EMBRYO CLONING

The technique to create **cloned embryos** is similar to that used to create Dolly the sheep, the world's first mammal **cloned** from a single adult cell. Scientists extract DNA from the cell of an adult patient and insert it into a hollowed out donor egg.

Somatic Cell Nuclear Transfer

Somatic cell nuclear transfer (SCNT), also called nuclear transfer, uses a different approach than artificial embryo twinning, but it produces the same result: an exact genetic copy, or clone, of an individual. This was the method used to create Dolly the Sheep.

What does SCNT mean? Let's take it apart:

Somatic cell: A somatic cell is any cell in the body other than sperm and egg, the two types of reproductive cells. Reproductive cells are also called germ cells. In mammals, every somatic cell has two complete sets of chromosomes, whereas the germ cells have only one complete set.

Nuclear: The nucleus is a compartment that holds the cell's DNA. The DNA is divided into packages called chromosomes, and it contains all the information needed to form an organism. It's small differences in our DNA that make each of us unique.

Transfer: Moving an object from one place to another. To make Dolly, researchers isolated a **somatic cell** from an adult female sheep. Next they removed the nucleus and all of its DNA from an egg cell. Then they **transferred**the **nucleus** from the somatic cell to the egg cell. After a couple of chemical tweaks, the egg cell, with its new nucleus, was behaving just like a freshly fertilized egg. It developed into an embryo, which was implanted into a surrogate mother and carried to term. (The transfer step is most often done using an electrical current to fuse the membranes of the egg and the somatic cell.)

The lamb, Dolly, was an exact genetic replica of the adult female sheep that donated the somatic cell. She was the first-ever mammal to be cloned from an adult somatic cell.

Watch these videos of enucleation and nuclear transfer.

Gene Cloning

Gene cloning, also known as Molecular cloning, refers to the process of making multiple molecules. Cloning is commonly used to amplify DNA fragments containing whole genes, but it can also be used to amplify any DNA sequence such as promoters, non-coding sequences and randomly fragmented DNA. It is used in a wide array of biological experiments and practical applications ranging from genetic fingerprinting to large scale protein production. To amplify any DNA sequence in a living organism, that sequence must be linked to an origin of replication, which is a sequence of DNA capable of directing the propagation of itself and any linked sequence. However, a number of other features are needed and a variety of specialized cloning vectors (small piece of DNA into which a foreign DNA fragment can be inserted) exist that allow protein expression, tagging, single stranded RNA and DNA production and a host of other manipulations.

Cloning of any DNA fragment essentially involves four steps

1. fragmentation - breaking apart a strand of DNA

2. ligation - gluing together pieces of DNA in a desired sequence

3. transfection - inserting the newly formed pieces of DNA into cells

4. screening/selection - selecting out the cells that were successfully transfected with the new DNA

Although these steps are invariable among cloning procedures a number of alternative routes can be selected, these are summarized as a 'cloning strategy'.

Therapeutic Cloning

Somatic-cell nuclear transfer, known as SCNT, can also be used to create embryos for research or therapeutic purposes. The most likely purpose for this is to produce embryos for use in stem cell research. This process is also called "research cloning" or "therapeutic cloning." The goal is not to create cloned human beings (called "reproductive cloning"), but rather to harvest stem cells that can be used to study human development and to potentially treat disease. While a clonal human blastocyst has been created, stem cell lines are yet to be isolated from a clonal source.

Therapeutic cloning is achieved by creating embryonic stem cells in the hopes of treating diseases such as diabetes and Alzheimer’s. The process begins by taking out the nucleus (containing the DNA) from an egg cell and putting in it a nucleus from the adult cell to be

cloned. In the case of someone with Alzheimer's disease, the nucleus from a skin cell of that patient is placed into an empty egg. The reprogrammed cell begins to develop into an embryo because the egg reacts with the transferred nucleus. The embryo will become genetically identical to the patient. The embryo will then form a blastocyst which has the potential to form/become any cell in the body.

In SCNT, not all of the donor cell's genetic information is transferred, as the donor cell's mitochondria that contain their own mitochondrial DNA are left behind. The resulting hybrid cells retain those mitochondrial structures which originally belonged to the egg. As a consequence, clones such as Dolly that are born from SCNT are not perfect copies of the donor of the nucleus.

