1. Gene Therapy

1.1. What is Gene Therapy?

- Gene therapy is a technique that **uses genes to treat or prevent disease.**
- This technique allows doctors to **treat a disorder by inserting a gene** into a patient's cells instead of using drugs or surgery.
- Researchers are testing several approaches to gene therapy, including:
    - Replacing a mutated gene that causes disease with a healthy copy of the gene.
    - Inactivating, or "knocking out," a mutated gene that is functioning improperly.
    - Introducing a new gene into the body to help fight a disease.

1.2. Approach and Technique

- Gene therapy is designed to introduce genetic material into cells to compensate for abnormal genes or to make a beneficial protein.
- If a mutated gene causes a necessary protein to be faulty or missing, gene therapy may be able to introduce a normal copy of the gene to restore the function of the protein.
- A gene that is inserted directly into a cell usually does not function. Instead, **a carrier called a vector is genetically engineered** to deliver the gene.
- Certain viruses are often used as vectors because they can deliver the new gene by infecting the cell. The viruses are modified so they can't cause disease when used in people.
- Some types of virus, such as retroviruses, integrate their genetic material (including the new gene) into a chromosome in the human cell.
- The vector can be **injected or given intravenously** directly into a specific tissue in the body, where it is taken up by individual cells.
- Alternatively, a sample of the patient's cells can be removed and exposed to the vector in a laboratory setting.
- The cells containing the vector are then returned to the patient. If the treatment is successful, the new gene delivered by the vector will make a functioning protein.
- Non-viral methods of gene transfer have also been explored in recent years. Methods for non-viral gene therapy include electroporation, the gene gun, sonoporation, magnetofection, the use of oligonucleotides, lipoplexes, dendrimers, and inorganic nanoparticles.

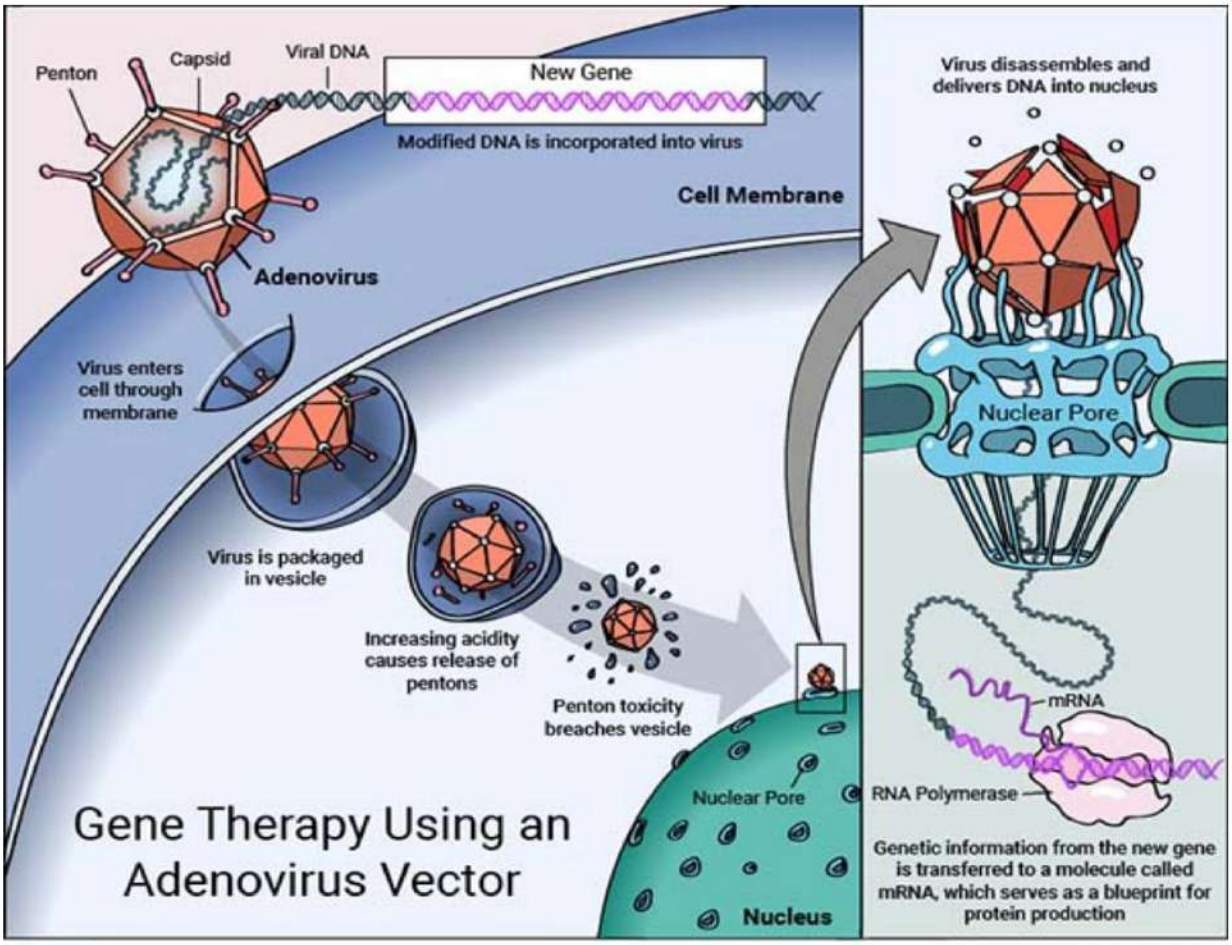

Figure.1. Virus based transfer of gene for gene therapy

1.3. Types for Gene Therapy

A. Depending upon the type of cells that are modified by the therapeutic genes:

Somatic Cell Gene Therapy

- In this type, genetic changes are directed towards somatic cells.
- As these cells are non-reproductive, the effect is not passed into future generations, making it safer.
- The disadvantage is the short duration of effects of somatic cell therapy as most tissues are be replaced by new tissues.

