UNIVERSITY OF CALCUTTA

Professor Sir Ian Wilmut and Dolly the Sheep.

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❖ INDEX

❖ INTRODUCTION

The word clone is used in many different contexts in biological research but in its most simple and strict sense, it refers to a precise genetic copy of a molecule, cell, plant, animal, or human being. Clones contain identical sets of genetic material in the nucleus of every cell in their bodies. Thus, cells from two clones have the same DNA and the same genes in their nuclei. True clones have identical DNA in both the nuclei and mitochondria, although the term 'clones' is also used to refer to individuals that have identical nuclear DNA but different mitochondrial DNA.

There are two types of cloning-

I. **Reproductive cloning** – It is defined as the deliberate production of genetically identical individuals. Each newly produced individual is a clone of the original. Monozygotic (identical) twins are natural clones.

II. **Therapeutic cloning**- It involves creating a cloned embryo for the sole purpose of producing embryonic stem cells with the same DNA as the donor cell. These stem cells can be used in experiments aimed at understanding disease and developing new treatments for disease. To date, there is no evidence that human embryos have been produced for therapeutic cloning.

The richest source of embryonic stem cells is the blastocyst. Stem cells are harvested from cloned embryos at this stage of development, resulting in destruction of the embryo while it is still in the test tube.

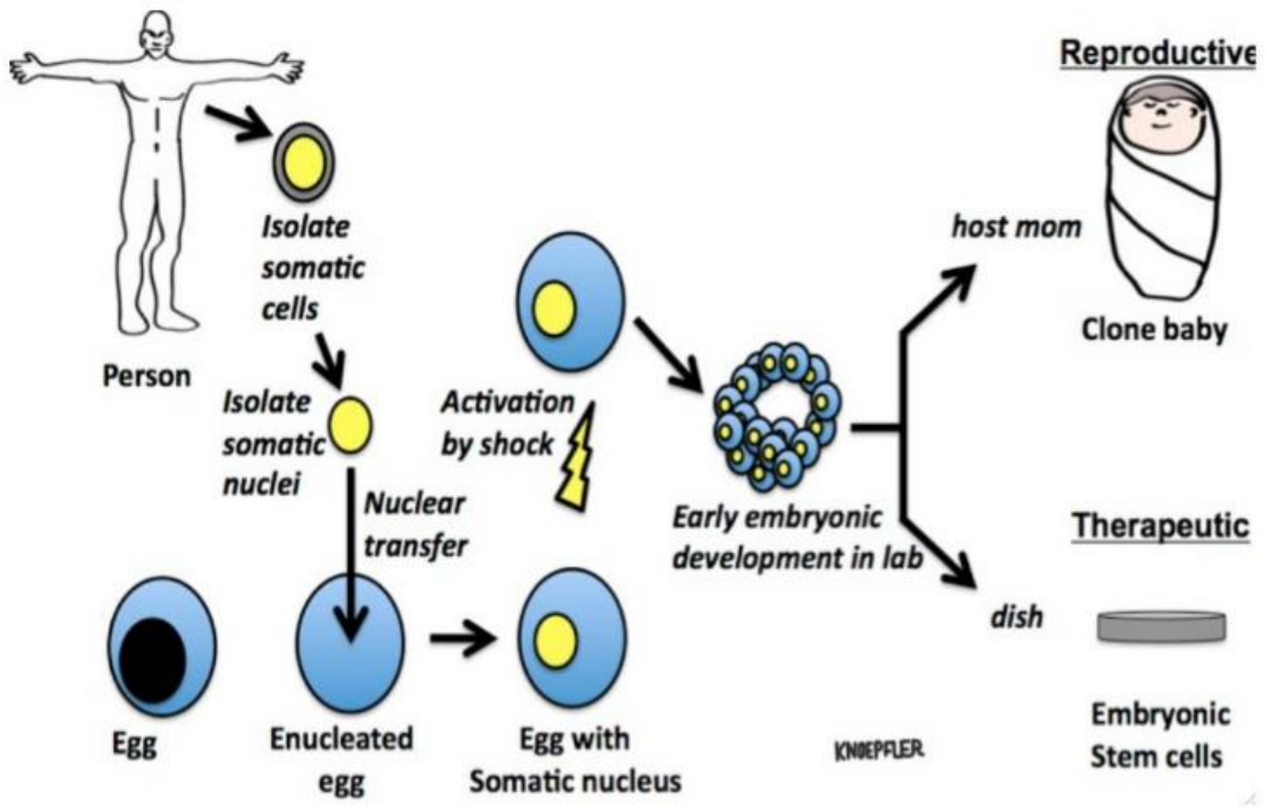

Fig 1- Types of cloning

❖ HISTORY OF 'DOLLY' , THE SHEEP

- On July 5, 1996, Dolly the sheep—the first mammal to have been successfully cloned from an adult cell—is born at the Roslin Institute in Scotland by a group of scientists led by Professor Sir Ian Wilmut.