Reproductive Cloning

Reproductive cloning (also known as Organism cloning) refers to the procedure of creating a new multicellular organism, genetically identical to another. In essence this form of cloning is an asexual method of reproduction, where fertilization or inter-gamete contact does not take place. Asexual reproduction is a naturally occurring phenomenon in many species, including most plants (vegetative reproduction) and some insects. Scientists have made some major achievements with cloning, including the asexual reproduction of sheep and cows.

Reproductive cloning generally uses "somatic cell nuclear transfer" (SCNT) to create animals that are genetically identical. This process entails the transfer of a nucleus from a donor adult cell (somatic cell) to an egg that has no nucleus. If the egg begins to divide normally it is transferred into the uterus of the surrogate mother. Such clones are not strictly identical since the somatic cells may contain mutations in their nuclear DNA.

Artificial embryo splitting or embryo twinning may also be used as a method of cloning, where an embryo is split in the maturation before embryo transfer. It is optimally performed at the 6- to 8-cell stage, where it can be used as an expansion of IVF to increase the number of available embryos. If both embryos are successful, it gives rise to monozygotic (identical) twins.

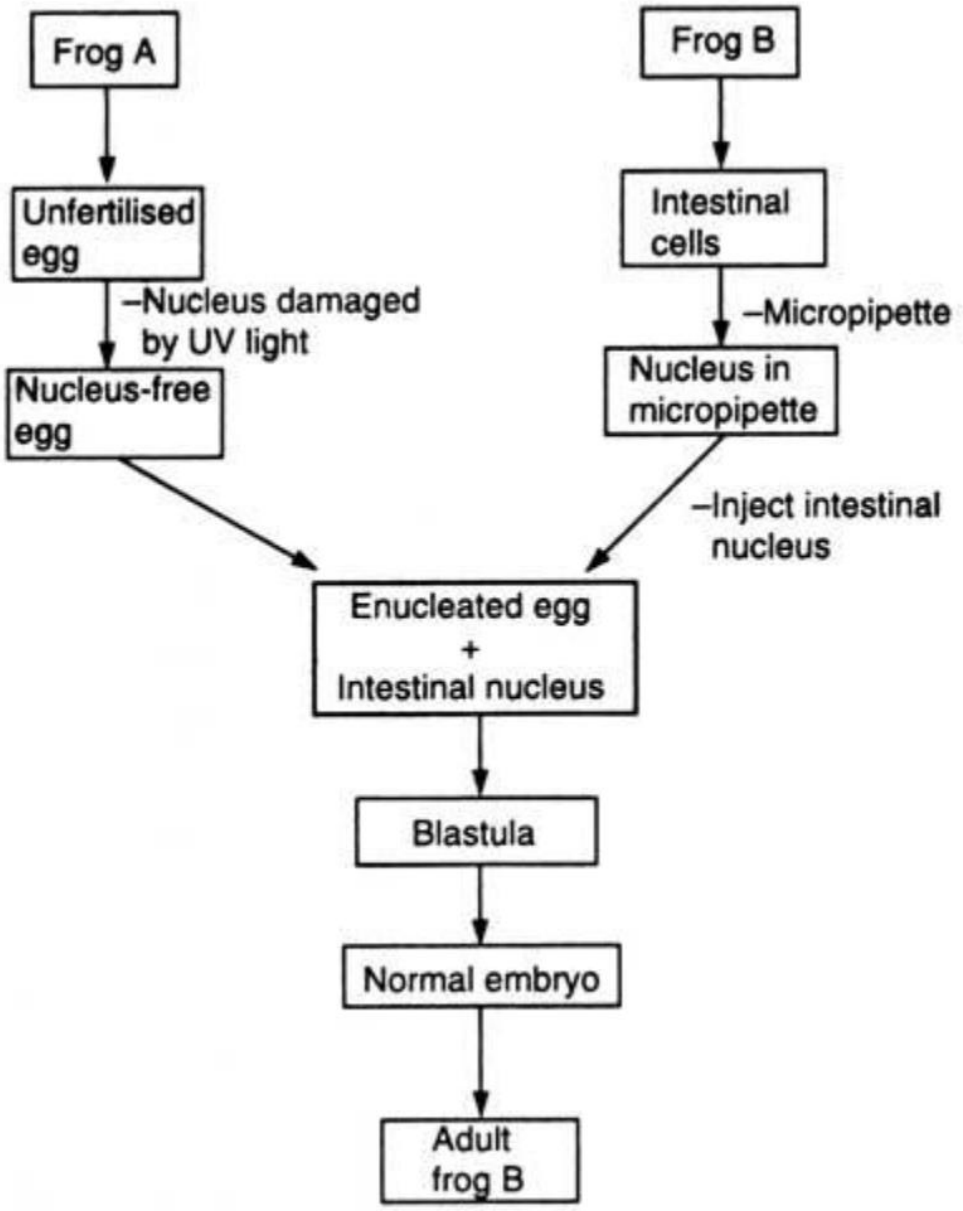
NUCLEAR TRANSFER:

A clone is a population of cells or organisms derived asexually from a single ancestor. They are genetically identical to each other to their common ancestor. Cloning means the production of exact genetic replica copies of an individual. They can not be considered as

an offspring but simply the copy of a given individual. Much work has been done on cloning in plants and microorganisms. However, the techniques used in plants can not be applied for animals. Moreover, many animals from a single genetically superior embryo can be produced. Still there is no method of finding out which embryos are capable of cloning. It is useless to clone an embryo if it is not superior.

Nuclear transplantation (also nuclear transfer) involves removal of a single blastomere from a cleavage stage embryo with a fine micropipette of glass, and placing it under the outer membrane of an unfertilized mature enucleated oocyte (whose haploid nucleus has been removed by using micropipette or destroyed by UV light). For the first time in 1955, Robert Briggs and Tom King at Cancer Research Institute, Philadelphia (USA) carried out nuclear transplantation experiment on embryonic cells of frog. They transferred nucleus of undifferentiated blastula (a stage soon after fertilization of egg) into an enucleated egg cell. They noticed the normal development of the embryo. When they performed serial transplantation of differentiated nucleus from late gastrula (a stage after blastula) into a nucleus-free unfertilized egg, abnormal embryos were formed. This shows that cell nucleus is differentiated with embryo development. In 1960s, J.B. Gurdon at Oxford University, U.K. transferred differentiated intestinal nucleus of a frog into nucleus-free unfertilized egg of different amphibian species*(Xenopuslaevis).* The embryo developed into tadpole and matured into frog (Gurdon, 1962). This new enucleated cell developed into normal embryo. Any damage to the donor nucleus during transplantation leads to abnormal development .

JOHN GORDAN"S EXPERIMENT:



- **DOLLY** - The first mammalian clone. 'Dolly', the worlds' first mammalian clone has been created from a fully differentiated non-reproductive cell of an adult sheep. It was born in February, 1996. The name Dolly has been given after an American country singer, Dolly Parton. In 1995, Ian Wilmut and his team of researchers at Roslin Institute, Edinburgh, Scotland, took udder (a fully differentiated tissue) from six year old sheep, Fin Dorset Ewe, and placed it in special solution that controlled cell cycle of cell division. The cell was deprived off certain nutrient. At the same time an unfertilized egg

was obtained from another adult sheep .Its nucleus was carefully removed leaving the intact cytoplasm in egg. The nucleus of udder cell was taken out and transferred into nucleus-free egg. This was facilitated by applying mild electric sock. The newly transplanted nucleus soon became functional according to the new cytoplasm in which it had been artificially transferred. This viable combination underwent cleavage like normal zygote. This so called embryo was then transplanted into the uterus of a third adult sheep (surrogate mother/foster mother) for its further development.