Germ Cell Gene Therapy

- This is the type of gene therapy, where germ cells, i.e. either sperm or ova are introduced with therapeutic genes, leading to the changes that are inheritable, i.e. changes in genes may affect future generations.

B. Based upon the technique of delivery of vectors to the target cell:

Ex-vivo Gene Therapy

- Ex -vivo gene therapy is where the defected cells are extracted out of the body and targeted with therapeutic genes.
- Once successfully modified, they are cultured ex-vivo and transferred back to the host, where now the corrected gene replicates.

In-vivo Gene Therapy

- In this modality, a vector that is capable of carrying the therapeutic gene, is used to inject host cells with normal genes.

C. The type of change brought out in the faulty gene:

Gene Replacement

- Gene replacement means replacement of defective genes with a corrected one.

Gene Addition Therapy

- Gene addition means restoration of normal function of a cell by addition of normal or functional copy of gene into the genome.
- This concept is primarily used in various gene therapy related research on cancer.

1.4. Success and Barriers

- Clinical trials of gene therapy in people have shown some success in treating certain diseases, such as:
    - Severe combined immune deficiency
    - Hemophilia
    - Blindness caused by retinitis pigmentosa
    - Leukemia
- But several significant barriers stand in the way of gene therapy becoming a reliable form of treatment, including:
    - Finding a reliable way to get genetic material into cells
    - Targeting the correct cells
    - Reducing the risk of side effects

1.5. Gene Therapy in India

- In India though interest in gene therapy took some time but with financial assistance provided by different government agencies, the country has shown rapid improvement in gene therapy-related research.
- India is at third place among the major Asian countries having gene therapy laboratories.
- The main aim is to develop new institutions for gene therapy research, strengthening existing institutions which have good expertise in this area in order to initiate work in molecular genetics for decreasing the burden of genetic disorders in the country.
- The pioneer of gene therapy-related research in India is **Advanced Centre for Treatment, Research and Education for Cancer** (ACTREC) where active work on gene therapy for head and neck cancer using synthetic vectors is being carried out.

1.5.1. Guidelines for Gene Therapy in India

- The Indian Council of Medical Research (ICMR), in December 2019, issued the National Guidelines for Gene Therapy Product Development and Clinical Trials, in consultation with experts and government agencies — including the Department of Biotechnology (DBT) and the Central Drugs Standard Control Organisation (CDSCO).
- The aim of the document is to ensure that gene therapies can be introduced in India and clinical trials for gene therapies can be performed in an ethical, scientific and safe manner.

Main features of the guidelines

- The national guidelines outline what encompasses gene therapy and GTPs. Gene Therapy Products (GTP) are defined as **any entity which includes a nucleic acid component being delivered by various means for therapeutic benefit** to patients.

- It brings new gene therapies under the umbrella of existing laws regulating drugs and medicinal practices in India — such as the Drug and Cosmetics Act, 1940 and the Drugs and Magical Remedies (The Objectionable Advertisements) Act, 1954.
- Under these guidelines, **germ-line gene therapy remains prohibited** in India.
- The national guidelines provide the general principles for developing GTPs for any human ailment and provide the framework for human clinical trials which must follow the established general principles of biomedical research for any human applications.

1.5.2. Gene Therapy Advisory and Evaluation Committee (GTAEC)

- The Gene Therapy Advisory and Evaluation Committee (GTAEC) is an independent body of experts representing diverse areas of biomedical research, concerned government agencies and other stakeholders.
- It was constituted and notified by the Department of Health Research, Ministry of Health and Family Welfare to oversee the activities in the field of gene therapy research in India.

Functions of the GTAEC

- To serve as an apex advisory body to Government of India for research and development in the field of gene therapy in India.
- To perform a comprehensive review of the pre-IND (Investigational New Drug) and IND applications of Gene Therapy Products.
- To formulate policies to inculcate scientific and ethical practices amongst stakeholders.
- To provide a forum for discussion of issues involved in basic and clinical research and progress in the field of gene therapy.
- To assess periodically the adequacy of the document in light of advancements occurring globally.
- To consider the unforeseen issues of public interests.

1.6. Mitochondrial Donation Treatment (MDT)

What is Mitochondrial Disease?

- Mitochondria are basically the **powerhouses of the cells,** they **generate the energy,** and thus are also **responsible for cell function** in the human body.
- Certain defects might occur impacting on the way the mitochondria produces energy for the cells (Specially in the ‘energy-hungry’ tissues of the brain, nerves, muscles, kidneys, heart, liver), and thereby impacting cell function.
- The diseases that arise out of such mitochondrial mutations are called mitochondrial diseases.
- When the mitochondria are impaired and do not produce sufficient energy, that affects how the organs function, leading to a broad assortment of symptoms across the body, including brain damage, organ failure and muscle wastage.
- Note: Mitochondrial diseases are **only passed on by the mother**.

