

ANIMAL CLONING: Industrialising Animals?



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Five years ago, it was announced that the first animal had been cloned from an adult cell. Produced in Scotland, the research that led to the creation of Dolly, the cloned sheep, was driven by a desire to make genetically modified animals more economically viable. There was a clear financial incentive in creating exact copies of sheep which produce a valuable therapeutic drug in their milk. Since then, attempts have been made to clone many different species. This briefing reviews animal cloning and considers what it means for the animals involved and our relationship with them.

What is cloning?

A clone is a genetically identical individual grown from a single cell of an embryo or an adult. Mice were successfully cloned from embryonic cells in the 1980s and frogs decades earlier¹. The first report of a clone from an adult somatic (non-reproductive) cell was in 1997 – Dolly the sheep - produced by nuclear transfer from an adult cell at the Roslin Institute in Edinburgh². Since then, pigs, cows, cats, goats, rabbits and mice have been cloned from either cultured foetal or adult cells^{3,4,5,6,7,8}.

Cloning has been achieved by nuclear transfer, where the nucleus (which contains the genetic material) of the cell to be cloned is inserted into an egg from which the nucleus has been removed (enucleated). An electric current is used to fuse the donor nucleus with the recipient cell and to start embryonic development. The embryo is transferred into the womb of a female and the animal that develops and grows from the embryo is a genetic copy of the animal from which the donor cell was taken.

PPL Therapeutics first reported producing cloned lambs from cultured embryo cells in 1996⁹, but Dolly represented a major

breakthrough as this was the first time that an animal had been cloned from an adult cell. This opened up much wider possibilities for the usefulness of cloning, especially because it could help to expand the use of GM techniques on animals.

Why clone animals?

There are two reasons for animal cloning:

1. To make copies of valuable animals which may have been conventionally bred or genetically modified. Cloning research on embryos was originally driven by the lure of producing large numbers of elite, identical animals at low costs¹⁰. As well as the aspiration to make copies of valuable farm animals for use in agriculture or GM animals that produce drugs in their milk, cloning is also being applied to make copies of pet animals and endangered species.
2. To facilitate the production of genetically modified animals. Cloning from cultured cell lines and adult cells holds the promise that GM technology could become much more efficient and that targeted manipulation could become possible^{11,12}. Genetic modification of normal embryos is difficult and inefficient because not all the embryo cells take up the injected DNA and the final animal is a mixture of some GM cells and some non-GM cells. Targeted genetic modification becomes possible if cells can be cultured before being transferred to enucleated eggs and a cloned animal produced. Cells could also be screened and those which are not transgenic, or which have integrated the introduced gene at the incorrect site, can be rejected before cloning. All the cells in the resulting cloned animal should therefore be genetically modified. Genetic modification and gene targeting in cultured

cells, followed by nuclear transfer, was reported in 2000¹³. A GM cloned animal produced in this way may then be bred naturally to produce a line of GM animals such as pigs to use as organ donors or sheep which produce a drug in their milk.

Who is involved in animal cloning?

Cloning is driven by an economic requirement for identical copies of valuable animals – it is seen as a way of imposing control and uniformity on animal production. Commercial interests are therefore at the forefront of the science. The companies involved in cloning animals for agriculture, as pets, for drug production and to increase numbers of endangered animals are summarised in Table 1. This does not include companies using cloning to produce GM animals as organ donors. These were reviewed in GeneWatch Briefing No 19: “*Animal Organs for Humans: The Science and Ethics of Xenotransplantation*”.

As well as commercial organisations, there is also a considerable amount of work involving cloned animals taking place in universities and institutes. The UK’s Roslin Institute leads the way in farm animal cloning, with the research that led to Dolly having been funded by the then Ministry of Agriculture Fisheries and Food (MAFF) and the Biotechnology and Biological Sciences Research Council. Many of the companies listed in Table 1 are spin-offs from universities, especially in the USA and Australia where there appears to be the greatest commercial interest. Geron and the Roslin Institute control most of the patents and intellectual property associated with cloning.

Cloning of mice to understand cell cycles and developmental biology is also being undertaken for medical research.

What effects does cloning have on animals?

There are many fundamental problems to be resolved before cloning is used outside a research context and many would argue that it should not be used at all. Cloned embryos tend to have severe abnormalities, resulting in an extremely high abortion rate,¹⁴ and the majority of those that are born alive seem to have some form of health defect^{15,16}. It has recently been reported that Dolly has developed arthritis of the hip and knee, which could be a result of genetic abnormalities from the cloning process¹⁷.