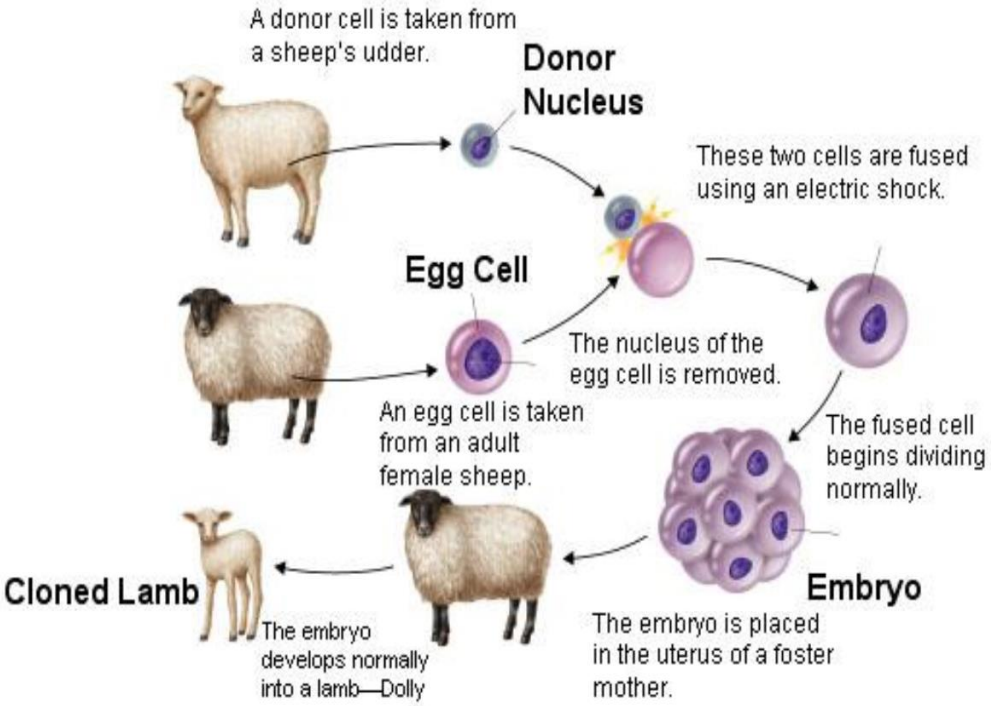
Finally, a normal healthy little lamb, Dolly was born in February, 1996 which was genetically similar to the *clone mother* from which nuclear DNA was taken out. It does not have any similarity with that sheep from which egg was taken out or surrogate mother because they did not contribute any genetic character (Wimut*et al.,* 1997). Thus, Dolly has only a single parent because she has born asexually, a characteristic feature found in lower forms of animal life, not in mammals.

