Important uses of genetic engineering

- 1- In virology
- 2- In agriculture
- 3- In environment
- 4- In health
- 5- In therapeutics
- 6- In animal field
- 7- In tissue engineering
- 8- In immunogenetics

In virology

- Mapping of most viruses
- Genetic engineered vaccines
- Gene probes & hybridization
- Copy of DNA (cDNA) from RNA viruses
- Early diagnosis of transformed cell

In agriculture

- Transgenic plants
- Genetically improved animals
- Waste management

In environment

- Production of toxins degradation bacteria
- Indicator bacteria (visible light bacteria)
- Treatment of wastes

In animals

- Animal cloning
- Gene cloning
- Controlling of animal diseases
- Gene therapy

Therapeutics

- Production of hormones (insulin , growth hormones) and important proteins (INF, cytokines , clotting factors)
- Gene therapy

Tissue engineering

- Production of biomaterials
  - Synthetic collagen fibers

Immunogenetics

- **Mapping of resistant genes in animal species**
- **Selection of naturally resistant animal species**

Animal cloning

- **Dolly the sheep may have been the world's most famous clone, but she was not the first.**
- **Cloning creates a genetically identical copy of an animal or plant.**
- **Many animals - including frogs, mice, sheep, and cows - had been cloned before Dolly.**

- **Plants are often cloned - when you take a cutting, you are producing a clone.**
- **Human identical twins are also clones.**

so why did she cause so much excitement and concern?

- **Because she was the first mammal to be cloned from an adult cell, rather than an embryo**
- **Since 1996, when Dolly was born, other sheep have been cloned from adult cells, as have mice, rabbits, horses and donkeys, pigs, goats and cattle.**

- **In 2004 a mouse was cloned using a nucleus from an olfactory neuron, showing that the donor nucleus can come from a tissue of the body that does not normally divide.**

How was Dolly produced?

- To produce Dolly, the scientists used the nucleus of an udder cell from a six-year-old Finn Dorset white sheep.
- The nucleus contains nearly all the cell's genes.
- They had to find a way to 'reprogram' the udder cells - to keep them alive but stop them growing – which they achieved by altering the growth medium (the ‘soup’ in which the cells were kept alive).

- Then they injected the cell into an unfertilized egg cell which had had its nucleus removed, and made the cells fuse by using electrical pulses. The unfertilized egg cell came from a Scottish Blackface ewe.
- When the scientists had managed to fuse the nucleus from the adult white sheep cell with the egg cell from the black-faced sheep, they needed to make sure that the resulting cell would develop into an embryo.

- **They cultured it for six or seven days to see if it divided and developed normally, before implanting it into a surrogate mother, another Scottish Blackface ewe.**
- **Dolly had a white face.**