- Dolly was cloned from a cell taken from the mammary gland of a six-year-old Finn Dorset sheep and an egg cell taken from a Scottish Blackface sheep. She was born to her Scottish Blackface surrogate mother on 5th July 1996. Dolly's white face was one of the first signs that she was a clone because if she was genetically related to her surrogate mother, she would have had a black face.

- Because Dolly's DNA came from a mammary gland cell, she was named after the country singer Dolly Parton. Originally code-named "6LL3,"

- Dolly was announced to the world on 22nd February 1997 to a frenzy of media attention. The Roslin team chose to make the announcement at this time to coincide with the publication of the scientific paper which describes the experiments that produced her. Dolly captured the public's imagination – no small feat for a sheep – and sparked a public debate about the possible benefits and dangers of cloning

Fig 2- Dolly at the National Museum of Scotland in Edinburgh

- The cells had been taken from the udder of a six-year-old ewe and cultured in a lab using microscopic needles, in a method first used in human fertility treatments in the 1970s. After

producing a number of normal eggs, scientists implanted them into surrogate ewes; 148 days later one of them gave birth to Dolly.

- Dolly's birth was announced publicly in February 1997 to a storm of controversy. On one hand, the cloning technology can lead to crucial advances in medicine, "therapeutic" cloning, to collect stem cells for use in the development of treatments for degenerative nerve diseases such as Alzheimer's and Parkinson's and as a possible way to preserve endangered species. On the other hand, the new cloning technology was seen as potentially unsafe and unethical, especially when it was applied to what many saw as the logical next step: human cloning.
- Over the course of her short life, Dolly was mated to a male sheep named David and eventually gave birth to four lambs. In January 2002 she was found to have arthritis in her hind legs, a diagnosis that raised questions about genetic abnormalities that may have been caused in the cloning process. After suffering from a progressive lung disease, Dolly was put down on February 14, 2003, at the age of six. Her early death raised more questions about the safety of cloning, both animal and human. As for Dolly, the historic sheep was stuffed and is now on display at the National Museum of Scotland in Edinburgh.

❖ METHOD OF CLONING DOLLY

I. The first successful cloning of a mammal was done at the Roslyn Institute in Scotland in 1997. The result was Dolly, the most famous sheep in the history of the world. What makes Dolly different from identical twins is that she was grown from a cell taken from an adult animal.

II. Dolly was developed through a technique known as Stem Cell Nuclear Transfer aka SCNT . SCNT cloning is the only technology available that enables generation of 99.8% genetically identical offspring from selected individuals of adult animals .

III. Dolly was cloned by transfer of a nucleus from a mammary (udder) cell of an adult sheep into an egg cell. This was the first demonstration of pluripotency (totipotency) of a nucleus of a differentiated adult cell.

➢ Steps of dolly production

1.The nucleus of an ovum is removed with a pipette.