The reprogramming required for an adult cell to revert to an undifferentiated state and then develop into a range of new cell types may offer an explanation for the large number of abnormalities associated with cloned animals. Cells from very early embryos are ‘undifferentiated’ – that is, they have the potential to develop into any of the cells in the body, and most of the 40,000 or so genes they contain still have the potential to be expressed. As the cell develops into a particular organ or tissue, genes are progressively ‘switched on’ or ‘switched off’ until only those genes required for the correct functioning of the differentiated cell will operate. Until the report of Dolly in 1997, it was thought that this progression was irreversible. Whilst this is obviously not the case, the processes involved are still not understood.

The efficiencies of cloning are extremely low, presumably as a result of the abnormalities in the developing embryos (see Table 2). The percentage of animals reaching adulthood per manipulated egg ranges from 0.5% in cows to 1% in sheep.

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Table 1: The companies involved in agricultural, pet and endangered animal cloning (see GeneWatch briefing No 19 for details of companies cloning animals for use in organ production)

| COMPANY | LOCATION/ PARTNERS | ANIMAL CLONING UNDERTAKEN | WEB SITE |
|-------------------------------|---|---|--|
| Advanced Cell Technology | USA | Agricultural animals (via Cyagra subsidiary and CyClone trademark), pet cell banking, endangered species, chickens (via subsidiary Cima Biotechnology), and mice. | www.advancedcell.com |
| Clone International | Melbourne, Australia | Exclusive licence (from Geron) to clone cattle and sheep in Australia and New Zealand and to clone animals in China. Also exploring cloning of horses. | www.cloneinternational.com.au |
| Genetic Savings and Clone | Works with Texas A&M University, based in Texas. | Targeting dogs, cats, cattle and horses. Also offer gene banking. | www.savingsandclone.com |
| Genetics Australia Ltd | With Monash University, Australia | Focusing on dairy cattle. | www.genaust.com.au |
| Geron | USA | Licenses out its nuclear transfer and gene targeting technologies for agricultural applications. Only works on medical applications itself. | www.geron.com |
| GTC Biotherapeutics | Formerly Genzyme Transgenics Corporation | Use of goats and other animals to produce pharmaceuticals in milk. | www.transgenics.com |
| Hematech | USA, collaborates with Kirin Brewery Co, Japan | Cloning cows with an additional chromosome using Kirin's 'TransChromo' technology to produce therapeutic human antibodies in their blood. | www.hematech.com |
| Infigen | USA | Agricultural animals via Genmark and 'Agriclone' trademarks. Recently cloned prize Guernsey cow. | www.infigen.com |
| Lazaron Biotechnologies | Louisiana, USA, working with Louisiana State University | Agricultural animals, pets, 'service animals', and endangered species. | www.lazaron.com |
| Nexia Biotechnologies | Montreal, Canada | Goats for use in production of pharmaceuticals and other products such as 'Biosteel'. | www.nexiabiotech.com |
| PerPETuate | Massachusetts, USA | Pet cloning company who contract Advanced Cell Technology and Cyagra to undertake cloning work. | http://perpetuate.net/ |
| PPL Therapeutics | Scotland & USA, working with Bayer in drug development | Applied to sheep and cattle for production of pharmaceuticals in milk. | www.ppl-therapeutics.com |
| ProLinia | With University of Georgia, based in Athens, GA | Specialise in pigs and cattle for improved production. Cloned calf from dead cow kidney cells. | www.prolinia.com |
| RAB Australia Animal Genetics | Albury, Australia; working with Clone International | Export of cloned dairy cattle to India, China and Vietnam to improve local stock. | www.rab.com.au/clone |

Table 2: Cloned animals reaching adulthood^{14,18}

| | EGGS MANIPULATED | LIVE BIRTHS | ANIMALS REACHING ADULTHOOD |
|--------------|-------------------------|--------------------|-----------------------------------|
| Sheep | 988 | 26 | 10 |
| Cow | 3524 | 24 | 17 |
| Pig | 511 | 6 | 6 |
| Mouse | 5354 | 65 | 32 |
| Goat | 285 | 3 | Not known |

A range of defects has been reported for cloned animals. In one report of 13 cloned calves, all 8 calves born live required oxygen and 2 subsequently died. The dead calves and aborted fetuses all showed cardiovascular and placental abnormalities. The maternal cows also underwent considerable hardship. 48 cows were impregnated, of which 18 became pregnant. 6 of these aborted, leaving 12 included in the study. 3 of the 12 aborted and died and 1 died after giving birth by caesarean¹⁶.