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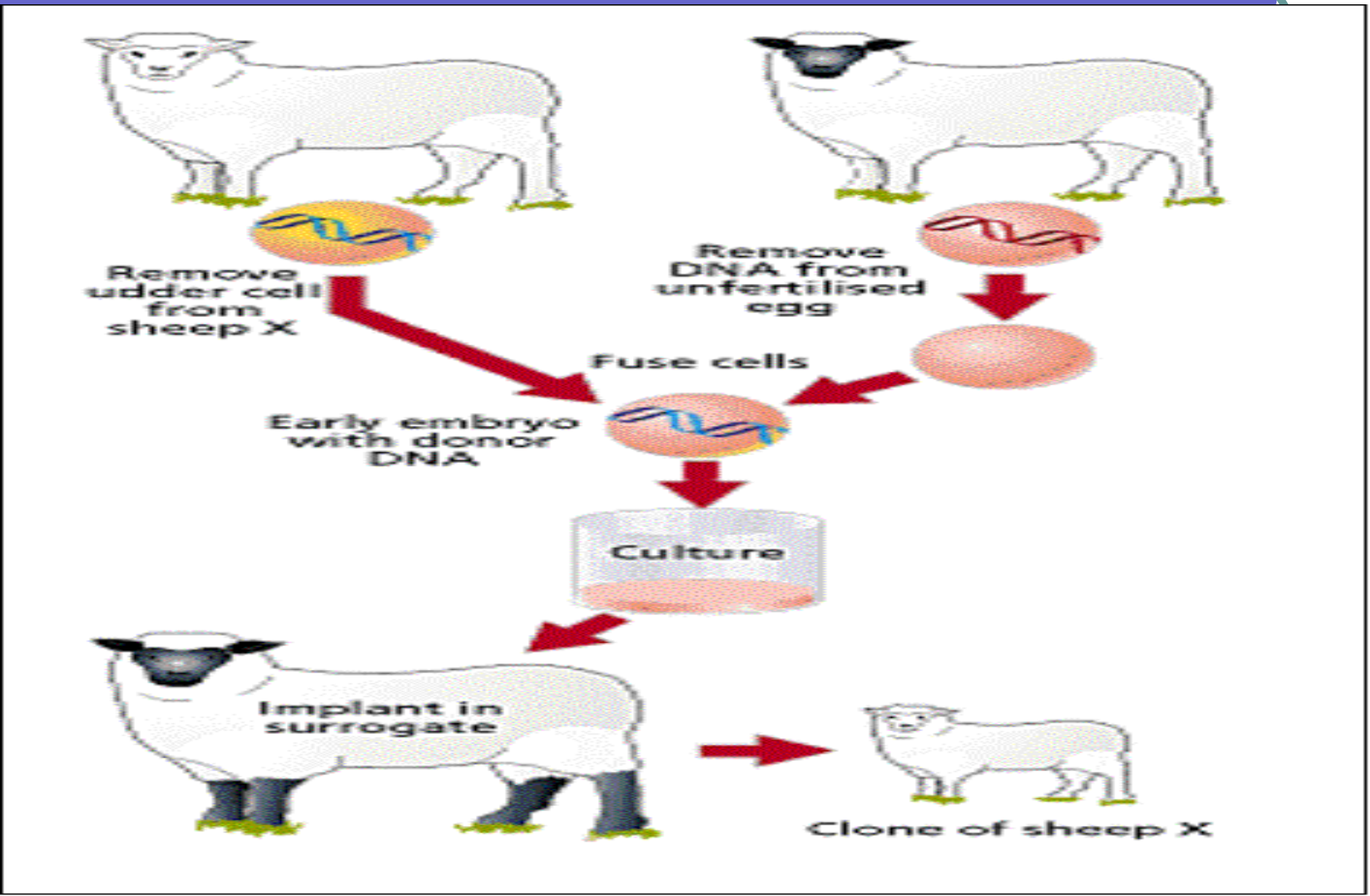
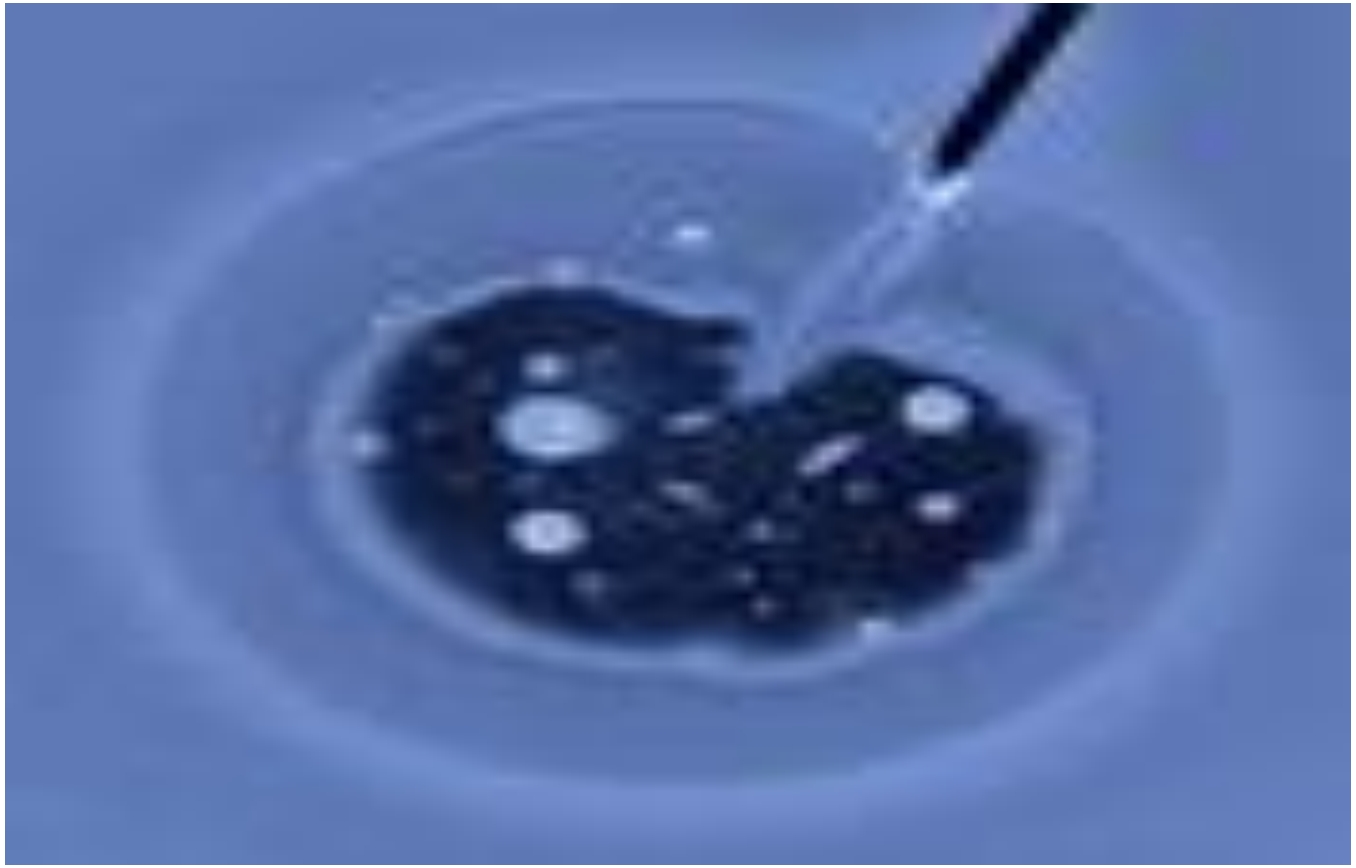
- **From 277 cell fusions, 29 early embryos developed and were implanted into 13 surrogate mothers.**
- **But only one pregnancy went to full term, and the 6.6kg Finn Dorset lamb 6LLS (alias Dolly) was born after 148 days.**