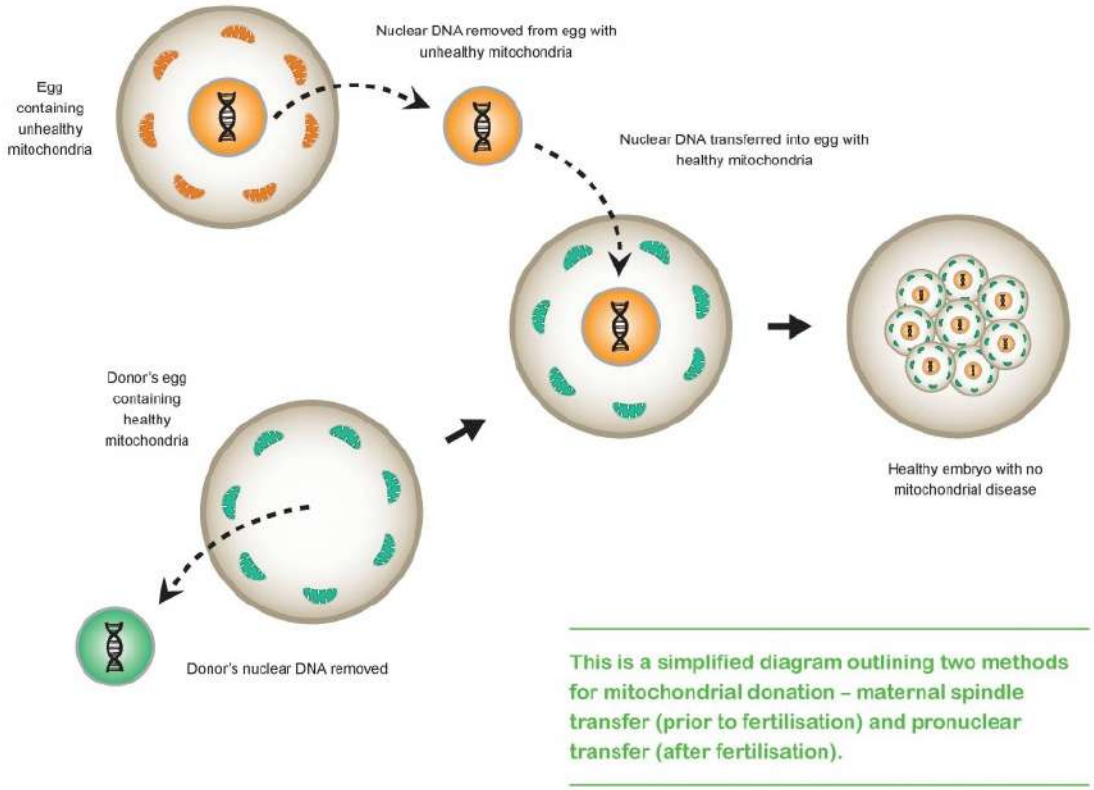

Figure.2. Mitochondrial Donation Treatment

Scientific Process

- Through an advanced In Vitro Fertilisation, the baby's **biological father's sperm was used to fertilize the eggs from the biological mother,** who has a mitochondrial disease, and **a third, female donor with clear mitochondria,** separately.
- Then, the nuclear genetic material from the donor's egg is removed and replaced with the genetic material from the biological parents'.
- The final product – the egg - which has the genetic material (DNA) from the parents, and the mitochondria from the female donor, is implanted in the uterus, and carried to full term to yield a baby who will be free from the mother's mitochondrial disease.
- The process is termed as Mitochondrial Donation Treatment (MDT).

Side Effects to the Procedure

- Sometimes it is possible that a small amount of the maternal mitochondria with errors may get passed on during the procedure. While largely helpful, the procedure is not without these minimal risks.

2. CRISPR and Gene Editing

- CRISPR (short for **"clustered regularly interspaced short palindromic repeats"**) is a technology that research scientists use to selectively modify the DNA of living organisms. It is a bacterial immune system that has been modified for genome engineering.

- The functions of CRISPR and CRISPR-associated (Cas) genes are **essential in adaptive immunity** in some bacteria, enabling the organisms to respond to and eliminate invading genetic material.
- These repeats were initially **discovered in 1987 in E. coli by Ishino,** but their function was confirmed in 2007 by Barrangou and co-workers.
- CRISPR-Cas9 enables geneticists and medical researchers to edit parts of the genome by removing, adding or altering sections of the DNA sequence.
- It is currently the simplest, most versatile and precise method of genetic manipulation.

2.1. Working Mechanism

- CRISPR consists of two components: **a "guide" RNA (gRNA) and a non-specific CRISPR-associated endonuclease (Cas9).**
- Cas9 acts as a **pair of 'molecular scissors'** that can cut the two strands of DNA at a specific location in the genome so that bits of DNA can then be added or removed.
- The gRNA is a short synthetic RNA composed of a sequence necessary for Cas9-binding and a **user-defined ~20 nucleotide "targeting" sequence** which defines the genomic target to be modified.
- Thus, one can change the genomic target of Cas9 by simply changing the targeting sequence present in the gRNA.

