719. Broom, D.M. 2018. Animal welfare and the brave new world of modifying animals. In *Are we Pushing Animals to their Biological Limits?* Eds T. Grandin and M. Whiting, 172-180. Wallingford: CABI.

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Introduction

Conventional breeding can have extreme negative effects on the welfare of animals; for example the breeding of dogs for cosmetic features and of broiler chickens and dairy cows for high levels of production. Any breeding that can be predicted to result in poor welfare should be illegal. Biotechnology can lead to much faster and greater change in the animals than can conventional breeding, so needs special legislation. Ethical consequences of the work should be considered whenever research in biotechnology is carried out. Genetic modification of animals and, to a lesser extent, cloning can have some positive effects on animal welfare. However, cloning procedures for farm animals have negative effects that are so great that current methodologies are never likely to be acceptable. Given the developments in the scientific assessment of animal welfare, the consequences of genetic modification can be evaluated. A checklist for animal welfare should take account of current scientific knowledge about assessing animal welfare and should be further developed for evaluating all genetically modified animals. When sold, every GM product for use with animals should have details of properly tested effects on animal welfare.

Domestication and Conventional Breeding of Animals

Animals kept and used by people have always been modified from their wild state, because only some of those that are kept survive, and hence this selection results in some genetic change in the population kept. In addition, people deliberately selected animals with certain characteristics. A definition of domestication (Broom and Fraser, 2015, modified after Price, 2002) is: the process, occurring over generations, by which a population of animals becomes adapted to man and to the captive environment by some combination of genetic changes and environmentally induced developmental events. The animals that have been domesticated, or, one might say, the animals that have allowed themselves to be domesticated or which have domesticated humans, are mainly social species with high levels of cognitive ability.

Selection of farm animals was initially limited to docility and manageability, but in the last 60 years, breeding programmes have focused on the genetic improvement of production traits, such as milk yield, growth rate and number of eggs, and were

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based on animals' phenotypes. A major advance in selection practice occurred in the mid-20th century with the advent of quantitative genetics based on principles of heredity and modern statistical theory (Hazel, 1943). The method is still based on phenotypic selection but more easily identifies variation at loci. Essentially, the statistical genetics method calculates an average of all genetic loci contributing to a trait as transmitted by the individual, and reports it as an estimated breeding value (EBV) (Lynch and Walsh, 1998). As a result, the animal production industry has undergone dramatic change during the last century (Ensminger and Parry, 1996).

Conventional breeding methods need not affect welfare but can change animals in such a way that they have more difficulty in coping or are more likely to fail to cope (Broom, 1995; 2008). One example of such an effect is the sensory, neurological or orthopaedic defects found commonly in certain breeds of dog. Others are the effects of the genes promoting obesity in mice, double muscling linked to parturition problems in cattle, and many examples of selection promoting fast growth and large muscles in farm animals. Modern strains of pigs have relatively larger muscle blocks, more anaerobic fibres and smaller hearts than their ancestral strains (Dämmrich, 1987). They are more likely to die or to become distressed during any vigorous activity, for example during transport. Modern broiler strains grow to a weight of 2– 2.5 kg in 35 days, as compared with 12 weeks 30 years ago. Their muscles and guts grow very fast but the skeleton and cardiovascular system do not. Hence many of the birds have leg problems, such as tibial dyschondroplasia or femoral head necrosis, or cardiovascular malfunction, often giving rise to ascites (Bradshaw *et al.*, 2002). Genetic selection of dairy cows for high milk production has led to increased leg disorders, mastitis and reproductive disorders, all of which are major welfare problems (Oltenacu and Broom, 2010). Any breeding that can be predicted to result in poor welfare should be illegal.