2. Cells from the mammary epithelium of an adult are grown in culture.

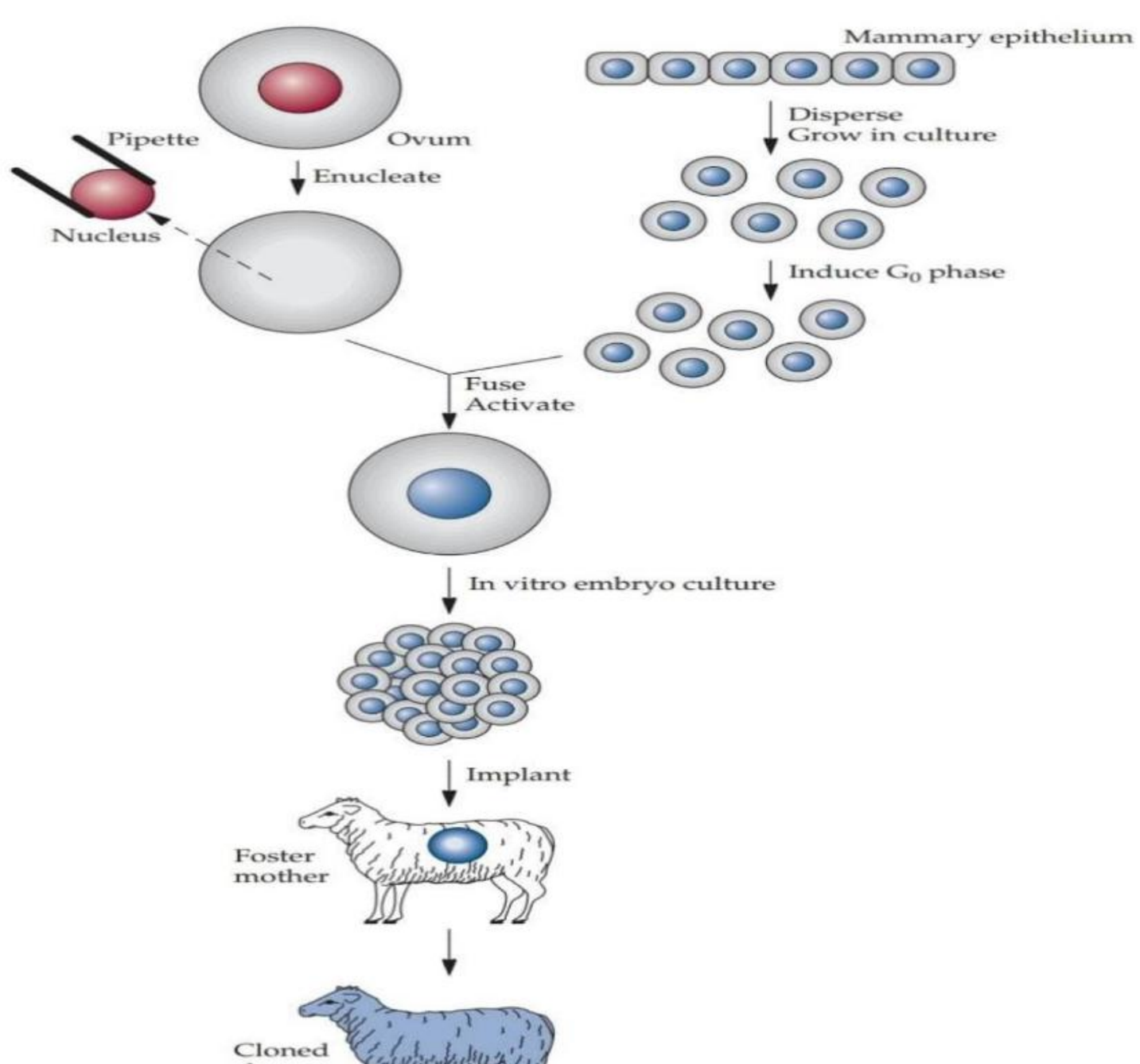

Fig 3- Process of cloning Dolly

3. The G0 (quiescent, nondividing) state is induced by inhibiting cell growth, by reducing the neutrient content of the cell culture media.

4. A G0 cell and an enucleated ovum are fused, and the renucleated ovum is grown in culture or in ligated oviducts until an early embryonic stage developed.

5. Then the developed embroy (in blastocyst stage) is implanted into a foster mother, where development proceeds to term.

In the experiment described by Wilmut et al. 277 enucleated ova were fused with G0 mammary cells, and 1 of 29 transferred early-stage embryos produced a live lamb

❖ ADVANTAGES OF NUCLEAR TRANSFER

- Cloning for Breeding Livestock

- In animal breeding, the rapid spread of certain traits within stocks of domestic animals is of obvious commercial importance and has very long historical standing.
- Artificial insemination and embryo transfer can increase the effective reproductive output of individual elite male and female animals and are widely used in the livestock industry.
- Nuclear transfer cloning, especially from somatic cell nuclei, could provide an additional means of expanding the number of chosen livestock. The ability to make identical copies of adult prize cows, sheep, and pigs is a feature unique to nuclear transfer technologies, and may well be used in livestock production, if the efficiencies of adult nuclear transfer can be improved.
- The net effect of multiplying chosen animals by cloning will be to reduce the overall genetic diversity in a given livestock line, likely with severe adverse long-term consequences.

- If this technique became widespread, efforts would have to be made to ensure a pool of genetically diverse animals for future livestock maintenance.