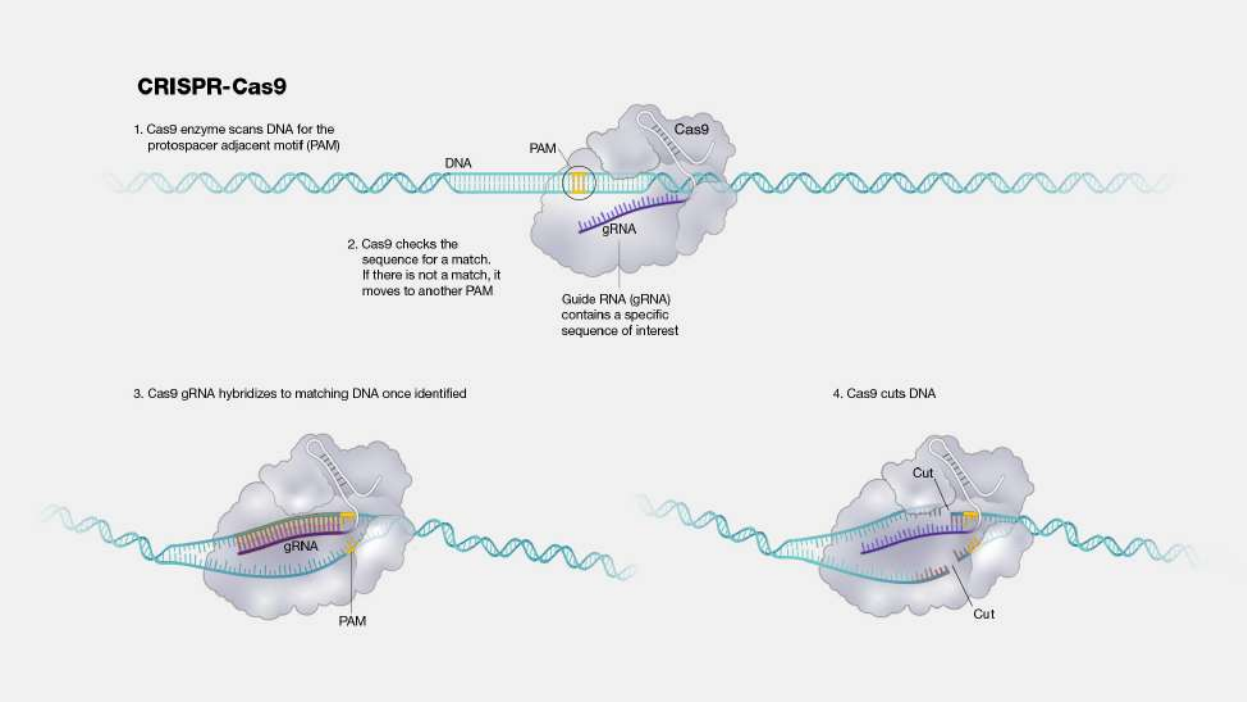

Figure.3. Method of CRISPR-Cas9

2.2. Applications

- **Targeted genome engineering:** The system can be used to facilitate a wide variety of targeted genome engineering applications. TheCas9 nuclease has enabled efficient and targeted genome modification in many species that have been intractable using traditional genetic manipulation techniques.

- **Rapid Generation of Cellular and Animal Models:** Cas9-mediated genome editing has enabled accelerated generation of transgenic models and expanded biological research beyond traditional animal model organisms.
- **Functional Genomic Screens:** The efficiency of genome editing with Cas9 makes it possible to alter many targets in parallel, thereby enabling genome-wide functional analysis to identify genes that play an important role in a phenotype of interest.
- **Transcriptional Modulation:** The technique may repress gene action which can be used in silencing the desired genes.
- **Epigenetic Control:** Complex genome functions are controlled by the highly dynamic process of epigenetic changes. CRISPR method can bring about desired epigenetic modifications.
- **Cas9 as a Therapeutic Molecule for Treating Genetic Disorders:** Cas9 can be used as a therapeutic technology for treating genetic disorders. For a monogenic recessive disorder due to loss-of-function mutations (such as cystic fibrosis, sickle-cell anemia, or Duchenne muscular dystrophy), Cas9 may be used to correct the causative mutation.

2.3. Prospect of Designer Babies

- Designer baby is a term that refers to the product of a genetically engineered baby. These babies are "designed" (fixed/changed) while still in the womb to achieve more desired health (being free from some diseases), looks, skills, or talents.

How can it be done?

- In medicine and (clinical) genetics pre-implantation genetic diagnosis (PGD or PIGD) (also known as embryo screening) refers to procedures that are performed on embryos prior to implantation, sometimes even on oocytes prior to fertilization.
- When used to screen for a specific genetic disease or for risk of getting a disease, its main advantage is that the method makes it highly likely that the baby will be free of the disease under consideration.
- This step can be followed by genetic manipulation of the egg or the zygote. Here, the standard recombinant DNA Technology is applied. After this, once again the genetic screening is done.
- If the desired genetic traits have successfully been introduced, then the zygote or the early embryo arising from it can be approved for implantation. Implantation can be done in a normal or surrogate mother.
- After the normal gestation, a "designer baby" with desired traits is born.

3. Cloning

3.1. What is Cloning?

- The term cloning describes a number of different processes that can be used to produce **genetically identical copies of a biological entity.**
- The copied material, which has the same genetic makeup as the original, is referred to as a clone.

3.2. Need for Cloning

Cloning Animal Models of Disease

- Animal models are genetically engineered **to carry disease-causing mutations** in their genes. Cloning could help reduce the time needed to make a transgenic animal model, and the result would be a population of genetically identical animals for study.

Cloning Stem Cells for Research

- Stem Cells transferred from one person to another are seen as foreign, and they usually trigger an immune response.
- Some researchers are looking at cloning as a way to create stem cells that are genetically identical to an individual. These cells could then be used for medical purposes, possibly even for growing whole organs.
- Also, stem cells cloned from someone with a disease **could be grown in culture and studied** to help researchers understand the disease and develop treatments.

Reviving Endangered or Extinct Species

- In 2009, scientists had their first near-success resurrecting an extinct animal.
    - Using goats as egg donors and surrogates, they made several clones of a wild mountain goat called the bucardo—but it died soon after birth.
- Cloning endangered species is easier than extinct species, because the surviving animals can donate healthy, living cells.
- However, the problem that endangered species face is the loss of genetic diversity, and cloning does nothing to address this problem.
- Cloning also does not address the problems of habitat destruction and hunting.

