Bio Factsheet

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# Artificial Cloning Techniques

This Factsheet describes:
- **modern techniques of cloning animals and animal tissues;**
- **modern techniques of cloning plants and plant tissues;**
- **applications of artificial cloning in plants and animals;**
- **ethical issues of cloning in animals.**

Natural cloning in nature and the use of bacterial and viral cloning in gene technology have been described fully in other Factsheets (referred to below) and are not repeated here.

## Introduction

The traditional definitions of a clone are:
- A population of genetically identical individuals descended from the same parent by asexual reproduction. This cloning process involves mitotic cell division but not meiosis and is natural (occurs in nature).

> Many flowering plants show asexual reproduction by producing structures such as runners, suckers, tubers, corms or bulbs which colonise the area around the parent. Fungi produce genetically identical offspring by asexual spores. Protoctistans, for example, *Amoeba,* reproduce asexually by binary fission. (For more information read Factsheet 95, Asexual Reproduction, Sept. 2001).

- A population of genetically identical cells produced by mitotic division from an original cell. This is also a natural process.

> This occurs in bacteria which reproduce asexually by binary fission. It occurs when body cells are replaced during normal growth and repair processes. For example, genetically identical blood cells are produced throughout life to replace aged cells. (Remember –although the cells are genetically identical they may develop different characteristics because different genes in their genetic make-up are activated or suppressed).

In recent years, because of the advances in genetic techniques, the word 'clone' has come to have additional meanings. For example:
- A clone can be considered as a group of identical genes or DNA molecules produced from an original length of DNA sequences. This is artificial cloning and is used during genetic engineering by recombinant DNA technology.

> For example, lengths of identical DNA can be produced by the Polymerase Chain Reaction (see Factsheet 67, Modern Techniques in Biology: Genetics, Apr. 2000). or genes can be reproduced by inserting into bacteria or viruses which then reproduce thus copying the genes. This is often referred to as '**molecular cloning** or **DNA cloning**'. (See Factsheets 13, Genetic Engineering, Sept. 1997, and 69, Genetic Engineering in Agriculture, Apr. 2000).

- The word 'clone' is used to refer to an individual organism, such as Dolly the sheep, which was produced by '**Nuclear Transfer**' techniques. Animal clones can also be produced by **embryo division**. These are artificial cloning techniques and are described below.

> Although whole organisms can be cloned by 'nuclear transfer' technology, it is not yet proved possible to clone individual tissues or organs without the rest of the body to go with it.