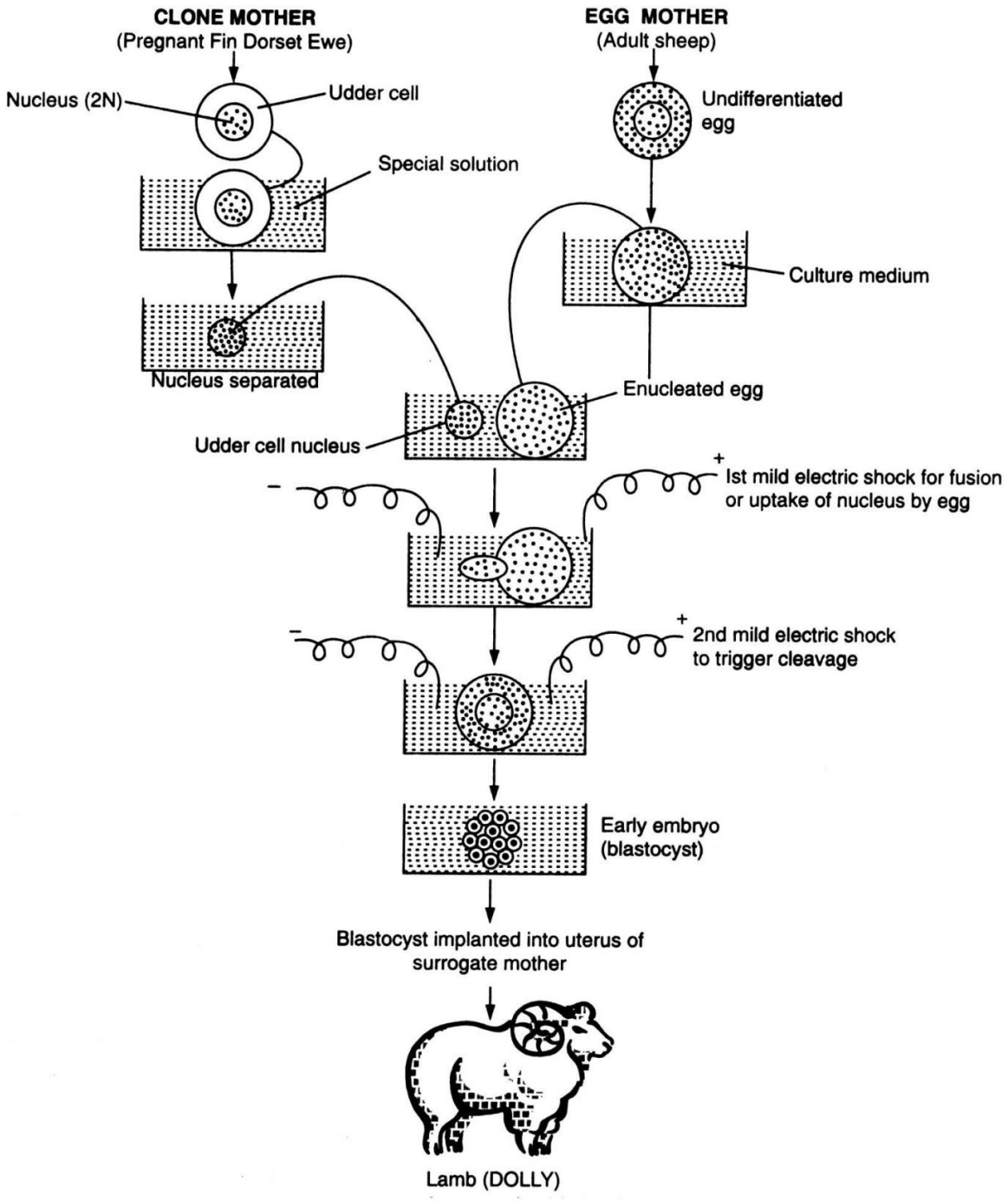
Although behind this great success the rate of success is very slow, yet it has given some hope to embryo-biotechnologists to bring about refinement. Out of 277 nuclei transferred singly to enucleated egg, only 29 eggs grew into embryos. Out of these, only 13 embryo could be successfully transplanted into surrogate mothers. Of these only one ewe was successful in giving birth to an offspring, Dolly (Wilmut *et al,* 1996).

The significant considerations that can be derived from this experiment are that:*(i)* the genes of differentiated cells have inherent totipotency, *(ii)* the interplay between the regulatory system of the genome in nucleus and the cytoplasmic factors of egg may make a cell totipotent, *(iii)* possibly the cytoplasm of enucleated egg makes the transplanted nucleus totipotent like

that of normal fertilized egg nucleus, *(iv)* the maternally derived information in egg cytoplasm has an important role in cleavage which usually occurs after fertilization.

Hence, it is the egg cytoplasm but not the nucleus that regulates cleavage. Because the udder cell nucleus has limited potential for mitosis; it is the egg cytoplasm that interacted intracellularly with udder cell nucleus and stimulated to undergo repeated mitosis, *(v)* carbon copy of the adult sheep could be produced without involving sperms from male partner, and *(vi)*the cloned animal produced via nuclear transplantation technique will be capable of restoring fertility as in 1998 Dolly gave birth to a little lamb named *Bonny.*





Potential Applications of Cloning

- SCNT is seen as a good method for producing agriculture animals for food consumption. It has successfully cloned sheep, cattle, goats, and pigs.
- Cloning is seen as a solution to the endangered species that are on the verge of going extinct.
- Cloning technologies are used widely in plant conservation.

- Reproductive cloning may enable researchers to make copies of animals with the potential benefits for the fields of medicine and agriculture for testing new drugs and treatment strategies.
- Embryonic stem cells which have the unique ability to generate virtually all types of cells in an organism can be used to grow tissues in the laboratory through therapeutic cloning that can be used to grow healthy tissue to replace injured or diseased tissues.
- It may be possible to learn more about the molecular causes of disease by studying embryonic stem cell lines from cloned embryos derived from the cells of animals or humans with different diseases.