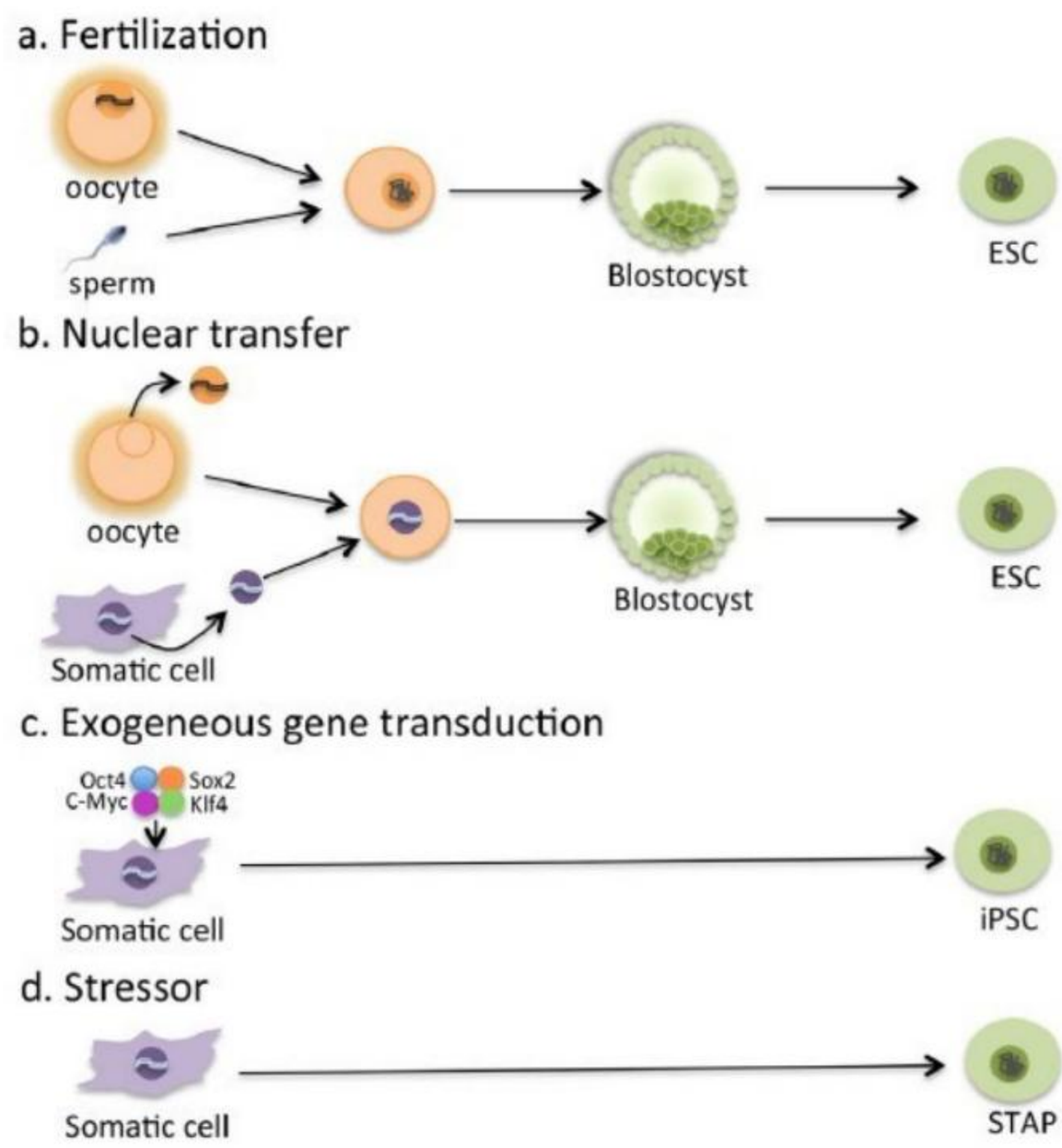

Fig 4 - Nuclear Transfer

➢ APPLICATIONS

I. MEDICAL APPLICATIONS

▪ Pharmaceutical products

1.One major application of animal transgenesis is the production of pharmaceutical products, also known as animal pharming.

2..Pharmaceutical proteins or other compounds can be produced in a variety of body fluids, including milk, urine, blood, saliva, chicken egg white and seminal fluid depending on the use of tissue specific promoters.

▪ Human therapeutic proteins

1. Human proteins are in great demand for the treatment of a variety of diseases. Whereas some can be purified from blood, this is expensive and runs the risk of contamination by AIDS or hepatitis C. Human proteins that have appropriate post-translational modifications can be produced in the milk of transgenic sheep, goats and cattle.
2. Output can be as high as 40 g per litre of milk and costs are relatively low.
3. PPL Therapeutics, one of the leaders in this field, recently announced that alpha-1-antitrypsin produced from a transgenic flock is now being used to treat cystic fibrosis patients in phase 2 clinical trials.
4. In the US, Genzyme Transgenics have focussed on goats and their lead product, tissue plasminogen activator, is also in clinical trials.
5. An immediate advantage of producing transgenic animals by nuclear transfer is that it uses less than half of the experimental animals than does pronuclear injection. Roslin Institute have already used this approach to produce Polly and Molly, two transgenic sheep that secrete the human blood clotting factor IX in their milk. At present such transgenic animals produce a human protein in addition to the normal complement of milk proteins.

Antithrombin III
α_1-Antitrypsin
Calcitonin
Erythropoietin
Factor IX
Factor VIII
Fibrinogen
Glucagon-like peptide
α-Glucosidase
Granulocyte colony-stimulating factor
Growth hormone
Hemoglobin
Serum albumin
Insulin
Insulin-like growth factor 1
Interleukin 2
α-Lactalbumin
Lactoferrin
Lysozyme
Monclonal antibodies
Nerve growth factor
Protein C
Superoxide dismutase
Tissue plasminogen activator

Fig 4 - some human proteins that have been expressed in human mammary glands

- **Protein based drug**

1. .Protein-based drugs differ from protein products synthesized in the blood in that they are produced in-vivo by other organs. This technology is even being applied to the development of complex proteins such as monoclonal antibodies as well as many other important human replacement proteins and protein drugs such as polyclonal antibodies and plasminogen activator .
2. Researchers recently created a line of transgenic swine that produce recombinant human erythropoietin a naturally occurring human hormone that boosts the body's production of red blood cells.