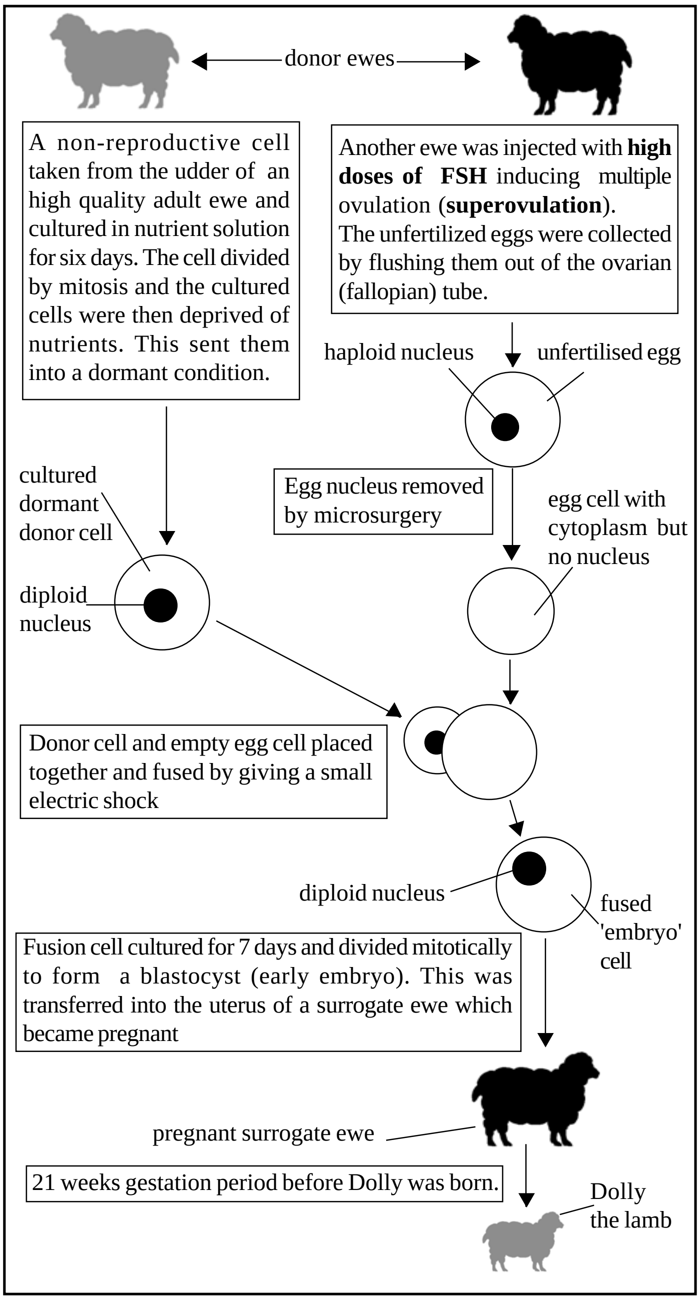
- Plant clones can be produced by **micropropagation** from plant tissue cultures. This is an artificial cloning technique and is described below.

> ***Exam Hint:*** *– read questions on cloning carefully and all through, to decide if they are concerned with natural or artificial cloning.*

## Artificial cloning of animals

**Cloning by nuclear transfer:** By this technique a complete animal is produced from a single cell of an adult animal. All the cells of the new animal are thus clones of the original cell. The first animal to be produced in this way was Dolly the sheep.

**Fig. 1. How Dolly the sheep was cloned**



277 fused ‘embryo’ cells were constructed of which 29 developed to the blastocyst stage. These were implanted into 29 Scottish Blackface ewes, but only one ewe produced a live lamb called Dolly, 148 days later on 5th July 1996. She was genetically identical to the original ewe who supplied the udder cell. During her life Dolly was mated, produced six normal lambs and was apparently genetically normal. However, when she was six years old she showed premature ageing, developed severe arthritis and a progressive lung disease. She was put to sleep at the age of six and a half years in February 2003.

A normal sheep lives for 11 to 12 years. The genetic material used to clone Dolly was taken from a six-year old ewe and so could be considered to be already six years old when transferred to Dolly.

**Possible applications of nuclear transfer:**
- It may eventually be of use to farmers to produce high genetic quality stock which are genetically identical. This would be useful to perpetuate high quality traits, for example, high milk yield, fast meat growth, low fat content, disease resistance.
- It may be of use to preserve rare breeds.
- It may be of use to produce genetically identical transgenic animals, for example, sheep to produce alpha-1 antitrypsin to treat cystic fibrosis or human insulin to treat sugar diabetes.

***Remember:*** *– a transgenic animal is one that has had a useful gene, for example, the human insulin gene, added to its genotype by genetic engineering. The adult animal will then produce the human insulin throughout its life. The insulin can easily be collected.*

**Cloning by embryo transfer (ET)**
Since its development in 1979 many sheep, goats, cattle, rabbits and toads, have been cloned by this method. For example, in sheep:

- the reproductive cycles in a flock of sheep are synchronised so that events occur at the same time in the different individual sheep. This is achieved by using ‘**progesterone- releasing sponges**’ which are placed in the vaginas of both donor and recipient sheep. The sponges contain GnRF (gonadotropin releasing factor) and a slow release synthetic progesterone. They are left in place for several days until oestrous occurs.
- donor ewes are given twice-daily injections of high dose FSH (follicle stimulating hormone) starting 72 hours before oestrous is expected. This increases the number of ovarian follicles which mature and results in superovulation (multiple egg release). These eggs will be fertilised by mating with the ram or by artificial insemination.
- embryos are collected from the uterus of a donor female a few days after fertilization.
- three day old embryos, which consist of only a few cells, are divided into individual cells.
- by incubating the cells, in a suitable culture solution, at 37,C, the individual cells are induced to develop into separate embryos.
- each embryo is transferred into a surrogate mother (synchronised to be at a similar stage in her reproductive cycle), and becomes implanted in the uterus establishing pregnancy. The embryos develop through pregnancy into normal offspring.
- alternatively the embryos can be stored in liquid nitrogen for transfer into recipients at a later date or in another place.

**Applications of embryo transfer:**
- ET enables the genetic composition of a flock of sheep or herd of cattle to be improved by using embryos from genetically superior donors.
- There is less risk of international disease transmission when embryos are transported rather than live adult animals for breeding programmes.
- Embryos can be stored frozen in liquid nitrogen and can be introduced into recipients at any convenient time.
- Rare breeds can be stored as frozen embryos and there is less risk of exposure to disease when these are transported than when adult animals are transported. The embryos can be considered as ‘gene banks’ because they contain valuable genes.
- In some cases, pathogen-free herds and flocks can be produced from infected donor animals. This is useful if the sick donors are genetically valuable.
- It may be of use to produce genetically identical transgenic animals, for example, sheep to produce alpha-1 antitrypsin, or humilin (human insulin).

*Exam Hint: – candidates sometimes mistakenly write that ‘all the animals used in cloning must be of high genetic quality’. This is not so - the transferred embryos must be of high genetic quality but the recipient animals can be of lower genetic quality because this will not reduce the genetic quality of the offspring.*

**Ethical issues of cloning in animals - Typical Exam Question**
- Embryo transplantation and nuclear transfer are being developed to produce animals that perpetuate good traits, such as high milk yield, high quality meat yield, longevity and disease resistance. If this produces more food for the world population it is ethically good, but if it causes animal suffering it is ethically bad. Many people think that animals should be given proper respect and should not be exploited by genetic modifications.
- Many people would consider the use of animal cloning (in conjunction with genetic engineering), to produce transgenic animals which can produce therapeutic proteins used for treating human or animal diseases to be ethically good, particularly as the substances made in this way cannot be made in adequate quantities by any other way. Thus cystic fibrosis sufferers can now be treated using readily available alpha-1 antitrypsin from transgenic sheep.
- Human cloning is considered unethical and is legally banned in Britain, the USA and most other countries of the world.
- Britain has made it legal, under licence to carry out stem cell research by cloning cells obtained from human embryos to produce replacement tissues for treatment of, for example, damaged heart muscle or damaged spinal cord. Stem cell research may also provide disease cures, for example, for Parkinson’s disease. Many people consider this to be ethical but many others think it is unethical to use embryos in this way. The embryos are those produced by fertility treatments, such as IVF, which are ‘surplus to requirements’.

## Artificial cloning of plants

Once a cell has differentiated to form part of a tissue it usually loses the ability to divide. However, the nucleus of any cell in a plant holds all the genes for making a complete plant. The genes that are required by the specific cell are active but the genes not required by a specific cell are repressed (inactive). In certain circumstances these inactive genes can be reactivated and cell division, by mitosis, will resume.

Single plant cells can be induced to divide by mitosis and grow and differentiate into complete plants. One technique is to treat a small piece of plant tissue, (called an **explant),** from a root or stem with hydrolytic enzymes, (for example, cellulases), to separate it into individual cells. The cells are then exposed to specific plant growth substances (auxins and kinins) at specific concentrations. This induces mitosis and the differentiation of roots, stems and leaves.