In another study of 40 cloned calves, 34 showed one or more of the following peri-natal abnormalities: hypoxia, hypoglycaemia, metabolic acidosis and/or hypothermia. 8 calves died before 14 weeks, 1 calf could not stand without external support, and 4 calves had minor limb deformities. Most calves did not suckle vigorously, did not display normal behaviour patterns and would be described as slow or weak. Some required tube feeding. Birth weight and other characteristics varied considerably even in clones from the same embryo¹⁵.

Of 80 genetically modified and cloned lamb embryos transferred to surrogates, only 14 lambs were born alive. All but 3 died before 12 weeks of age with abnormal kidneys, brain or liver¹³.

A recent study compared the expression of various genes in mice cloned from embryonic cells. It found expression in the cloned mice was extremely disturbed and varied wildly. Some clones survived to adulthood despite widespread disruption of gene regulation, showing that even apparently normal animals may have subtle abnormalities. This uncontrolled defective gene regulation can (at least in mice) be transmitted to offspring¹⁹. The use of nuclear transfer techniques for genetic modification may introduce another element of unpredictability into the genetic outcome even while offering a route to more targeted manipulations.

Many reports have recorded abnormally long gestation periods and high birth weights followed by difficult births as well as peri-natal deaths^{20,21}. These effects are also found in bovine pregnancies resulting from *in vitro* fertilisation and it has been suggested that the *in vitro* process could be the cause²¹. While this may be a contributory factor, abnormalities have been found to be markedly greater in clones produced from adult cells rather than embryos with the same *in vitro* processes. The act of cloning from differentiated cells seems to be causal²².

It is not only the cloned animals that are affected by the process. Because the technique involves collecting eggs and implanting the cloned embryos into females to develop, a large number of surgical and non-surgical interventions are carried out on other animals. Female 'donor' animals are induced to superovulate (produce more eggs than normal) by drug administration and the

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eggs are then harvested, which may be surgical or may – in the case of mice, for example – be achieved by killing the animals. (65 slaughtered cows are needed to extract sufficient eggs to produce 10 cloned calves¹⁰.) Fertilisation of the harvested eggs occurs *in vitro* and is followed by embryo implantation in surrogate mothers.

The creation of ‘pseudo pregnancies’ in the surrogate mothers is also achieved with drugs and, in the case of mice, by mating with vasectomised or sterile males. Laparotomy or laparoscopy may be required both for egg extraction and/or embryo implantation. All of these procedures are likely to cause stress and/or pain to the animal. The donor females may also be mated when extremely young.

A very high proportion (87-95%) of implanted embryos in animals other than mice and rats are not carried to term²³. This means that a large number of animals go through miscarriages or stillbirths. Many offspring die soon after birth. It is hard to know how severely this affects each species but certainly larger farm animals are known to suffer distress at miscarriages²⁴. A high proportion of surrogate mothers may suffer ill effects: in one study of nuclear transfer where pregnancy was confirmed in 18 cows, 6 aborted and a further 4 died as a result of the pregnancy¹⁶.

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Farm animals

There are many companies working on cloning with the intention of using it as a means of reproducing expensive animals in agriculture (see Table 1). Cattle cloning is being advertised commercially in the US and Australia. Genetics Australia Ltd is working with Monash University to this end and considers the cost at which cloned embryos could be sold is approaching that of artificial insemination costs – although the success rates (pregnancies and live births) are still unacceptably low¹⁰. High-performing bulls have been cloned under commercial licence in Australia for sale to China and elsewhere²⁵.

Cloning of pigs and cattle is being promoted to increase productivity by ProLinia²⁶, a company formed by University of Georgia scientists, which recently announced the production of a calf cloned from a dead cow’s kidney cell. They claim that their work on pigs is: *“helping them to replicate their choicest pigs to produce better chops and slabs of bacon”*.

Advanced Cell Technology have established a company called Cyagra which is *“in the cloning business to give mother nature a hand in producing duplicates of the very best and most profitable animals in the dairy and beef industries while eliminating costly trial and error breeding. The technology is here. It is called CyCloning!”*²⁷

Farm animals are also being cloned to facilitate their genetic modification, usually to produce drugs in their milk but also in blood, urine or sperm. Recently, calves that produce human antibodies in their blood for therapeutic use with an additional mini-chromosome have been cloned by Hematech²⁸.

As well as the animal suffering involved in the production of clones, the use of cloned farm animals would further narrow the gene pool, which could seriously undermine future breeding efforts. In the longer term, if problems arose such as unexpected susceptibility to disease, the consequences could be disastrous for farmers. There are also other systems such as animal, plant or human cell cultures which could be used to produce therapeutic proteins.

