FEDERATION OF VETERINARIANS OF EUROPE

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FVE Review Paper
Animal Cloning

Executive Summary

The cloning of mammals is a relatively new technology that is starting to be applied in the breeding of elite cattle, pigs, goats and sheep, to restore dead pets particularly dogs and cats, to conserve rare breeds and even to resurrect extinct species, as well as in research using a variety of species. There is little data on which to base a robust opinion, and as with many developing technologies, research studies constantly change the situation. At present there are serious prenatal losses of cloned embryos and fetuses, especially in cattle and other ruminants. Losses are still considerable during the neonatal period and serious welfare problems can still occur up to 3-6 months. These losses will impact on the welfare of the surrogate dam when there is a high incidence of dystocia and Caesarean section due to 'Large Offspring Syndrome' in ruminants.

There is no evidence that food safety is adversely affected, but nevertheless the public are concerned for ethical and other reasons, such as: poor welfare due to a focus on agricultural productivity; a slippery slope towards human cloning; a reduction in genetic diversity, and environmental impact.

In terms of farm animal health and welfare there is a mixture of potential for good and bad depending on the genetic traits selected. If cloning was undertaken only from animals with a proven sound conformation and good productivity and as a consequence with low levels of disease and good welfare, then cloning could be a force for good, providing the current welfare and efficiency problems associated with cloning are overcome. On the other hand, if cloning is simply to be used to promote productivity in an already welfare compromised farmed species it could be misused, and an opportunity lost to improve animal health and welfare.

For the other uses of animals, cloning is highly contentious and the harms and benefits need to be carefully weighed, as well as whether the objectives are realistic. However, the use of animals in research e.g. to study the impact of epigenetic variability on phenotype and genotype-environment interactions would be approved under other legislation.

The role of the FVE could be to help evaluate the harms and benefits in Europe from a veterinary viewpoint, including whether the technique of cloning itself is unethical.

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1. Introduction

The early ground-breaking work in animal cloning was in amphibia in the 1950s and ’60s looking at fundamental questions in developmental biology concerning the ability of cells to differentiate into all other cell types (totipotency and pluripotency) and when and how this ability was lost. More recently, it is the cloning of mammals by SCNT (Somatic Cell Nuclear Transfer) that holds the most interesting opportunities for research and potential practical applications.

This report sets out the present situation in regard to the cloning of animals, mainly for food production but also for other purposes. The scientific data on the impact of cloning on animal health and welfare are summarised, as well as areas of uncertainty, and the Opinion concludes with the potential roles for the veterinary profession. It also includes summaries of: public perceptions and ethical issues, impact on genetic diversity, biodiversity and the environment, and on food safety. Several other reports on cloning have been recently produced, and a list of them and other key references are given at the end.

2. Terminology and technologies involved

Cloning is a process by which animals are reproduced asexually. The word cloning is applied to different techniques (Royal Society 1998) but this opinion concentrates on cloning by somatic cell nuclear transfer (SCNT). In the future it can be expected that SCNT will be the commonest technique to be used.

SNCT can be used in combination with other techniques, for example transgenesis, genetically modified stem cells, in which case the impact on the animals will be a combination of the cloning technology and the transgene or other manipulation.

In this document the term ‘clone’ is used to describe an animal born using SCNT where the nucleus of an unfertilised oocyte is replaced by the nucleus of a somatic cell derived from fetal or adult tissue, and the resultant ‘embryo’ is then activated to develop without fertilisation.

The ‘source animal’ is the animal from which the somatic cell (occasionally stem cells are used) was taken and the ‘surrogate dam’ is the recipient into which the cloned embryo is transferred. ‘Clone progeny’ refers to the offspring born by sexual reproduction where at least one of the parents was a clone.