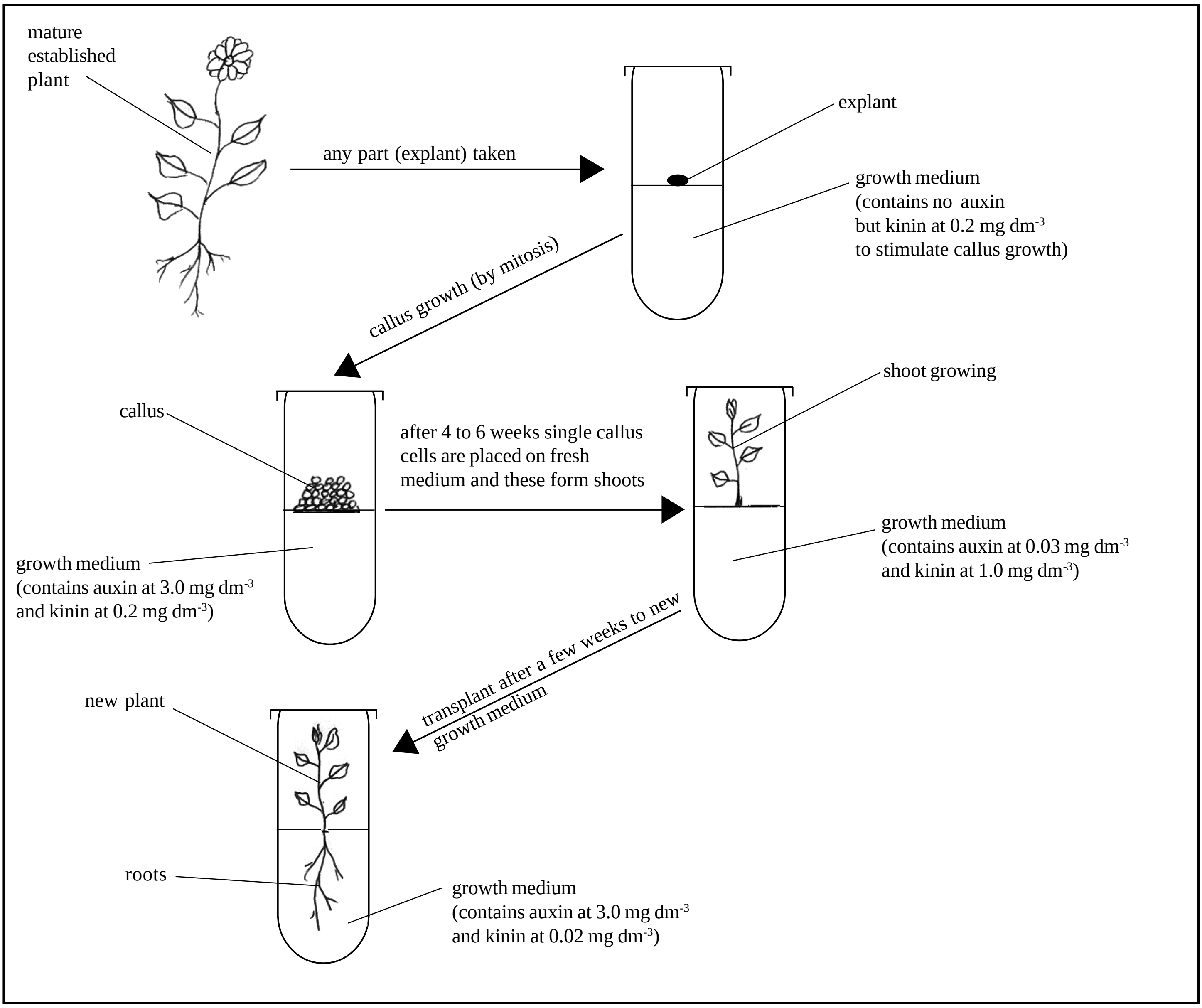
Another techniques is to start with a small explant and place it on a nutrient growth medium. Cells in the tissue start to divide and produce many cells which form a shapeless undifferentiated mass called a **callus.** When the callus is treated with the auxins and kinins at the appropriate concentrations, it develops into a complete plant. The auxins and kinins are incorporated into the growth medium.