- **Xenotransplantation**

1.Xenotransplantation is the transplantation of organs or cells from one species to another.

2. Pig is considered the preferred candidate for xenotransplantation because of physiological compatibility and breeding characteristics. Large numbers of pathogen free pigs can be raised to provide organs for transplantation in to humans

3. Through the use of genetic engineering and cloning, scientists have created pigs which are deficient for αGalT (a sugar that causes tissue rejection during transplantation in humans)and do not cause further medical complications.

- **Increase animal disease resistance**

1.Genetic engineering of agricultural animals has the potential to improve disease resistance by introducing specific genes into livestock by identification of single genes in the major histocompatibility complex (MHC).

2.One specific example where transgenesis has been applied to disease resistance in livestock is the attempt to produce cattle resistant to mastitis. Transgenic dairy cows that secrete lysostaphin(an antimicrobial peptide that protects mammary glands against infection) into their milk have been produced to address the mastitis issue.

- **Cell therapy**

1. Intact cells are already used to treat patients suffering from a number of diseases, including leukemia and Parkinson's disease. In most cases these cells have to be obtained from close relatives to avoid problems of immune rejection.
2. The fact that Dolly was cloned from a cell taken from an adult ewe shows that even differentiated cells can be 'reprogrammed' into all the cell types that make up an intact animal.
3. The only way that we can perform this dedifferentiation step now is by 'incubating' cells in the cytoplasm of an unfertilised egg but when we know more about the mechanisms involved, then it may be possible that human cells can be reprogrammed without the use of a human egg. This would allow the patient's own cells to be used in cell therapies, thereby avoiding the time, expensive and uncertainty of tissue matching.

II. COMMERCIAL APPLICATIONS-

- **Enhance growth and meat trait-**

1.The possibility of introducing beneficial fats such as the omega-3 fatty lipoprotein receptor gene and hormones like leptin are potential targets that would decrease fat and cholesterol in animal products .

2.The use of genetic engineering to improve feed efficiency and/or appetite could profoundly impact livestock production and deliver significant benefits to producers, processors, and consumers. Increased uptake of nutrients in the digestive tract, by alteration of the enzyme profiles in the gut, could increase feed efficiency.

3.The ability to introduce enzymes such as Phytase or xylanase into the gut of species where they are not normally present, such as swine or poultry, is particularly attractive The use of phytase transgenic pigs in commercial pork production could result in significantly decreased environmental phosphorus pollution from livestock.

- **Improve wool production-**

The manipulation of the quality, length, fineness and crimp of the wool and hair fiber from sheep and goats has been examined using transgenic methods. Transgenic methods also allow improvements to fiber elasticity, surface and strength. Decreasing the surface interactions between fibers could decrease shrinkage of garments made from such fibres.

- **Improving milk quality-**

There are a number of potential opportunities for altering the nutritional content of milk. For example, cow's milk is ideal for calves but not for premature infants.

Gene targeting using nuclear transfer will allow milk to be produced in which one or more of the normal cow's proteins have been replaced by human proteins, thereby improving its nutritional quality for these special 'consumers'.

Other people have a immune response to specific proteins in milk or are intolerant of lactose and gene targeting would allow the creation of herds of cows that produced milk lacking the problem components.

Organism	Annual milk yield (liters)	Estimated recombinant protein per female (kg/year)
Rabbit	5	0.02
Pig	300	1.5
Sheep	500	2.5
Goat	900	4
Cow	10,000	60

- **Rapid multiplication of desired livestock-**

1. In addition to providing a route to gene targeting in livestock, nuclear transfer could be used to deliver what is the popular image of cloning: that is the production of, at least in principle, unlimited numbers of genetically identical animals.
2. Non-surgical means would be needed for embryo transfer and success rates would have to be dramatically improved. Studies suggest that it may be 10-20 years before this could be possible Nevertheless, the main advantage is the more rapid dissemination of genetic progress from elite herds to the commercial farmer.
3. At present this is achieved through artificial insemination - which supplies only half the genes- and by limited use of embryo transfer. This process is not that efficient and in dairy cattle, the performance of the average cow is probably some 10 years behind the best.
4. Farmers would choose cloned embryos for high merit beef bulls or dairy cows from catalogues that described the genetic merit for a series of economically important traits, including fertility, health and longevity. The cloned embryo would be delivered to the farm .
5. A major risk would be loss of genetic diversity but this could be avoided by systems that ensured that breeding companies produced a limited number of clones of each genotype and restricted the number of each of the clones that could be sold to any one producer.

III. CONSERVATION PURPOSES

- **Animal conservation-**

  Cloning can be used along with other forms of assisted reproduction to help preserve indigenous breeds of livestock, which have production traits and adaptability to local environments that should not be lost from the global gene pool. In some situations, it may be used to aid the conservation of some exotic species. At the very least, it is appropriate to consider the cryopreservation of somatic cells from these endangered animals as insurance against further losses of diversity or possible extinction of Wildlife .

