriya Agarwal), oct 1978.

Mitochondrial Replacement Therapy / Three Parent Baby

Mitochondrial replacement therapy (MRT) is a new form of reproductive invitro fertilization (IVF) which works on the principle of replacing a women's abnormal mitochondrial DNA (mt-DNA) with the donor's healthy one.

Process –

1. **Identifying Suitable Candidates:** The procedure is specifically intended for couples who wish to have their genetic child but do not want to use a donor egg.
2. **Selection of Donor and Biological Parents:** The biological mother, who has a mitochondrial disease, provides her eggs, which are fertilized by the biological father's sperm.
   - ✓ Female donor with healthy mitochondria is also involved.
3. **Mitochondrial Replacement:** The genetic material (DNA) from the donor's egg is extracted and replaced with the genetic material from the biological parents.
   - ✓ This creates an embryo with the parents' DNA and the donor's mitochondria.
4. **Implantation and Pregnancy:** The modified embryo is then implanted in the uterus resulting in the birth of a baby free from the mother's mitochondrial disease.

The resulting baby has DNA from the mother and father as usual, plus a small amount of genetic material (Mt DNA) – about 37 genes – from the donor. So called "three-parent babies", though more than 99.8% of the DNA in the babies comes from the mother and father.

Uses- The development of healthy baby free from genetic disorders and to terminate the lethal mitochondrial disorders.

Surrogacy

A surrogate, is a woman who conceives, carries and gives birth to a child for another person or couple (intended parent/s). The surrogate agrees to give the child to that person or couple after the birth.

Traditional Surrogacy - In this method sperms of the father/person willing to have a baby are fertilized with ovum of surrogate mother using IVF technique & then embryo is transplanted in uterus of surrogate mother.

Gestational Surrogacy – In this method ovum and sperm of the couple willing to have a baby are fertilised using IVF technique & then embryo is transplanted in uterus of surrogate mother.

The baby born contains genetic effects of both of his biological parents

Use : To get a baby when -

a. Couple is not able to have a baby.
b. Conception is fatal for women.
c. Single person wants his/her baby

Surrogacy (Regulation) Act 2021

Regulation of Surrogacy:

- The Act prohibits commercial surrogacy, and allows altruistic surrogacy.
- The Act permits surrogacy when it is:

  (i) for intending couples who suffer from proven infertility;

  (ii) altruistic;

  (iii) not for commercial purposes;

  (iv) not for producing children for sale, prostitution or other forms of exploitation; and

  (v) for any other condition or disease specified through regulations.
- Eligibility Criteria for Surrogate Mother: To obtain a certificate from the appropriate authority, the surrogate mother has to:

  (i) be a close relative of the intending couple;

  (ii) be an ever-married woman having a child of her own;

  (iii) be 25 to 35 years old;

  (iv) not have been a surrogate mother earlier; and

  (iv) have a certificate of medical and psychological fitness.
- Registration of Surrogacy Clinics: Surrogacy clinics cannot undertake surrogacy or its related procedures unless they are granted registration by the appropriate authority.
- National and State Surrogacy Boards: The central and state governments shall constitute the National Surrogacy Board (NSB) and the State Surrogacy Boards (SSBs), respectively.
- Offences & Penalties:
  - ✓ The Act creates certain offences which include:

    (i) undertaking or advertising commercial surrogacy;

    (ii) exploiting the surrogate mother;

    (iii) selling or importing human embryo or gametes for surrogacy, and

    (iv) abandoning, exploiting or disowning a surrogate child/
  - ✓ These offences will attract a penalty of up to 10 years and a fine of up to 10 lakh rupees.

Cloning

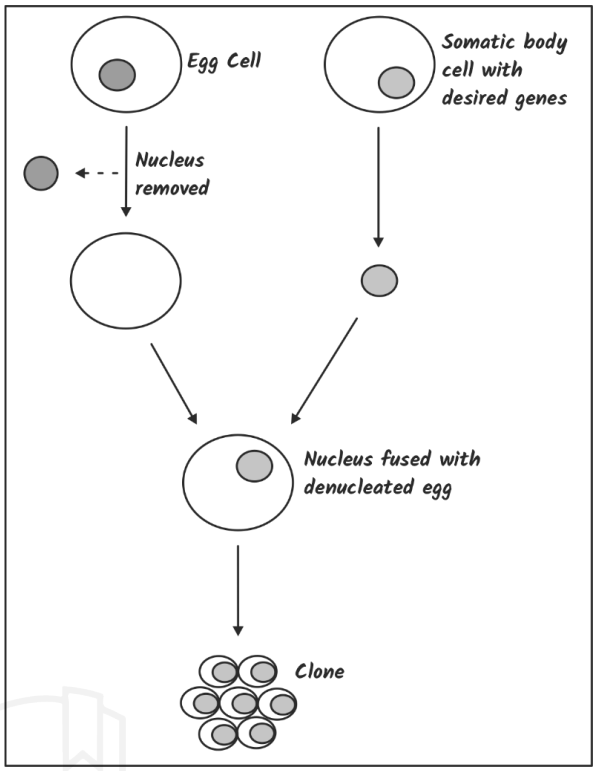
Cloning means an exact copy of something. Cloning is a technique that creates genetically identical copies of organisms, cells, or DNA.