Cloning of Dolly



Pick up of nuclear materials



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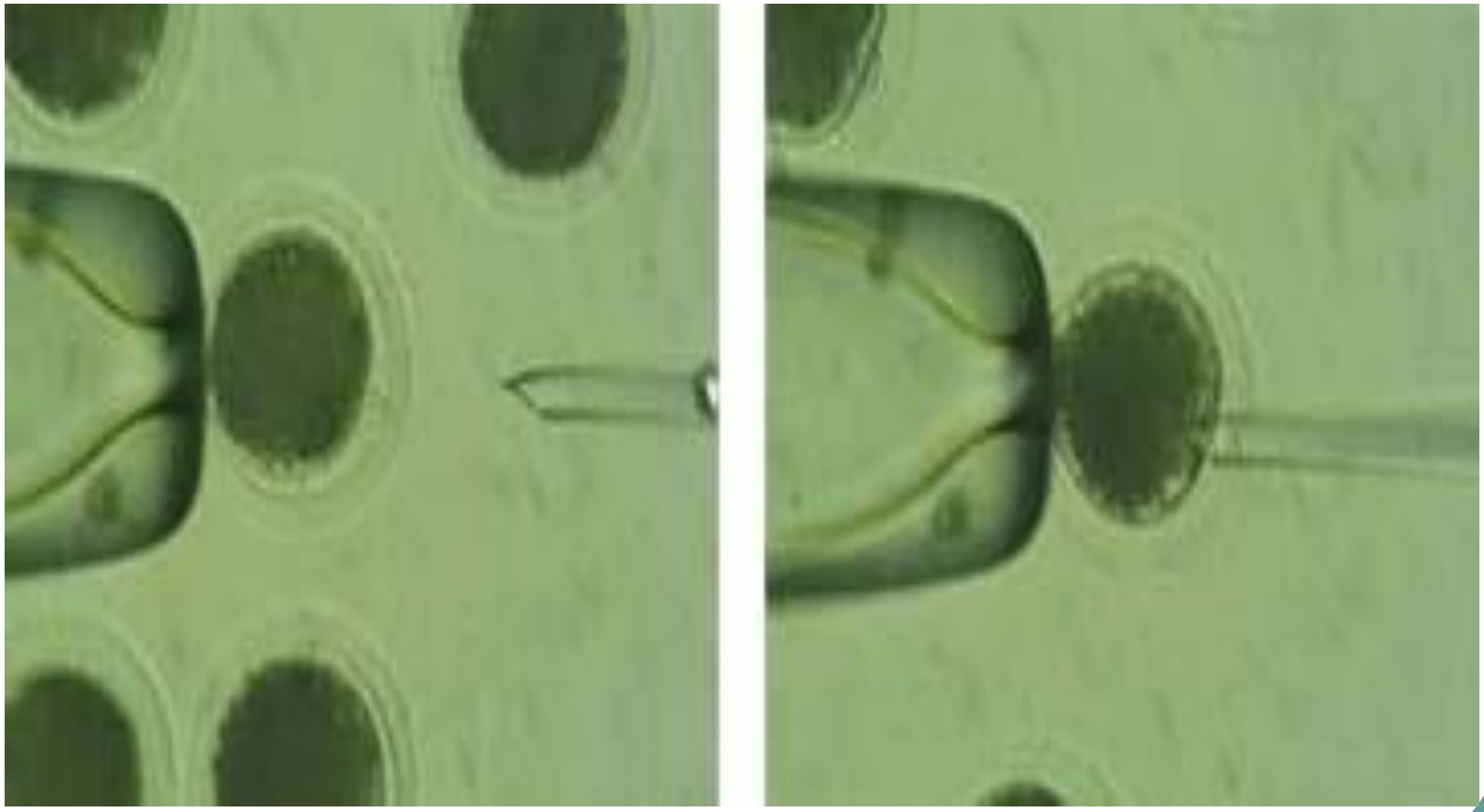