- **Genetic conservation**

1. Although cloning is associated in most people's minds with a loss of genetic diversity, the techniques that were used to produce Dolly will also provide new approaches to genetic conservation.
2. With increasing commercial pressures, many indigenous breeds adapted to local conditions are under threat from imported breeds that are being reared in intensive farming systems.
3. The local breeds may contain valuable genes that confer heat tolerance or disease resistance and there is an urgent need to prevent their extinction.
4. Current methods of conservation involve storage of frozen semen or embryos but are time consuming and costly. As a consequence, the future of only a small proportion of endangered breeds is being addressed. The new techniques developed at Roslin may provide much simpler and more effective means of conserving breeds.

IV. **RESARCH PURPOSES**-

- **Research model** –
  Sets of cloned animals could be effectively used to reduce genetic variability and reduce the numbers of animals needed for some experimental studies. This could be conducted on a larger scale than is currently possible with naturally occurring genetically identical twins .

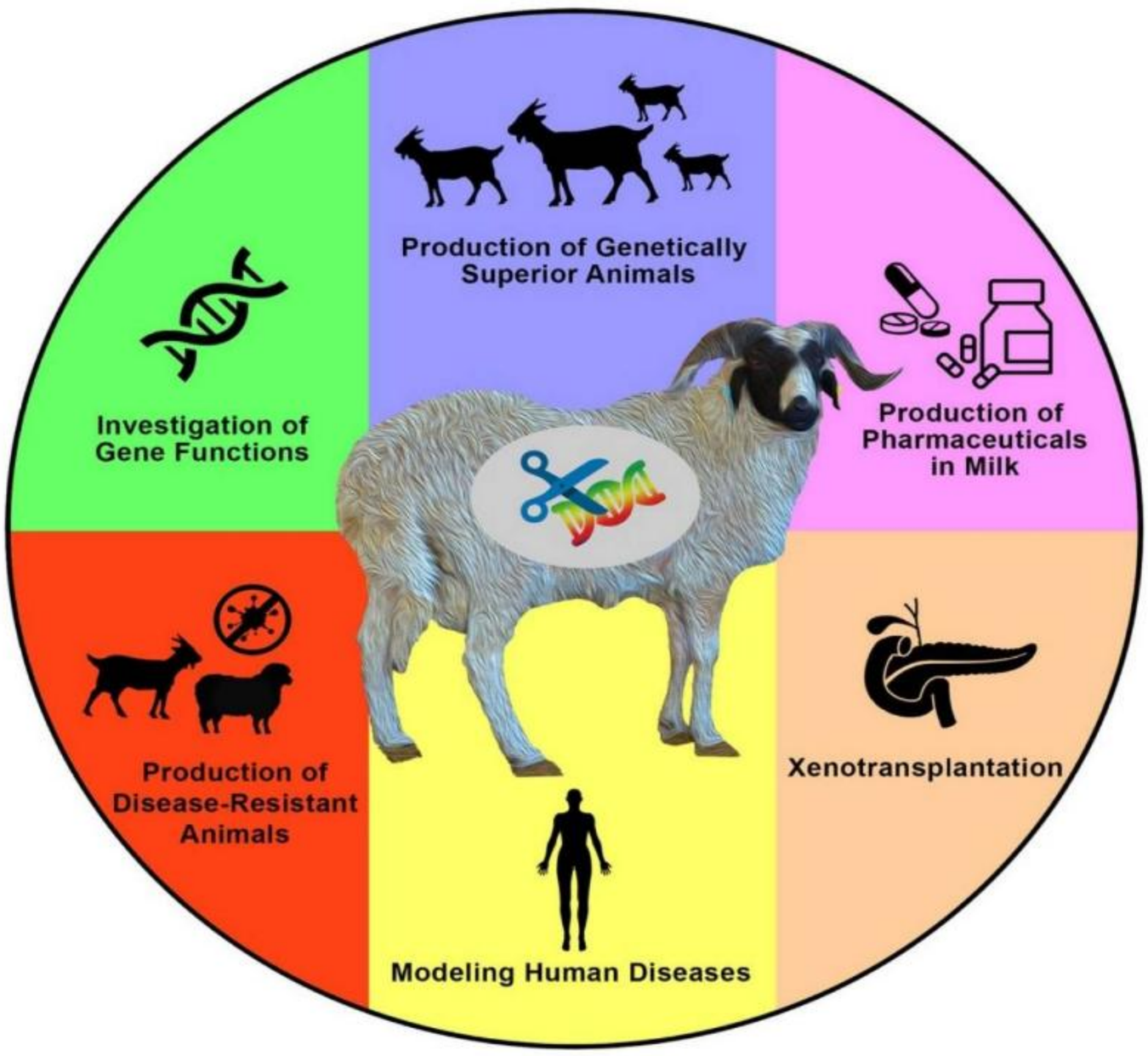

Fig 5 - Applications of cloning

❖ ADVANTAGES OF CLONING

1. Cloning animals would allow us to balance environmental habitats.
If an animal becomes extinct or disappears because their habitat does not support life in needed ways, then the outcomes in the local region can be very dramatic.Cloning could encourage us to restore this equilibrium by replacing or even reintroducing cloned animals that are either extinct or endangered.