Techniques of Cloning

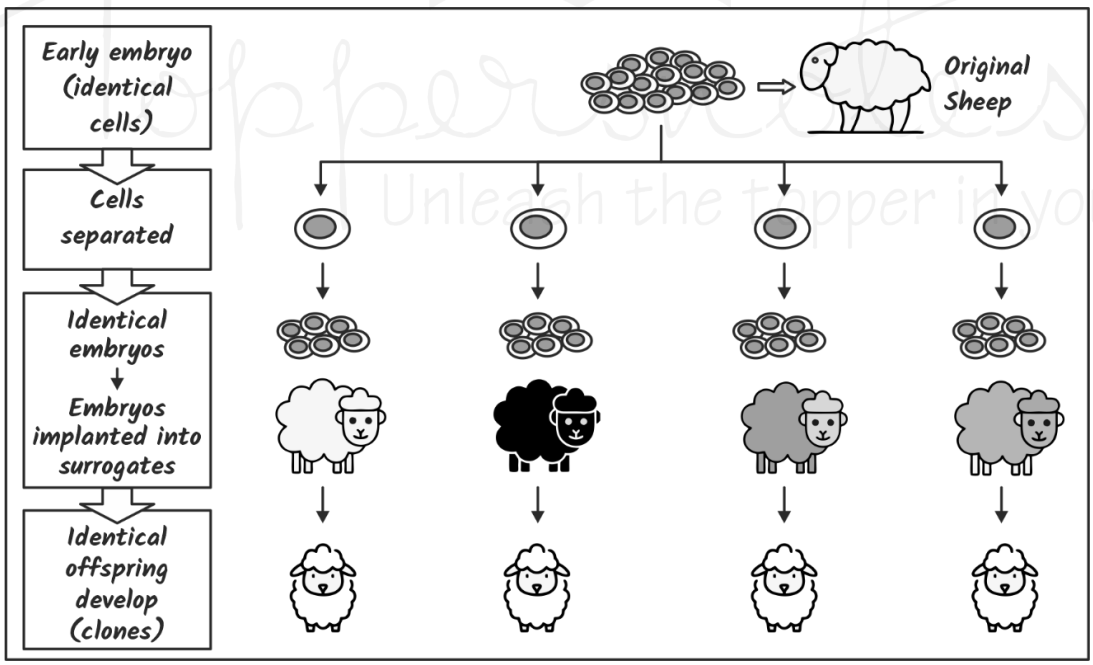
1. Gene cloning, which creates copies of genes or segments of DNA.
2. Reproductive cloning, which creates copies of whole animals.

- ✓ In this we actually reproduce not organ but entire being(donor) from where we got genetic information.
- ✓ Example: Dolly the sheep (1996).

3. Therapeutic cloning, which creates embryonic stem cells.
   - ✓ In therapeutic cloning, the aim is to clone cells that make particular organs or types of tissue.
   - ✓ It is also called somatic cell nuclear transfer or research cloning.
   - ✓ Focuses on producing stem cells for medical treatment.
   - ✓ Example: Cloning human embryos to generate tissue or organs for transplants.



4. Embryo Cloning (Artificial Twinning)
   - ✓ Mimics natural identical twinning by splitting a fertilized embryo.
   - ✓ Produces genetically identical embryos.



NOTE-

- ✓ World's First Animal Clone - Dolly Sheep(1996).
- ✓ India's Animal Clone - Garima, Mahima (Buffalo clone).
- ✓ Ganga- India's first cloned Gir female calf. The Karnal-based National Dairy Research Institute (NDRI) has cloned, for the first ever in India, a female calf of the desi breed Gir that was capable of producing over 15 litres of milk per day.

Non Genetic Biotech

Bio – Informatics

Bioinformatics is a hybrid science that combines biological data with techniques for information storage, distribution, and analysis to support a wide range of scientific research, including biomedicine. Branch of Biology in which computer science and information technology are used for compilation, storage & analysis of biological data.

Uses –

1. *Protein sequence analysis.*
2. *Finding the gene responsible for deadly diseases.*
3. *Drug designing.*
4. *Biological Data Visualisation: It involves the application of graphics and data representation and incorporates sequences, genomes, alignments, phylogenies, macromolecular structures, microscopy, and other imaging information.*
5. *Genome informatics: It is the application of bioinformatics tools to process the outputs of genome-wide assays and technologies, facilitating the interpretation of data and linking them to function to analyse biomolecules. For example – Human Genome Project.*

NOTE –

Human Genome Project

- *A publicly funded international collaborative research project Launched in 1990.*
- *Aim: To determine sequence of chemical base pairs making up human DNA, & identifying & mapping all of genes of human genome.*
- *Mapping of human genome involves sequencing multiple variations of each gene.*

Genome India Project

- *Initiated by Department of Biotechnology (DBT) in 2020*
- *Based on Human Genome Project.*
- *Aim: To collect 10,000 genetic samples from citizens across India, to build a reference genome.*
- *Involves 20 leading institutions of country with Centre for Brain Research of Indian Institute of Science (IISc) Bangalore – nodal point.*

IndiGen– India's Genome Sequencing Project

- *Conducted by Council of Scientific and Industrial Research (CSIR)*
- *A "whole-genome sequence" of 1,008 Indians that belonged to diverse ethnicities.*
- *A precursor to the Genome India Project" (GIP).*