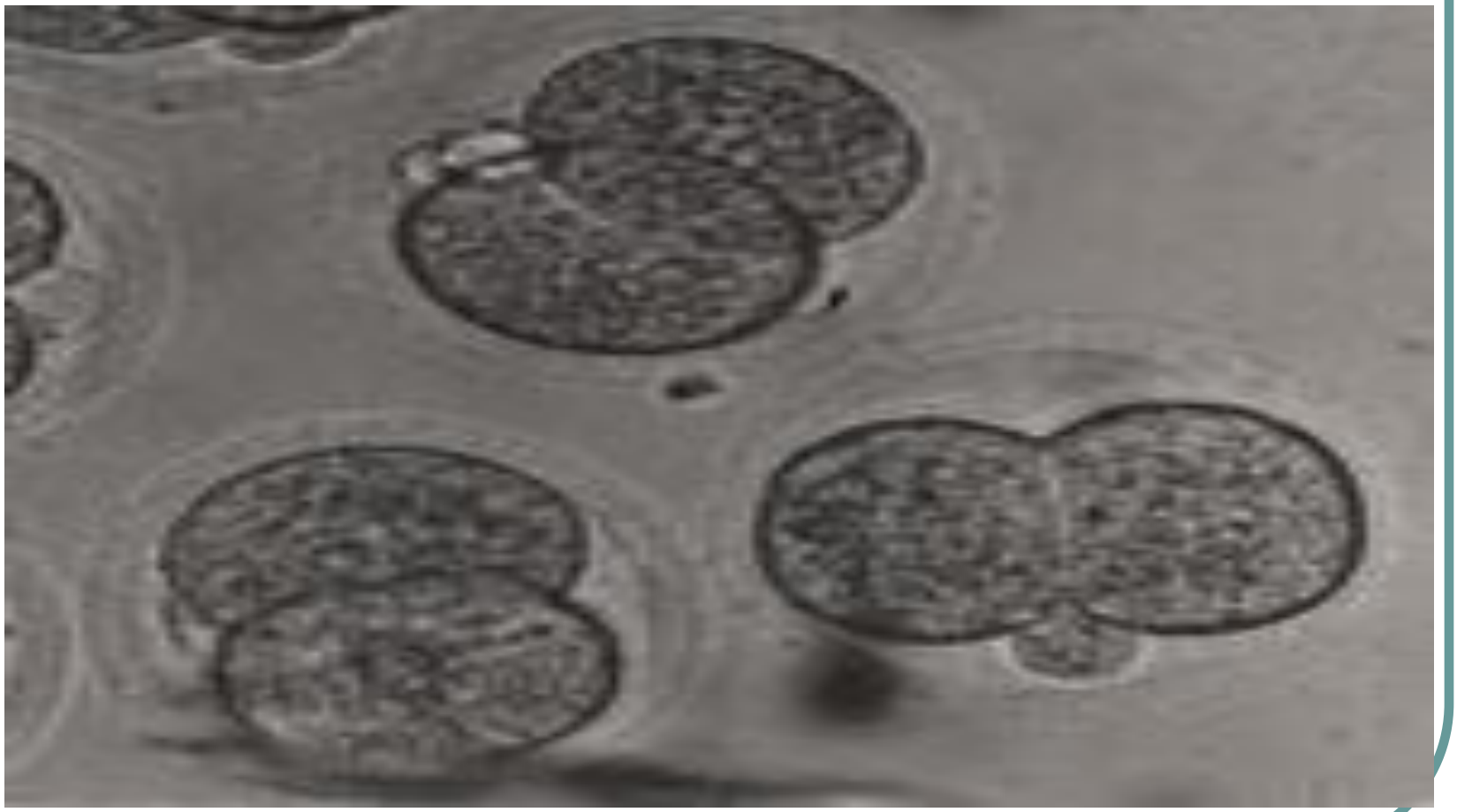
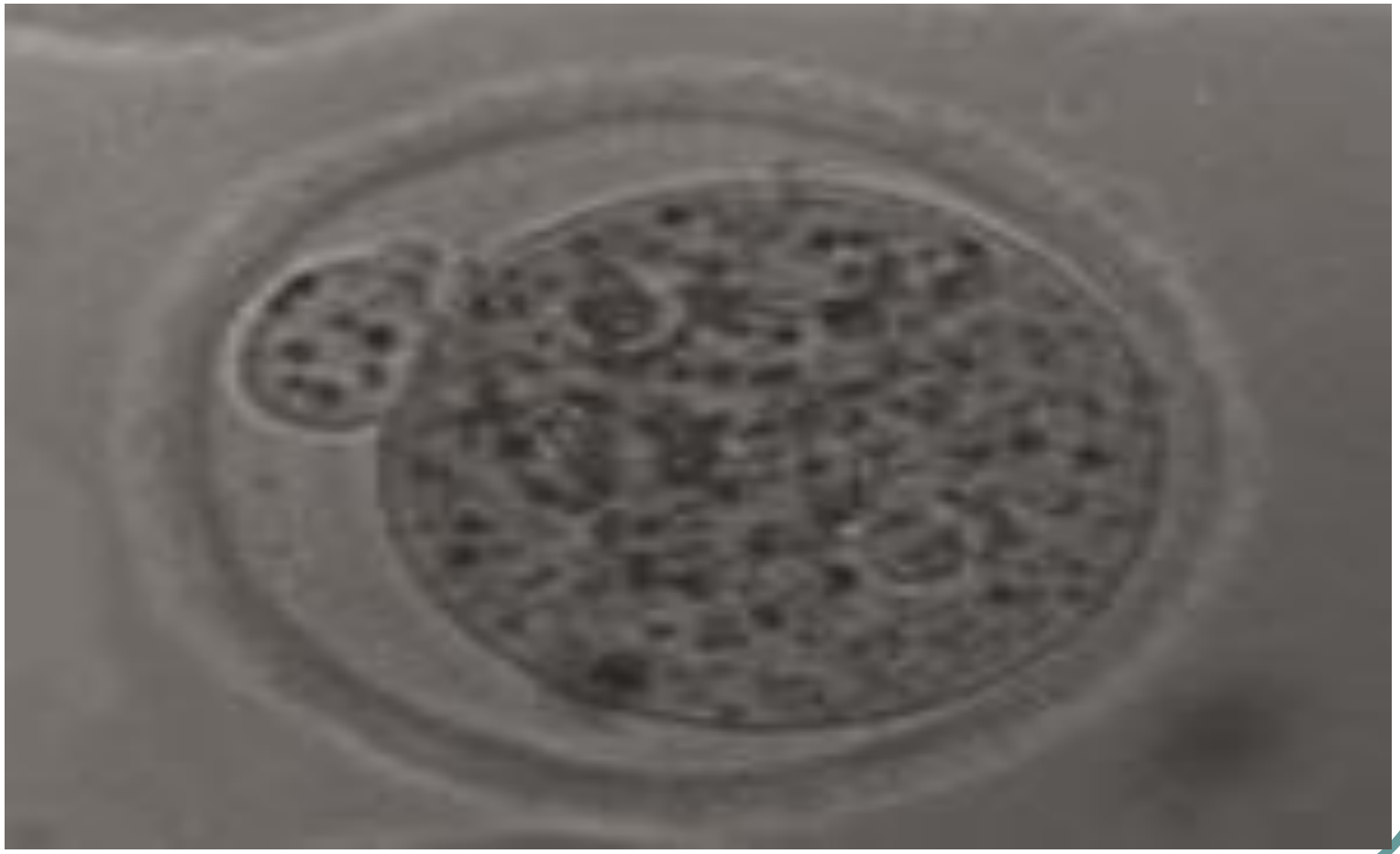