2. Cloning animals would create more security in the global food supply.
Cloning animals is a reasonable approach that could help to stabilize our supply of animal-based proteins.

Although this advantage won't solve hunger by itself, a higher level of food availability will reduce conflicts, encourage innovation, and push scientists toward the innovative results that are likely necessary for the future.

3. Cloning animals could advance scientific discoveries in other fields.
The scientific processes that allow us to clone animals could be useful in the duplication of specific cells found throughout the body. We could potentially take the techniques discovered in this field to produce new tissues or organs as needed.

4. Cloning animals could help pet parents find greater comfort.

The processes of cloning animals would allow people to protect their memories of a beloved companion with an identical animal created by scientists.

5. Cloning animals allows us to preserve endangered species.
Researchers were able to save the Przewalski's horse only because 13 horses were captured from a wild herd and kept in a zoo in the 1940s. Even then, two of the horses were hybrids. Standard breeding practices helped to save the species, which now counts in the thousands of individuals.

6. Cloning animals gives us the opportunity to produce the most desirable traits.
When scientists work on animal cloning, they are doing what others have done through selective breeding for more than 1,000 years. The outcomes are similar to what we can achieve through natural reproductive processes that include human interference. This work is a chance to create precise, desired traits in animals.

We might use animal cloning to produce dairy cows that offer more milk, cloning specific hens as a way to improve egg production,breeding livestock animals to produce more meat per carcass..

7. Cloning animals could help us to reduce human disease.
The flu virus originates in birds, pigs, and other animal species. Our animal cloning processes could work to stop its development by creating more resiliency against its activity when it forms. It's an opportunity to stop the adverse impacts of disease before it even gets a chance to begin.

8. Cloning animals would not impact the quality of the food supply.
Rulings in 2008 from research at the time found that it is safe for people to consume animal products from cloned species. The FDA ruled that any livestock species can enter the commercial food chain.

❖ DISADVANTAGES OF CLONING ANIMALS

1. Cloning animals is the least effective way to produce offspring.
The success rate of the nuclear transfer method for animal cloning currently stands at 1%. That means about one embryo out of every 100 will be in a quality suitable enough for implantation. Once scientists reach this stage, the viability of the offspring is still questionable, with many of the embryos spontaneously aborting during the pregnancy.

2. Cloning animals is expensive.

3. Cloning animals reduces the genetic diversity of that species.
The likelihood of genetic disease and other health issues rises when parents with similar genetics have offspring.When there isn't genetic variation, then a population loses the ability to respond to changing environmental variables.

4. Cloning animals would eventually slow the rate of reproduction.
Animals that have the highest levels of genetic similarity tend to have the lowest rate of reproduction. We already see this disadvantage taking place with cheetahs, who as a species shares 99% of their genome with other individuals. Cloning animals would produce a similar outcome

5. Cloning animals has a history of creating abnormal pregnancies.
Almost half (45%) of the pregnancies that animal cloning techniques produce fail by the third trimester. When compared to the experiences from natural reproduction methods, the results are more significant and place the mother's life in more danger. Even when a pregnancy comes to term using a cloned embryo, it is more common for a C-section to be necessary. Dystocia and development abnormalities also appear more often, creating a higher risk of losing the mother during the birthing process.

6. Cloning animals can create long-term health issues to manage.
Dolly the Sheep had shorter telomeres than others of her species, and this genetic trait may have been a contributing factor to her living six years instead of the expected nine.

Cloned animals can experience life-threatening health issues at any time because the cell programming can alter their genetic profile. This process can reduce reproductive capabilities in some species, and some outcomes don't have a known cause.

7. Cloning animals could result in sterility issues.
When scientists create a clone, then there is a higher risk that the animal will not have the capability of producing offspring. If that impairment doesn't exist, the risk factors stay higher than they do with natural reproduction for the next generation.

8. Cloning animals could result in unforeseen consequences.
Restoring life to our planet seems like a worthy goal on paper, but it could produce more harm than good in some situations. An extinction from centuries ago altered the natural habitats so that the world could adjust to the change. Introducing wooly mammoths back into society could create massive problems with animal management in the wild. Even small introductions, like the dodo bird, could create problems with homeostasis.

We have no way to know how modern diseases would impact the ancient creatures we might try to revive through cloning. It could cause viruses to mutate, introduce new pathogens, or expose people to harmful bacteria.