3. Public opinion in Europe

The Eurobarometer Poll (2008) on cloning showed that a large majority of EU citizens (80%) knew the definition of animal cloning, but 84% had concerns about the unknown long-term effects on ‘nature’. Many respondents were concerned that animal cloning might lead to human cloning (77%), and that the process of cloning itself raised moral issues (61%) in that it might lead to decreasing genetic diversity. Furthermore, 69% of the interviewees responded that animal cloning risked treating animals as commodities (instrumentalisation) rather than creatures of inherent value that had feelings. Some were willing to accept cloning for some purposes e.g. to preserve rare breeds (67%); for improving disease resistance in animals (57%), but not for increasing food production (58% against). Furthermore, if food products from the offspring of cloned animals become generally available 83% would want special labelling of these products.

Conclusion: In 2008 a majority of EU citizens have concerns about animal cloning and a majority is not willing to accept animal cloning for food production purposes. Furthermore, if food products from the offspring of cloned animals become available they would require them to be labelled.

4. The present situation

Species that have been cloned include mainly laboratory and farmed animals (cattle, pigs, goats, sheep, buffalo and, more recently, fish), companion animals (dogs, cats), and horses. Other species include those that have been threatened with extinction and there is some speculation that it may be possible to help conserve rare breeds and even to retrieve animals that have become extinct.

Farmed animals particularly sheep and cattle, but soon followed by pigs, goat, rabbits and horses, were amongst the first mammalian species to be cloned. These species were chosen because of their economic importance and also because of already existing and well-developed assisted reproduction techniques, such as large numbers of oocytes from the slaughterhouse, *in vitro* production of embryos and their transfer into surrogate dams.

The first mammalian clone in 1996, was a sheep called Dolly, but even today the efficiency of SCNT is still low and variable, with many species differences. The reasons for failure are still being unravelled but seem to be related to the source of nuclei, their genetic reprogramming, abnormal gene expression due to epigenetic effects, and with the techniques of micromanipulation.

A list of health and welfare issues involved in the cloning process is given in Table 1 (page 9).

- **Cattle**

The pregnancy rates are acceptable and few animals are culled for this reason. The viability of the pregnancies is associated with early post-implantation losses in the first trimester at the time of placental development (30-70 days). This can be detected by ultrasound using crown rump measurements and fetal heart rate. Later in pregnancy, hydrops of the fetal membranes and fetal overgrowth/oedema give rise to so-called Large Offspring Syndrome (LOS). This can be detected by rectal examination, abdominal circumference, fetal death and transabdominal ultrasound (hyperechogenic placentomes, speckled allantoic fluid). LOS, unsurprisingly, is associated with a higher rate of dystocia and Caesarean section (although many CSs were elective for fear of losing a clone at natural birth). The success rate, measured as the percentage of all embryos implanted resulting in a live offspring at birth, can vary from 1 to 20% but typically is 1 to 5%. Perinatal deaths or euthanasia are due to umbilical infection, enlarged internal organs, sudden death, reluctance to suckle, and difficulties in breathing and standing (flexor tendon contraction, joint laxity), hypoglycaemia, paradoxical hyperthermia, infections, heart abnormalities, bloating, and internal haemorrhage. Overall, up to 40% or more of clones are fatally affected before 6 months of age.

While the fetus is unlikely to experience any adverse effect if it dies *in utero*, the impact on the surrogate dam has to be considered. This may involve her carrying an overweight fetus and placenta, resulting in dystocia and an eventual Caesarean section.

The welfare of cattle clones that are born alive and survive to weaning may be seriously compromised and they also take longer to reach a normal homeostasis, the impact of which is not known. After weaning there is little evidence that their health and welfare are affected within their production lifetime. Longer term studies that might be relevant for breeding animals are not yet available.

- **Pigs**

In the pig, late losses in pregnancy appear to be far less frequent and occur at 35-45 days, and also between 60 and 70 days. Retardation of growth *in utero* has been recorded. Reduced, litter size is observed (range 1-12 with an average of 4-6) but can be compensated through transferring more embryos. Neonatal problems are rare.

- **Goats**

Have similar pre-natal problems to cattle but there is little quantitative data.

- **Sheep**

Have similar pre-natal problems to cattle (sporadic losses of fetuses during the second and third trimesters, with major placental and hydrops conditions) and pregnancy toxaemia. Fetal and neonatal animals have had abnormalities of the renal, cardiac, hepatic and musculo-skeletal systems. Again there is little quantitative data.