Potential Drawbacks of Cloning

- Many conservation biologists and environmentalists vehemently oppose cloning endangered species—mainly because they think it may deter donations to help preserve natural habitat and wild animal populations. The "rule-of-thumb" in animal conservation is that, if it is still feasible to conserve habitat and viable wild populations, breeding in captivity should not be undertaken in isolation.
- In a 2006 review, David Ehrenfeld concluded that cloning in animal conservation is an experimental technology that, at its state in 2006, could not be expected to work except by pure chance and utterly failed a cost-benefit analysis.

- The human consumption of meat and other products from cloned animals was approved by the FDA on December 28, 2006, with no special labeling required. Such practice has met strong resistance in other regions, such as Europe, particularly over the labeling issue.
- Cloning from a single specimen could not create a viable breeding population in sexually reproducing animals.
- If males and females were to be cloned, the question would remain open whether they would be viable at all in the absence of parents that could teach or show them their natural behavior.

- As the procedure currently cannot be automated, and has to be performed manually under a microscope, SCNT is very resource intensive.
- The biochemistry involved in reprogramming the differentiated somatic cell nucleus and activating the recipient egg is far from being well-understood.
- The mitochondria in the cytoplasm also contains DNA and during SCNT this mitochondrial DNA is wholly from the cytoplasmic donor's egg, thus the mitochondrial genome is not the same as that of the nucleus donor cell from which it was produced. This may have important implications for cross-species nuclear transfer in which nuclear-mitochondrial incompatibilities may lead to death.
- Many religious organizations oppose all forms of cloning, on the grounds that life begins at conception.
- Researchers have found several abnormalities in cloned organisms. The cloned organism may be born normal and resemble its non-cloned counterpart, but majority of the time will express changes in its genome later on in life.

QUADRIPARENTAL-HYBRIDS:

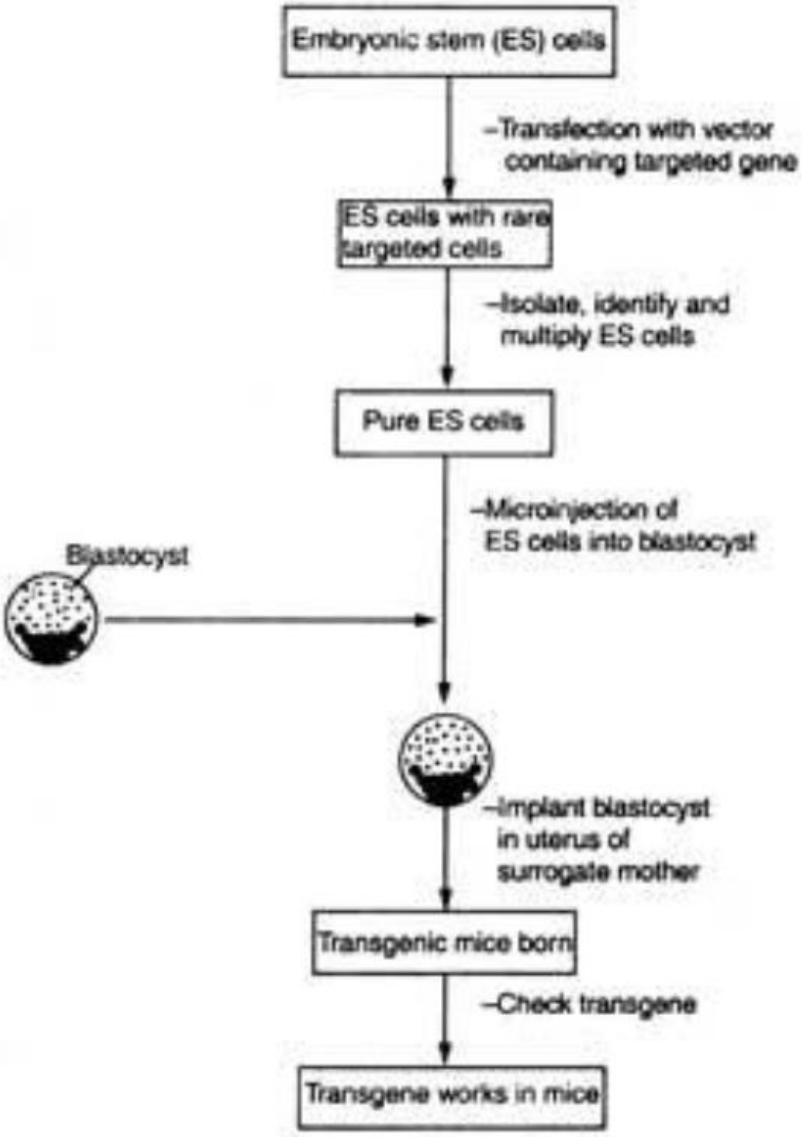
During 1960s, Beatrice Mintz at Cancer Research Institute, Philadelphia (USA) demonstrated the interesting experimentation. She carried out fusion of embryos of two