Pharming for Drug Production

- Farm animals such as cows, sheep, and goats are being genetically engineered to produce drugs or proteins that are useful in medicine.
- Just like creating animal models of disease, cloning might be a faster way to produce large herds of genetically engineered animals.

Cloning Humans

- The prospect of cloning humans is highly controversial, and it raises a number of ethical, legal, and social challenges that need to be considered.
- Even though many species have been cloned successfully, the process is still technically difficult and inefficient.
- The success rate in cloning is quite low: most embryos fail to develop, and many pregnancies end in miscarriage.
- Current efforts at human cloning are focused on creating embryonic stem cells for research and medicine.

3.3. Types of Cloning

There are three different types of cloning:

- **Gene cloning:** creates copies of genes or segments of DNA.
- **Reproductive cloning:** creates copies of whole animals.
- **Therapeutic cloning:** produces embryonic stem cells for experiments aimed at creating tissues to replace injured or diseased tissues.

3.4. Methods Used for Reproductive Cloning

Cloning Using Somatic Cell Nuclear Transfer (SCNT)

- The procedure starts with the **removal of the chromosomes** from an egg to **create an enucleated egg.**

- The chromosomes are replaced with a nucleus taken from a somatic (body) cell of the individual or embryo to be cloned. This cell could be obtained directly from the individual, from cells grown in culture, or from frozen tissue.
- The egg is then stimulated, and in some cases it starts to divide. If that happens, a series of sequential cell divisions leads to the **formation of a blastocyst,** or preimplantation embryo.
- The blastocyst is then transferred to the uterus of an animal.
- The successful implantation of the blastocyst in a uterus can result in its further development, culminating sometimes in the birth of an animal.
- This animal will be a clone of the individual that was the donor of the nucleus.
- SCNT was the method used to create Dolly the Sheep.

Cloning by Embryo Splitting

- This procedure begins with **in vitro fertilization** (IVF). The zygote divides into two and then four identical cells.
- At this stage, the cells can be separated and allowed to develop into separate but identical blastocysts, which can then be implanted in a uterus.
- The limited developmental potential of the cells means that the procedure cannot be repeated, so embryo splitting can yield only two identical individuals and probably no more than four identical humans.

3.5. The Case of Dolly

- In 1996, Ian Wilmut and his colleagues at the Roslin Institute in Edinburgh, Scotland had successfully cloned a sheep named Dolly. Dolly was the first cloned mammal.
- Wilmut and his colleagues transplanted a nucleus from a mammary gland cell of a Finn Dorsett sheep into the enucleated egg of a Scottish blackface ewe.
- The nucleus-egg combination was stimulated with electricity to fuse the two and to stimulate cell division.
- The new cell was divided and was placed in the uterus of a surrogate mother blackface ewe to develop. Dolly was born months later.
- The lamb, Dolly, was an exact genetic replica of the adult female sheep that donated the somatic cell nucleus to the egg. She was the first-ever mammal to be cloned from an adult somatic cell.