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Pets

There are proposals to clone pets and to produce genetically modified pet animals. The first domestic cat was cloned in the US in 2002²⁹. Of 82 cloned embryos transferred into 8 recipient cats, there was one failed pregnancy and one live clone delivered by caesarean section. The project at Texas A&M University was funded by the owners of a dog, Missy, as a stage towards producing a clone of her. Missy has recently died and her owners are reported to be upset that she was not able to meet her clone and they are investing more millions to mechanise and make cloning more amenable to mass production³⁰.

One company anticipates that the cost for cloning a pet will be approximately \$25,000 and that they may be cloning dogs as early as 2003

The potential profit in cloning pets is already being exploited (see Table 1). The pet cloning programme instigated by Missy's owners has led to the development of a company, Genetic Savings and Clone³¹, which works with Texas A&M University. They already offer a service for cryo-storage of tissue from pets in anticipation of the time when cloning will be possible – for a fee. They anticipate that the cost for cloning a pet will be approximately \$25,000 and that they may be cloning dogs as early as 2003, a service which they plan to offer commercially as soon as they can³².

Genetic Savings and Clone are also offering a gene banking service for cattle, sheep, goats, pigs and horses. They are developing cloning for horses and for assistance and rescue dogs. The company names its “big four” as dogs, cats, cattle and horses.

The drive behind the cloning of pets appears to be the desire to ‘replace’ an animal that had special attributes or was greatly loved. This is built upon a misapprehension that genes alone will determine how an animal looks or behaves. The first cloned cat had a different coat colour and pattern than the animal it was cloned from because of environmental factors in the womb. An animal's behaviour is strongly influenced by its early experiences – whether it socialised with others of the same species or people, for example. Cloned animals may not live up to people's expectations of them and, in developing the techniques, many of the same species will have had to suffer. It is difficult to understand how this can be morally justified.

Extinct and endangered species

There have been a number of attempts to clone extinct and endangered animals, including the Asian gaur³³ (an endangered wild ox), the mouflon lamb³⁴ (a rare breed of sheep), the woolly mammoth³⁵, and the panda³⁶. Only the gaur and the mouflon were born live, and only the mouflon has survived for more than a few days. There are also plans to clone the Indian cheetah, which became extinct 50 years ago³⁷. To overcome the problem of the limited availability of genetic material - resulting from the small size of remaining populations - scientists also hope to use genetic material from dead animals³⁸. As with pets, commercial interests are already anxious to become involved (see Table 1). Advanced Cell Technology produced the gaur clone and Genetic Savings and Clone are researching cloning of wildlife and endangered species and plan to start a gene banking service soon.

Given the considerable problems with producing healthy offspring even in well known species, cloning extinct animals is extremely unlikely to be successful

Given the considerable problems with producing healthy offspring even in well known species, cloning extinct animals is extremely unlikely to be successful. Not only will it be difficult to assess whether live offspring properly represent their species, but the technology ignores the fact that a species is a product of an interaction between genes and environment. A cloned extinct animal will not have the opportunity to develop normally as it will have none of its own species

from which to learn and, in most cases, will not be able to live in the environment in which it evolved. These factors are likely to have a considerable negative impact on its welfare.

Using cloning to 'rescue' endangered species is a bizarre strategy. The major factor which renders a species endangered is habitat loss. In the same habitat as every endangered large animal - the ones that are usually noticed - there are almost always many other species of animals, insects and plants that will be lost. Cloning the 'headline' species does nothing to preserve the habitat or the associated species. The considerable resources used for cloning would be better spent contributing to more effective habitat management and preservation. Even if clones of extinct animals were successful, there would usually be no habitat left in which they could survive. In addition, endangered species have drastically reduced gene pools and it is certainly possible that cloning will introduce further genetic weakness.

Lastly, there is the danger that proposing cloning to 'save' endangered species will actually undermine efforts to protect habitats by giving people the false impression that extinctions are reversible.

Conclusions

At present, cloning technology is fraught with problems and each cloned animal is produced at great expense to the welfare of many others. The reasons for embryo abnormalities and peri-natal death are poorly understood, but the problems have appeared in all species which have been cloned. GeneWatch does not believe that cloning of animals is justifiable because of the suffering involved for the individual animals in the short and long term and the wider dangers that it brings. Further narrowing of gene pools in agricultural animals will not improve animal welfare nor increase world food security. The production of designer pets will involve yet more animal suffering. Cloning extinct animals encourages the pretence that endangered species can be saved. Cloning also normalises a technology which could at some point be extended to humans. None of this can be justified by the narrow commercial interests at stake.

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GeneWatch



**The Mill House, Manchester Road, Tideswell, Buxton, Derbyshire, SK17 8LN, UK
Phone: 01298 871898 Fax: 01298 872531 E-mail: mail@genewatch.org**

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