**Applications of plant micropropagation:**

- large numbers of plants (for commercial sale) can be produced cheaply from small pieces of plant tissue.
- it can be used to produce offspring from particularly good or valuable plants.
- it produces plants free from fungal and bacterial infections, and almost certainly free from viral infections.
- it produces plants that are clones because they were all produced from one parent plant, thus the good qualities of the parent plant are perpetuated.
- genetically modified plants can be produced by introducing new useful genes into the genome at the callus stage, using genetic engineering techniques.

***Exam Hint**:– a common error is to think cloning always involves genetic engineering to produce genetically modified plants or animals. Remember that cloning can be used without genetic engineering and its main application is to multiply valuable plants and animals as clones.*

**Fig.2. Micropropagation of plants by tissue (callus) culture**



## Practice Questions

1. Read through the following passage which describes how Dolly the sheep was cloned.
   Then fill in the spaces with appropriate words or phrases.

Dolly the sheep was cloned using the technique of .....……………… Scientists took a ……………………cell from the ……… of an adult ……………… ewe and cultured it in a ………………………. for six days. Another ewe was treated with ……………………. drugs to cause ………………………………. and the eggs were collected from the …………………… by a flushing method. The eggs were then subjected to microsurgery to remove their ………………. .

The donor cells and the empty eggs were put together and given an …………………….. to combine them. The eggs, each of which now contained a donor nucleus, began dividing as fertilized eggs would and became embryos. These were allowed to grow in a nutrient solution for several days.

Individual embryos were then ……………………… through a fine catheter passed through the …………………. into the uterine horns of a recipient ewes. These ewes acted as ………………. mothers. One ewe carried Dolly through pregnancy to birth.

**Total 13**

2. (a) (i) What is superovulation? **1**

   (ii) How can superovulation be induced? **2**

   (iii) Why is superovulation useful in animal cloning techniques? **1**

   (b) (i) How may breeding cycles be synchronised in sheep? **3**

   (ii) Why is it necessary to synchronise breeding cycles when cloning sheep? **1**

**Total 8**

3. (a) In the micropropogation of plants explain the meaning of the terms
   (i) explant, and **2**

   (ii) callus. **2**

   (b) Comment on the use of plant growth substances in micropropagation of plants. **3**

**Total 7**

**Answers**

1. nuclear transfer; non-reproductive; udder; donor; nutrient solution; fertility; superovulation; ovarian/fallopian tubes; nuclei; electric shock; implanted; cervix; surrogate;

**Total 13**

2. (a) (i) when more ovarian follicles mature than normal and so release more eggs at ovulation than normal; **1**

   (ii) by giving injections of 'high dose' FSH;
   two injections per day for three days before oestrous; **2**

   (iii) so plenty of eggs are available, for producing plenty of embryos for embryo transfer/for nuclear transfer; **1**

   (b) (i) by using intra-vaginal sponges which are left in place until oestrous;
   these contain slow-release progesterones;
   and GnRF; **3**

   (ii) donor and recipient sheep/all recipient sheep need to be at a similar stage of the oestrous cycle for the embryos to implant and develop successfully; **1**

**Total 8**

3. (a) (i) a small piece of plant tissue used as a source of cells for micropropagation;
   can be taken from any part of the plant; **2**

   (ii) a mass of undifferentiated cells;
   formed by mitosis from an explant cell; **2**

   (b) ref to auxins and kinins;
   used together at specific concentrations;
   relative concentrations control whether callus, roots or shoots form; **3**

**Total 7**

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