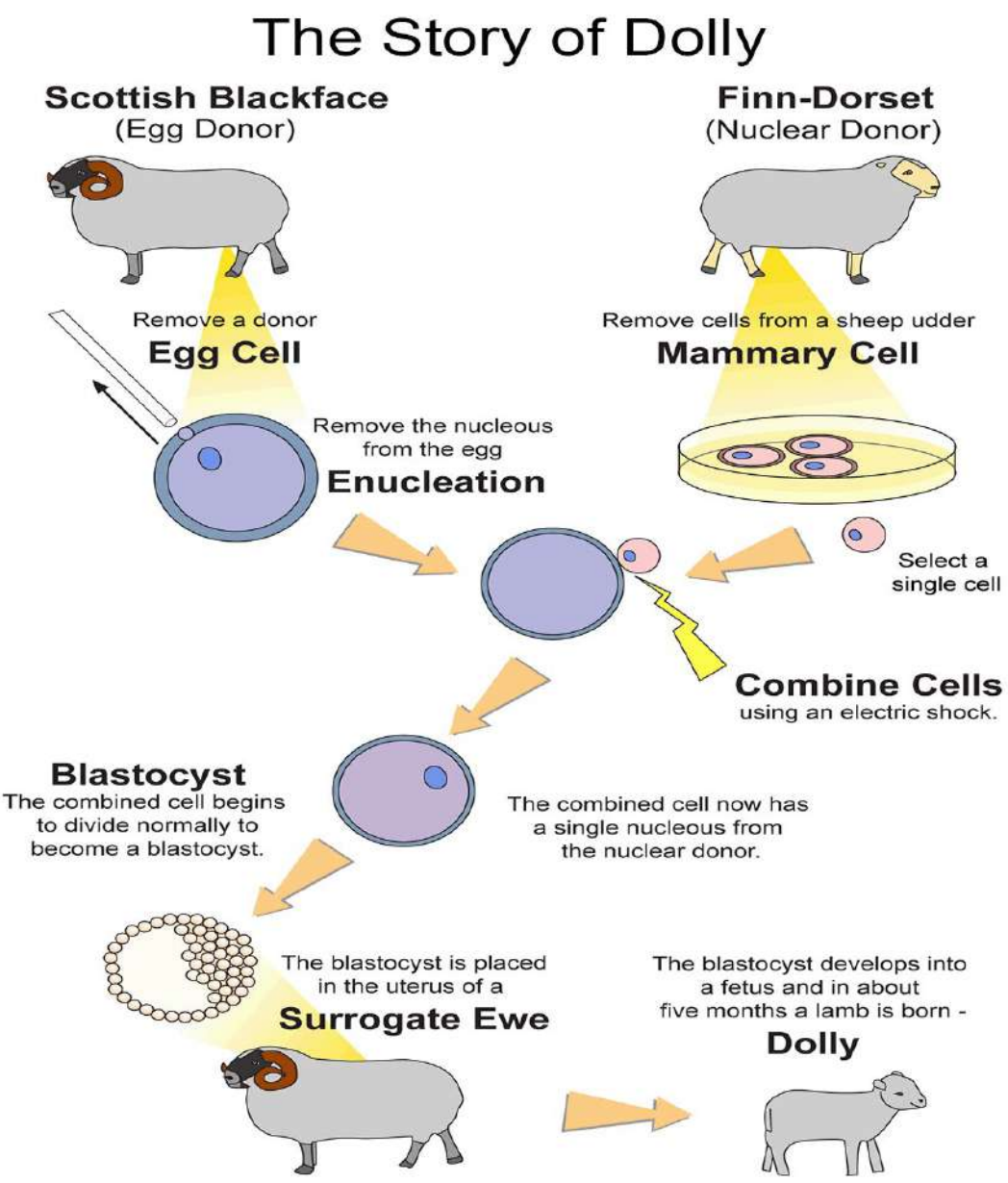

Figure.4. Production of Cloned Sheep Dolly

- **Note:** Scientists at India's National Dairy Research Institute produced the first cloned buffalo in 2009; however, the buffalo died a few days later.

3.6. Risks of Cloning

High failure rate due to one of the following reasons

- The enucleated egg and the transferred nucleus may not be compatible.
- Implantation of the embryo into the surrogate mother might fail.
- The pregnancy itself might fail.
- Problems during later development.

Large Offspring Syndrome

- Cloned animals that do survive tend to be **much bigger at birth than their natural counterparts.** This condition is called "Large Offspring Syndrome" (LOS).

- Clones with LOS have abnormally large organs. This leads to breathing, blood flow and other problems.

Telomere differences

- Cloned animals show differences in telomere length compared to naturally born animals. Therefore, they **tend to age faster.**

3.7. Ethical Issues Related to Cloning

- Reproductive cloning would present the potential of creating a human that is genetically identical to another person who has previously existed or who still exists.
    - This may conflict with long-standing religious and societal values about human dignity, possibly infringing upon principles of individual freedom, identity and autonomy.
- Therapeutic cloning, while offering the potential for treating humans suffering from disease or injury, would require the destruction of human embryos in the test tube.
    - Opponents argue that using this technique to collect embryonic stem cells is wrong, regardless of whether such cells are used to benefit sick or injured people.

India's First Cloned Desi Gir Female Calf

- Recently (March 2023), the National Dairy Research Institute (NDRI) in Karnal, Haryana, has produced India's first cloned female calf of the desi breed Gir, named 'Ganga.'
- The NDRI had initiated a project to clone indigenous cow breeds such as Gir and Sahiwal to increase milk production.
- Three animals were used for producing this calf: Oocyte was taken from the Sahiwal breed, a somatic cell from the Gir breed, and a surrogate animal was a crossbreed.

4. Therapeutic Cloning

4.1. What is Therapeutic Cloning?

- Therapeutic cloning is a technique **to produce clonal stem cells.**
- In this method, the transfer of nuclear material isolated from a somatic cell is carried out into an enucleated oocyte. This produces a clonal cell from where embryonic cell lines can be produced with the same genome as the nuclear donor.
- These embryonic cells are harvested and used for stem cell based therapies.
- While the goal of reproductive cloning is the creation of a person, the purpose of therapeutic cloning is to generate patient-specific cell lines isolated from an embryo not intended for transfer in utero.
- Therapeutic cloning offers great promises for regenerative and reproductive medicine, and in gene therapy, as a vector for gene-delivery.