- **Horses**

Late pregnancy losses are rare and this may be because it has a similar type of placenta to the pig, and also be the reason for the difference with ruminants.

- **Dogs and cats**

In companion animals the work is complicated by the paucity of oocytes to work with and especially in dogs by the fact that matured oocytes can only be obtained after *in vivo* ovulation. In dogs and cats the success rate is even lower than with farm animals with a high rate of stillborn animals after cloning.

- **Research**

All cloning is, of course, at a research stage but cloned rodents, and many other vertebrate species, are being used.

Conclusions:

At present there are only a limited number of quantitative studies available (in cattle and pigs) for analysis for the assessment of the health and welfare of cloned animals over their production lifetime. In addition, there are many confounding and causal factors within the reported studies that could influence any assessment. Nevertheless, it can be concluded that the process of cloning is very inefficient and has a high failure rate with fatal outcomes.

Cloning efficiency remains significantly lower than other assisted reproductive techniques, but there are notable species differences that make the outcomes significantly different. However, as mechanisms are increasingly understood and possible remedies discovered, it is likely that the efficiency of SNCT will improve, and so the spectrum of potential applications will broaden.

5. Reasons for cloning

Breeding stock: One of the main reasons at present to clone farm animals is to preserve the breeding capacity of genetically elite animals (proven through progeny testing), particularly males and to insure against loss of valuable genetic and characteristic features (as well as an insurance against future loss). This would apply to germplasm products such as oocytes and particularly semen as the monetary return from a male clone can easily write off the cost of producing it.

Pig cloning involves the use of valuable boars for two reasons. First, because billions of sperm are required for artificial insemination, and so any given boar can serve only a small number of females compared with bulls. Second, the most effective way to evaluate the genetic quality of a pig is through a detailed analysis of the carcass. Cloning offers the opportunity to evaluate the quality of a boar by slaughtering it and then using numerous copies of those individuals whose carcasses meet the required standards. In females similar reasons can be given and so oocytes can also be valuable.

For other animals it may be to restore their breeding capacity e.g. after injury, disease, old age, or that they have been previously castrated (horses, dogs, cats).

European farm animal breeders are in the business of selecting the best parents for the next generation from the diverse and distinct European livestock in a market that is highly competitive. While there is no practical benefit, at present, at farm level, it is being employed by breeding companies at the forefront of global research and development. Cloning can increasingly be expected to be a worthwhile strategy to produce high value breeding lines that can be used to expand elite pedigree stock. The perceived increase in 'value' may be agricultural: increased performance (food conversion, growth rate); improved health and welfare (e.g. resistance to infectious disease, lower incidence of non-infectious disease such as lameness and mastitis); good conformation (reduced disease); and aesthetic value (good appearance).

Pet replacement: Some owners want to have a replica of their pet animals in the belief that they can 'replace' that loss.

To preserve a rare or extinct breed. The cloning of the Gaur and the Mouflon are examples where the enucleated oocytes of the cow and the sheep, respectively, were used to produce a cloned embryo. However, in these cases it was possible to use surrogate dams of the rare species. In the case of extinct breeds cryopreserved DNA, naturally occurring samples or preserved tissues could possibly be used providing an appropriate surrogate dam can be found.

In research: cloning is being increasingly used in developmental biology; in the aetiology and pathophysiology of disease; in biotechnology for the production of therapeutic agents e.g. pharmaceutical proteins, organ development, in xenotransplantation; and possibly in safety testing.

6. Future developments using cloning

Notwithstanding the current inefficiencies in cloning and the poor welfare of many of the animals involved, there is considerable potential for improving the health and welfare of farm animals in the future. To date, much of the breeding of farmed animals has concentrated on their productivity to the exclusion of genetic traits involving health and welfare. However, there are clear signs that this is now changing and efforts are being made to incorporate welfare traits associated with reducing the incidence of diseases such as lameness in pigs, poultry and dairy cows, mastitis in dairy cows, and aggression in pigs, even though this may mean reducing some of the productivity indices.