Fusion of the two cells



Formation of fertilized cell



Dolly

Dolly &its mother

Cloning of cat

Cloning of horse

What happened to Dolly?

- She mated and produced normal offspring in the normal way, showing that such cloned animals can reproduce.
- Born on 5 July 1996, she was euthanased on 14 February 2003, aged six and a half.
- Sheep can live to age 11 or 12, but Dolly suffered from arthritis in a hind leg joint and from sheep pulmonary adenomatosis, a virus-induced lung tumour to which sheep raised indoors are prone.

- On 2 February 2003, Australia's first cloned sheep died unexpectedly at the age of two years and 10 months. The cause of death was unknown and the carcass was quickly cremated as it was decomposing.
- Dolly's chromosomes were are a little shorter than those of other sheep, but in most other ways she was the same as any other sheep of her chronological age.
- However, her early ageing may reflect that she was raised from the nucleus of a 6-year old sheep.

- Study of her cells also revealed that the very small amount of DNA outside the nucleus, in the mitochondria of the cells, is all inherited from the donor egg cell, not from the donor nucleus like the rest of her DNA. So she is not a completely identical copy.
- This finding could be important for sex-linked diseases such as hemophilia, and certain neuromuscular, brain and kidney conditions that are passed on through the mother's side of the family only.

Why are scientists interested in cloning?

- Their research aimed at producing medicines in the milk of such animals.
- Researchers have managed to transfer human genes that produce useful proteins into sheep and cows, so that they can produce, for instance, the blood clotting agent factor IX to treat hemophilia or alpha-1-antitrypsin to treat cystic fibrosis and other lung conditions.

- **Cloned animals could also be developed that would produce human antibodies against infectious diseases and even cancers.**
- **‘Foreign’ genes have been transplanted into zebra fish, which are widely used in laboratories, and embryos cloned from these fish express the foreign protein.**
- **It is already being used alongside genetic techniques in the development of animal organs for transplant into humans (xenotransplantation). Combining such genetic techniques with cloning of pigs (achieved for the first time in March 2000) would lead to a reliable supply of suitable donor organs.**

- The use of pig organs has been hampered by the presence of a sugar, alpha gal, on pig cells, but in 2002 scientists succeeded in knocking out the gene that makes it, and these ‘knockout’ pigs could be bred naturally.
- However, there are still worries about virus transmission.
- Greater understanding of the development of the embryo and of ageing and age-related diseases.

Cont.

- It could even enhance biodiversity by ensuring the continuation of rare breeds and endangered species.
- Cloning could be used to create better animal models of diseases, which could in turn lead to further progress in understanding and treating those diseases.

Ethical concerns and regulation

- Most of the ethical concerns about cloning relate to the possibility that it might be used to clone humans.

Difficult of human cloning

- It would have to involve women willing to donate perhaps hundreds of eggs, surrogate pregnancies with high rates of miscarriage and stillbirth
- The possibility of premature ageing and high cancer rates for any children so produced

Regulation

- In the USA, President Clinton asked the National Bioethics Commission and Congress to examine the issues, and in the UK the House of Commons Science and Technology Committee, the Human Embryology and Fertilization Authority and the Human Genetics Advisory Commission all consulted widely and advised that human cloning should be banned.

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- The Council of Europe has banned human cloning: in fact most countries have banned the use of cloning to produce human babies (human reproductive cloning).
- Therapeutic cloning (or cell nucleus replacement) for tissue engineering, in which tissues, rather than a baby, are created.

Therapeutic cloning

- In therapeutic cloning, single cells would be taken from a person and 'reprogrammed' to create stem cells, which have the potential to develop into any type of cell in the body. When needed, the stem cells could be thawed and then induced to grow into particular types of cell such as heart, liver or brain cells that could be used in medical treatment. Reprogramming cells is likely to prove technically difficult.

Cont.

- Therapeutic cloning research is already being conducted in animals, and stem cells have been grown by this method and transplanted back into the original donor animal.
- In humans, this technique would revolutionise cell and tissue transplantation as a method of treating diseases. However, it is a very new science and has raised ethical concerns.

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- The Human Fertilization and Embryology Act was amended in 2001 to allow the use of embryos for stem cell research and consequently the HFEA has the responsibility for regulating all embryonic stem cell research in the UK.

Human cloning : go ahead

- However, in 2004 South Korean scientists announced that they had cloned 30 human embryos, grown them in the laboratory until they were a hollow ball of cells, and produced a line of stem cells from them. Further news is awaited.

How does cloning affect the DNA of animals?

- Cloning does not change DNA and clones are not genetically modified organisms (GMO's). It is simply assisted reproduction.

How does cloning relate to other reproductive technologies?