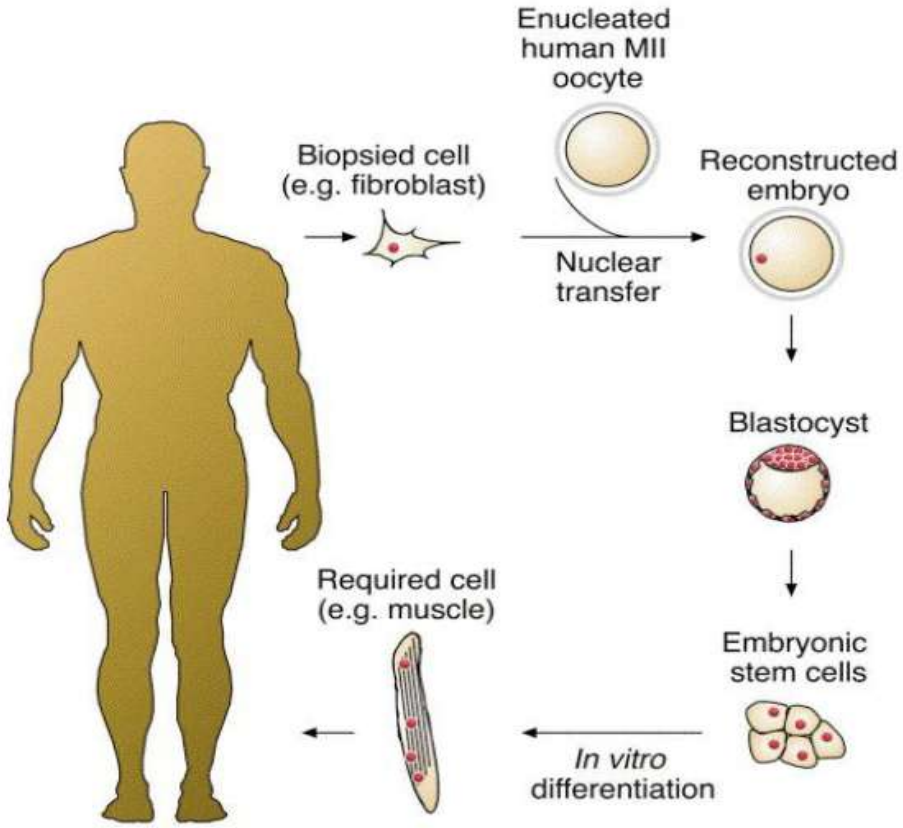

Figure.5. Therapeutic Cloning

4.2. Procedure of Therapeutic Cloning

- Nucleus is extracted from a sick person.
- The Nucleus is then inserted into an enucleated donor egg.
- The egg then divides like a typical fertilized egg and forms an embryo.
- Stem cells are removed from the embryo.
- Any kind of tissue or organ can be grown from these stem cells to treat various ailments and diseases.

4.3. Benefits

- A major benefit of therapeutic cloning is that the cells removed are pluripotent. Pluripotent cells can give rise to all cells in the body.
    - This means that pluripotent cells can potentially **treat diseases in any body organ** or tissue by replacing damaged and dysfunctional cells.
- Another advantage is that the **risk of immunological rejection is alleviated** because the patient's own genetic material is used.
- In addition, the procedure would allow scientists to create stem cell therapies that are patient specific and perfectly matched for the patient's medical condition.
- Some of the diseases that could benefit from stem cell transplants are:
    - **Parkinson's disease** - replacing destroyed brain cells with healthy ones.
    - **Type I diabetes** - providing viable functioning stem cells for the pancreas.
    - **Retinal diseases** - transplanting stem cells to replace those in the retina that have been damaged by disease.

4.4. Potential Drawbacks

- Some experts are concerned about the striking similarities between stem cells and cancer cells.
- Both cell types have the ability to proliferate indefinitely and some studies show that after 60 cycles of cell division, stem cells can accumulate mutations that could lead to cancer.
- Therefore, the relationship between stem cells and cancer cells needs to be more clearly understood if stem cells are to be used to treat human disease.

5. Stem Cells

5.1. What is a Stem Cell?

- A stem cell is a cell that is **capable of extensive proliferation,** creating more stem cells (self-renewal) as well as more differentiated cellular progeny.
- They are, in effect, a population of embryonic cells, continuously producing cells that can undergo further development within an adult organism.
- Development of the cells derived from the stem cells gives rise to the differentiated cell types.
- The stem cells possess two fundamental characteristics:
    - **Self-renewal** - the ability to go through numerous cycles of cell division while maintaining the undifferentiated state.
    - **Potency** - the capacity to differentiate into specialized cell types.

5.2. Main Potency Types of Stem Cells

The stem cell types are known on the basis of their potency. Potency means the **differentiation potential** (the potential to differentiate into different cell types) of the stem cell. The following major types of stem cells are described:

- **Totipotent stem cells** can differentiate into embryonic and extraembryonic cell types.
    - Such cells can construct a complete, viable, organism. Such cells are mostly zygotic, produced from the fusion of an egg and sperm cell. Cells produced by the first few divisions of the fertilized egg are also totipotent.
- **Pluripotent stem cells** are the descendants of totipotent cells and can differentiate into several unrelated types of cells.
- **Multipotent stem cells** can differentiate into a number of cells, but only those of a closely related family of cells.
- **Oligopotent stem cells** can differentiate into only a few cells, such as lymphoid or myeloid stem cells.
- **Unipotent cells can** produce only one cell type, their own, but have the property of self-renewal which distinguishes them from non-stem cells (e.g. muscle stem cells).