This welcome change in direction needs to come about as quickly as possible to improve the current health and welfare status of farmed animals. One way would be to use the opportunities presented in the selection of genetic traits of the female lines as well as the male lines through cloning in the pedigree herds and flocks. This will enable high welfare quality animals to feed down from these pedigree lines though the existing market structures. In this way agricultural practices are more likely to be sustainable with good agricultural productivity in a variety of environmental and farm conditions, and with the production of marketable welfare friendly products acceptable to the general public.

7. Genetic diversity and environmental impact

The evolutionary success of mammals relies on sexual reproduction making the species more adaptable to changing environments. This is in contrast with cloning procedures where genetic variation is reduced to multiply selected elite genotypes. The reduction of genetic variation may cause an increased susceptibility to new or emerging diseases that affect a single genotype. Animals could also be selected on the basis of their environmental impact to control for example green gas emissions, reduced pollution in the underground water.

8. Food safety

All reports and research to date have shown that there are no significant physiological differences between clones, their offspring and conventional animals that would threaten food safety. While some biochemical differences in body tissues have been noted in cloned animals, again, they do not seem to pose any threat to food safety, and they seem not to be present in the offspring of clones. However, further studies are needed and this necessitates that animals and their products are traceable.

There are few long term research studies on these animals (cattle and pigs at the moment but in the future rabbits, horses) to know if there are health or welfare issues that might affect animals kept for longer periods e.g. for breeding.

9. Ethical aspects

Given that all animals are used (some would say exploited i.e. instrumentalisation) by humans in a variety of ways it is important to respect their intrinsic or inherent value so that they are treated humanely and not caused avoidable suffering. That is that humans have a duty of care for the animals that they own or produce for society. This demands that attention is paid to their health and welfare and that the integrity and dignity of animals is respected.

Health and welfare issues already exist in modern day farming and, theoretically, it is possible to use cloning to promote better animal welfare as opposed to producing animals whose welfare may be poor. This is what ought to occur.

10. Role of the veterinary profession

Veterinarians are involved in various ways in cloning technology through being scientists, animal welfare officers regulating the welfare of experimental animals in some way, being involved in commercial companies rolling out this technology, being general practitioners on the farms, and in protecting the safety of food for the public.

The opportunity to promote good welfare and to reduce poor welfare should not be lost and the profession could and should play a role in ensuring that both these ethical goals are achieved.

KEY REFERENCES

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Table 1: **Health and welfare issues involved in the cloning process**

Species	Source animal (if alive)	Sourcing of oocytes	Surrogate dam	Pregnancy abnormalities	Clones	Post natal mortality	Progeny of clones
Cattle Buffalo	Limited discomfort during biopsy	Slaughterhouse	Non-surgical transfer, possible hydrops, Caesarean sections	Hydrops, late abortions, LOS[1]	Enlarged umbilical cord, higher stillbirth rate, LOS	Respiratory problems, immune suppression	Normal
Sheep	Limited discomfort during biopsy	Slaughterhouse	Surgical transfer, possible hydrops Caesarean sections	Hydrops, late abortions, LOS	Enlarged umbilical cord, higher stillbirth rate, LOS	Respiratory problems, immune suppression	Normal
Goat	Limited discomfort during biopsy	Slaughterhouse	Surgical transfer, possible hydrops Caesarean sections	Hydrops, late abortions, LOS	Enlarged umbilical cord, higher stillbirth rate, LOS	Respiratory problems, immune suppression	Normal
Pig	Limited discomfort during biopsy	Slaughterhouse	Surgical transfer	Rare, low rate of abortion	Small litter size, variable size of piglets	None	Normal
Horse	Limited discomfort during biopsy	Slaughterhouse	Non-surgical transfer,	Rare, low rate of abortion	None	None	Normal
Cat	Biopsy under general anaesthesia	Spayed ovaries	Surgical transfer Caesarean sections	Rare, low rate of abortion	High still birth rate	Respiratory problems, immune suppression	N A
Dog	Biopsy under general anaesthesia	Surgical recovery of ovulated oocytes	Surgical transfer Caesarean sections	Rare, low rate of abortion	moderate stillbirth rate	Respiratory problems, immune suppression	N A

[1] Large Offspring Syndrome