different species of mouse. This resulted in the formation of a single embryo which finally developed into a normal healthy animal having four parents. Embryo A was derived from the cross of male x female of one species, and embryo B derived after cross of male x female of the other species. The embryo A and B were united together and produced a single mass. In this experiment Mintz removed zonapellucida membrane of two early embryos and placed them in a suitable culture medium. The embryonic cells of the two embryos of blastula stage united randomly into a single mass of double sized embryo (blastocyst). A fresh membrane developed around the embryo. Then the embryo was transferred into the uterus of a foster mother. The foster mother was mated with a sterile male to bring her into proper stage for implantation. The first offspring with four parents was born in 1965. Similarly, exciting experiments have been done on another mammal. Following the same technique a hybrid of goat and sheep named geep was produced (Joshi, 1998).

- At present two types of techniques for embryo cloning viz., nuclear transplantation (transfer) and embryonic stem cells, are being developed.

Embryonic stem cells (ES cells) are pluripotent stem cells derived from the inner cell mass of a blastocyst, an early-stage preimplantation embryo. Human embryos reach the blastocyst stage 4–5 days post fertilization, at which time they consist of 50–150 cells. Isolating the embryoblast or inner cell mass (ICM) results in destruction of the blastocyst, which raises ethical issues, including whether or not embryos at the pre-implantation stage should be considered to have the same moral or legal status as more developed human beings.

- Human ES cells measure approximately 14 μm while mouse ES cells are closer to 8 μm.



Embryonic Stem (ES) cells

- Cloning of mice could not be done as in sheep via nuclear transplantation. This was due to acceleration of developmental programmes of embryo. However, it is evident that before first embryonic division the cell has started its process of differentiation. Therefore, for cloning of mice an alternative approach has been made *i.e.* the use of ES cells. A blastocyst of mouse is place in culture condition. The inner cells that form future foetus continue to divide and remain in undifferentiated totipotent state as ES cells. There is a peptide growth factor known as leukaemia inhibitory factor (LIF) which establishes and maintains ES cell lines. The ES cell lines will be very useful in the area of production of transgenic animals *(see* preceding sections). However, the ES cells are used in two different ways: a small number of ES cells can be injected into blastocoel space of a blastocyst (Fig. 7.5). The ES cells get mixed with inner mass of cells of blastocyst to produce a chimera mouse which is a mixture of two cell genotype having the patches of different colored fur. Crossing of male and female chimera will allow selection of homozygous mice derived from ES cells (Read and Smith, 1996).

Pluripotent

Embryonic stem cells of the inner cell mass are pluripotent, that is, they are able to differentiate to generate primitive ectoderm, which ultimately differentiates during gastrulation into all derivatives of the three primary germ layers: ectoderm, endoderm, and mesoderm. These include each of the more than 220 cell types in the adult body. Pluripotency distinguishes embryonic stem cells from adult stem cells found in adults; while embryonic stem cells can generate all cell types in the body, adult stem cells are multipotent and can produce only a limited number of cell types. If the pluripotent differentiation potential of embryonic stem cells could be harnessed in vitro, it might be a means of deriving cell or tissue types virtually to order. This would provide a radical new treatment approach to a wide variety of conditions where age, disease, or trauma has led to tissue damage or dysfunction.

References and further reading

Tissue Engineering--Current Challenges and Expanding Opportunities by Linda G. Griffith and Gail Naughton (2002)

Textbook of Animal Biotechnology, by B Singh (Author), S K Gautam (Author), M S Chauhan (Author)

Animal Biotechnology (3rd Edition), by M.M. Ranga (Author)