- Livestock cloning is the most recent evolution of selective breeding in animal husbandry, which dates back to the dawn of time
- Arab sheikhs first used artificial insemination in horses as early as the 14th century (Arab chieftains first use artificial insemination to produce superior horses). .

- **1891**
  Walter Heap performs the first successful Embryo Transfer in England with rabbits
- **1894**
  Hans Dreisch creates the first cloned animals by blastomere transfer. He isolates blastomeres from 2- and 4-cell sea urchin embryos and observes their development into small larvae.
- **1952**
  Briggs and King use nuclear transfer of adult donor cells to clone frogs

- **1963**
  In China, embryologist Tong Dizhou clones
- **1977**
  Somatic cell nuclear transfer used to produce cloned frogs.
- **1979**
  A sheep is cloned by embryo splitting.
- **1983**
  First mammal produced by embryonic nuclear transfer.
- **1984**
  Creation of sheep "identical twins" by embryo splitting.

- **1987**
  Embryonic cell nuclear transfer in cattle.
- **1989**
  Sheep and cow embryos cloned, thus pointing out that existing reproductive technology would open the way for large scale cloning in livestock.
- **1993**
  Repeated cycles (multiple generations) of nuclear transfer procedures used to produce a large number of identical animals.

- **1996**
  First report of a mammal cloned from an embryo-derived cell culture. All prior nuclear transfer had used cells from embryos rather than from cell lines established from embryos. This made it possible to easily clone an unlimited number of animals from a single embryo.
- **1996**
  The Roslin Institute in Scotland produces a sheep, Dolly, the first mammal cloned from a cell of an adult animal.

- **1997**
  Infigen, Inc. produces Gene, the first cloned cow, from a fetal cell.
- **1998**
  Genzyme Transgenic Corporation and Tufts University produce Mira, the first goat cloned from an embryonic cell.

  University of Hawaii clones three generations of mice from nuclei of adult ovarian cumulus cells.

  Noto and Kaga, the first cows cloned from adult cells, are produced by the Ishikawa Prefectural Livestock Research Center

- **2000**
  The University of Teramo in Italy clones the first mouflon, a rare type of sheep, from an adult cell.

  Researchers at PPL Therapeutics produce Millie, Christa, Alexis, Carrel, and Dotcom, the first pigs cloned from adult cells.
- **2001**
  Noah, a gaur and the first of an endangered species to be cloned, is produced by Advanced Cell Technologies.

  CC, the first female cat cloned and the first clone of a domestic animal, is produced by Genetic Savings & Clone.

- **University of Georgia and Prolinia clone a cow from a kidney cell drawn from a carcass**

- **2003**

Trans Ova Genetics and Advanced Cell Technologies produce the first bantengs (an endangered species) cloned from adult cells.

Ditteaux, the first African wildcat cloned from an adult cell, is produced by the Audubon Center for Research of Endangered Species.

Dewey, the first deer cloned from an adult cell, is produced by ViaGen and Texas A&M shortly before Christmas.

Idaho Gem, the first mule cloned from a mule fetus, is produced by the University of Idaho.

- **2004**
  Tabouli and Baba Ganoush are the first cats cloned using chromatin transfer technology (CT)
- **.2005**
  ViaGen, Inc. clones three calves from rare Prime Yield Grade 1 and 2 beef carcasses.

  Snuppy, the first clone of a dog, is produced at Seoul National University in South Korea.

  Audubon Center for Research of Endangered Species naturally breeds unrelated African wildcat clones, which then gave birth to the first offspring of unrelated clones of a wild species.

- In the last fifty years, techniques such as embryo transfer, in vitro fertilization, embryo splitting, and blastomere transfer have become commonplace - providing farmers and ranchers powerful tools for breeding their best animals.
- Livestock cloning is the most recent evolution of selective breeding in animal husbandry, which dates back to the dawn of time

Cont.

- Cloning accelerates the birth of the best possible stock by allowing farmers to be certain of the genetic make-up of a particular animal.