5.3. Occurrence of the Stem Cells

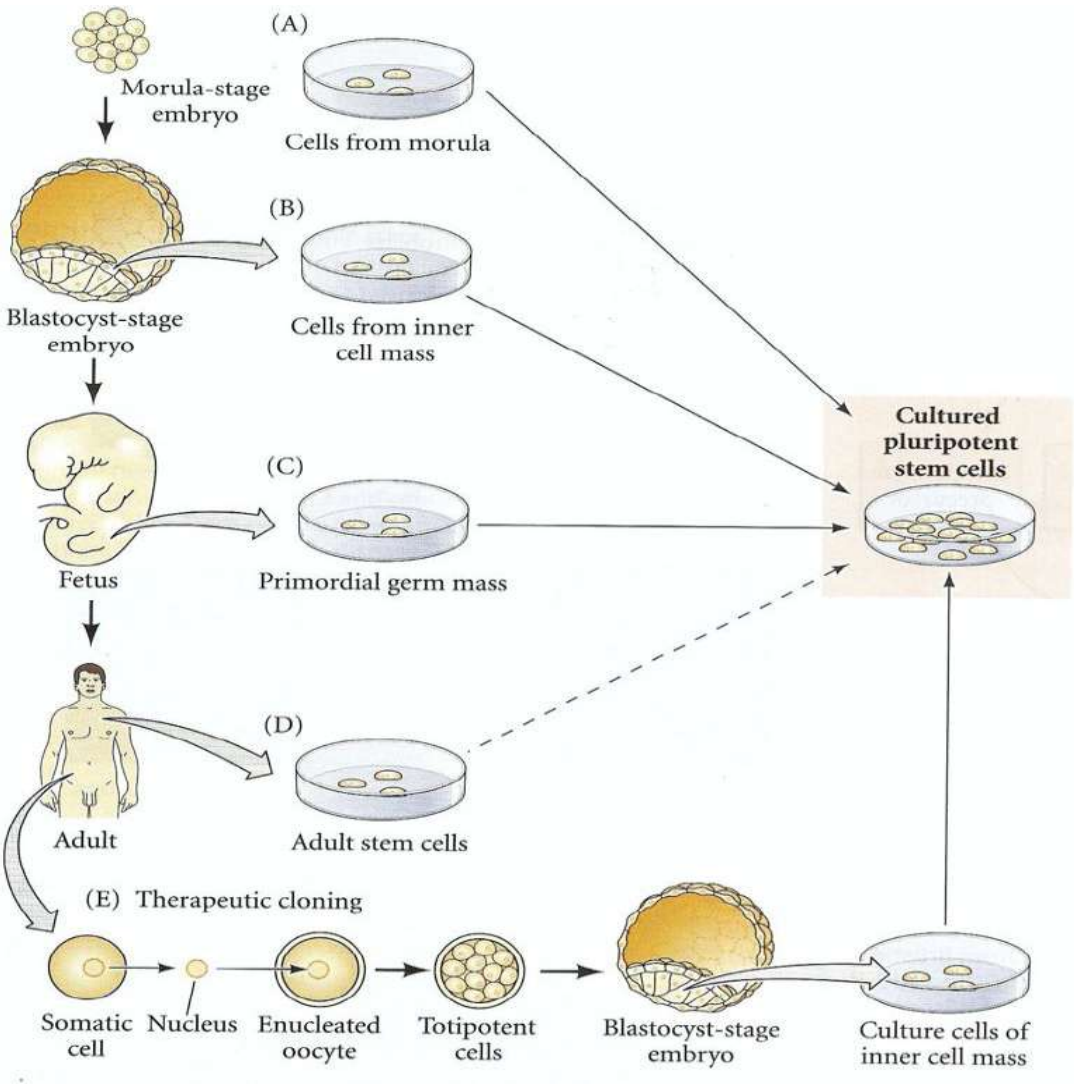

Figure.6. Sources of Stem Cells

Stem cells are found in the embryo as well as in the adult body. The important sources of stem cells are as follows:

- **Embryo:** Embryonic stem cells are derived **from the inner cell mass of a blastocyst.** They are pluripotent.
- **Foetus:** Foetal stem cells are mainly derived **from germ cells derived** from spontaneously aborted foetuses. Pluripotent stem cells are also found in the foetal brain.
- **Adult Body:** Stem cells can also be **found in small numbers** in various tissues in the adult body including brain and muscle tissues.
- **Other Sources:** Stem cells can also be obtained from other sources, for example, the **umbilical cord of a newborn baby** is a source of blood stem cells.

5.4. Induced Pluripotent Stem Cells (iPS cells)

- Induced pluripotent stem (iPS) cells are a new type of pluripotent cells that are **created by inducing the specialized cells** to express genes that are normally present in embryonic stem cells and that control cell functions.

- Embryonic stem cells and iPS cells share many characteristics, including the ability to become the cells of all organs and tissues, but they are not identical.
- iPS cells are a powerful method for creating **patient- and disease-specific cell lines** for research. These are not adult stem cells, but rather **reprogrammed cells with pluripotent capabilities.**
- iPS cells are useful tools for drug development and modeling of diseases, and scientists hope to use them in transplantation medicine.
- The Nobel Prize in Physiology or Medicine 2012 was awarded jointly to John B. Gurdon and Shinya Yamanaka for the discovery that **mature cells can be reprogrammed to become pluripotent stem cells.**

5.5. Application of Stem Cells

Treatment of Brain Diseases

- Stem cells can treat diseases such as Parkinson's disease and Alzheimer's. These can help to replenish the damaged brain cells.

Increase Understanding of How Diseases Occur

- By watching stem cells mature into cells in bones, heart muscle, nerves, and other organs and tissue, researchers may better understand how diseases and conditions develop.

Test New Drugs for Safety and Effectiveness

- Before using investigational drugs in people, researchers can use some types of stem cells to test the drugs for safety and quality.
- This type of testing will most likely first have a direct impact on drug development for cardiac toxicity testing.

Tissue Regeneration

- The stem cells can be used to grow a specific type of tissue or organ. This can be helpful in kidney and liver transplants.
- The doctors have already used the stem cells from beneath the epidermis to develop skin tissue that can repair severe burns or other injuries by tissue grafting.

Blood Disease Treatment

- The adult hematopoietic stem cells are used to treat cancers, sickle cell anemia, and other immunodeficiency diseases.
- These stem cells can be used to produce red blood cells and white blood cells in the body.

5.6. National Guidelines for Stem Cell Research

In 2017, India drafted the National Guidelines for Stem Cell Research. It was a collaborative effort of the Indian Council of Medical Research and Department of Biotechnology. The guideline focuses on:

- Monitoring mechanism and regulatory pathway for basic, clinical research and product development based on categories of research and level of manipulation.
- Procurement of gametes, embryos and somatic cells for derivation and propagation of any stem cell lines, their banking and distribution.
- Other important areas like international collaboration, exchange of cell/lines and education for stakeholders and advertisement.