9. Cloning animals could eventually lead to cloning humans.
There is no supporting scientific evidence to suggest that a cloned human embryo exists, although genetic alteration reports have come from China in 2019. Cloning humans is more complicated than it is for other mammals because of the location of spindle proteins on the chromosomes.

Removing the nucleus removes these proteins. That process causes interference with cell division. As our technologies improve, the science that leads to better animal cloning could lead to improved human cloning techniques.

10. Cloning animals could result in more cancer-related issues..

❖ ETHICAL ISSUES-

I. HISTORY OF BIOETHICS-

- During the seventies, people considered that the term "Bioethics" was coined by an American Biochemist Van Rensselaer Potter.
- In his article, he linked Mankind, nature, culture, science, and values .He had a prominent role in the formation of today's Bioethics which led to the development of new discipline to address the ethical issues raised in the field of medical and life sciences.
- But according to the literature, it appears that the word bioethics has been referred to earlier in 1927 by German protestant, Fritz Jahr in his article "Bio-Ethik.

II. PROBLEMS

- Ethics of cloning Animal cloning may give rise to two different types of moral problems

1. It may create some negative impacts on animals, human beings and the environment;

2. It may disobey various important moral principles .

- A large number of literature citations have shown high rates of spontaneous abortion, premature death, stillbirth, genetic disorders, chronic illness in cloned animals, Due to the above mentioned problems the efficiency of animal cloning has drastically reduced to about 12% which means only 2 out of 100 embryos get successfully implanted in surrogate animals and become offspring

- **RISK TO CLONE ANIMALS** –
  The animals involved in animal cloning experiments are subjected to high risks adversely affecting them which includes abnormal placentation, pregnancy toxemia, and hydroallantois. In one study it was found that 5 out of 10 cloned pigs died between 3-130 days of age from sickness like chronic diarrhoea, congestive heart failure and decreased growth rate which results in a 50% mortality

Percent efficiency in animal cloning =(Number of live offspring produced /Total number of transferred embryos) * 100

- **PHILOSOPHICAL VIEWS** –
  Several debates have held over a period of time to discuss the validity of animal cloning. It was concluded to ban the use of products from cloned animals and to prevent the use of animal cloning. For example, the U.S. Humane society which is an animal protection organization has requested to ban on products coming from cloned animals and their offspring.

- **RELIGIOUS VIEWS-**

Many religions have various guidelines or principles on doing things in the right way, on how should we live. According to the Islamic religion research, one has to ultimately find the truth and to observe the signs of Allah's glory in order to understand natural phenomena and the hidden story behind the universe .In the early Christian era, St. Augustine (AD 354 - 430) a theologian and Neoplatonic philosopher was highly influential. He mentioned that the percept "Thou shalt not kill" is not applicable to animals, because as they do not object and are unlike humans. According to him, God created an animal for the betterment of humans so people should also protect animals for their own uses.

- **USE OF CLONED ANIMALS FOR FOOD AND HEALTH-**

The impact of food production from cloned livestock arose many questions. One part of the question is ethics and the other part is science. The next question arises about the safety of such food to eat. However, in late October 2003, the US FDA stated that no evidence could be found against the safety of the food and milk derived from cloned animals . But in another statement, they claimed that due to lack of sufficient information it was not possible to justify that cloned food was safe.

- **SAFETY MEASURES-**

1. The manipulation of living organisms by human race cannot go further without regulation.Some ethical standards are required to evaluate the morality of all human activities that might help or harm living organisms.

2. The Indian Government has set up GEAC(Genetic Engineering Approval Committee), which makes decisions regarding the validity of GM research snd the safety of introducing those organisms for public services.

- **BIOPATENTS-**

A patent is the right granted by a government to an inventor to prevent others from commercial use of his invention. Biopatent is a patent granted by the government to the inventor for biological entities and for products obtained from them. For example, basmati rice and neem based products are biopatents of India.

❖ CONCLUSIONS

The term "clone" has many meanings but in its simplest and most scientific sense it means the making of identical copies of molecules, cells, tissues, and even entire animals. The latest news about cloning Dolly the sheep involved somatic cell nuclear transplant cloning. In this process the nucleus from an adult somatic cell is transplanted into an enucleated ovum to produce a developing animal that is a "delayed" genetic twin of the adult.

There are many applications that nuclear transfer cloning might have for biotechnology, livestock production, and new medical approaches. Work with embryonic stem cells and genetic manipulation of early embryos in animal species (including nuclear transfer) is already providing unparalleled insights into fundamental biological processes and promises to provide great practical benefit in terms of improved livestock, improved means of producing pharmaceutical proteins, and prospects for regeneration and repair of human tissues. Potential risks include those known to be associated with the manipulation of nuclei and eggs and those yet unknown, such as the effects of aging, somatic mutation, and improper imprinting. These effects could result in high rates of failed attempts at pregnancy as well as the increased likelihood of developmentally and genetically abnormal embryos.

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