Biotechnology Definitions and Methods

Those aspects of modern biotechnology that are having, or are likely to have, the greatest impact on animal welfare are the use of genetically modified animals (GM animals) and cloning by nuclear transfer. The term 'GM animals' is used here to refer to animals whose genetic material has been altered using a method that does not occur naturally, but excluding chemical or physical mutagenesis. Cloning is not genetic modification but it means producing more than one genetically identical individual. Cloned amphibians were produced by Gurdon and collaborators in the 1970s (see review by Gurdon and Byrne, 2003), and many mammals have now been cloned. The first GM animal, a mouse, was made in the early 1980s (Gordon *et al.*, 1980; Palmiter, 1986) and this technology has been successfully applied to most mammals including cattle, pigs and sheep (Hammer *et al.*,1985; Simons *et al.*, 1988), and to poultry (Love *et al.*, 1994) and fish (Devlin *et al.*, 2001).

Microinjection was the earliest method of making GM animals. Electroporation has also been used. Both of these lead to mosaics, i.e. genetic variability in the cells of the animal. Sperm mediated gene transfer (SMGT) is the injection of a transgene vector, often viral, for GM. Androgenesis, gynogenesis and embryonic cell nuclear transplantation (ECNT) are used for cloning. Somatic cell nuclear transfer (SCNT) is used for cloning or for GM.

Effects of Biotechnology Procedures on Animal Welfare

Animal welfare varies from very good to very poor and can be evaluated scientifically using a wide range of measures (Broom and Fraser, 2015). The effects of biotechnology procedures on animal welfare might be: (i) to improve it; (ii) to have no effect on it; or (iii) to make it poorer. Members of the public express positive and negative views about GM and cloning. Some of the issues raised by scientific studies and public comments are listed in Boxes 12.1 and 12.2.

Examples of benefits from the effects of genetic modifications of animals are: to benefit the animals by conferring disease resistance (Behboodi *et al*., 2005); to help to treat human disease by producing a blood clotting factor in sheep's milk, (Houdebine, 2005); to develop new products for other purposes (Niemann and Kues, 2003); and to increase efficiency of animal production (Wheeler, 2003). Some people would find none of these to be acceptable whilst others might accept them all. Many people would accept some with qualifications, and a major reason for rejection is that animal welfare may be poorer in the modified animals than in those that are not modified. A major problem in relation to GM research is the complete failure of the community of researchers in this area to investigate the welfare of the animals produced. In a

Box 12.1. Possible negative effects of cloning and GM.

1. Welfare problems – effects of the procedure, e.g. SCNT can lead to placental or foetal abnormality.
2. Welfare problems – effects of the transgene, e.g. insertion of the human growth hormone gene into pigs has caused major growth abnormalities.
3. Genetic uniformity in the population produced could increase the risk of disease epidemics.
4. There could be effects on the safety of transgenic animal products for human consumption.
5. There could be effects of transgenic animals on wild animal populations, for example those of fish that escape from captivity.
6. Ethical issues and societal issues such as equity of access to products by consumers, or freedom to make ethical consumption choices.

(modified after Broom, 2014)

Box 12.2. Possible positive effects of cloning and GM.

1. Improved welfare for the transgenic animals, for example due to deliberately enhanced disease resistance.
2. Reduction in the number of animals required for breeding programmes – cloning allows copying of individuals so fewer are needed.
3. GM change could enhance the nutritional value of animal products.
4. Decreased pollution if GM increases animal digestion ability.
5. Reduced cost of food, increased production of food.
6. Engineering of animals suited to arid or other harsh environments.

(modified after Broom, 2014)

recent review of developments in transgenic animal production, Murray and Mega (2016) wondered why no transgenic animal had been taken up for animal production, but at no point in their paper did they mention the welfare of the animals produced.

Effects of Cloning Procedures on Animal Welfare

There are effects of cloning procedures on animal welfare. Cloned common carp and rainbow trout are more variable and some do not survive well. A proportion of the cloned fish offspring are haploid and non-viable whilst diploid hatchlings appear to have normal survival. Birds cannot be fully cloned at present but there has been primordial germ cell transplantation, involving some cloned cells, in domestic chicks. The hatching rate of these birds was reduced by about 60% and survival of hatched to adulthood was reduced by 20%. Bovine clones have a high level of mortality, particularly *in utero*, where only 27% of pregnancies survive to term (e.g. Chavatte-Palmer *et al*., 2012). There is also increased mortality in early life, and cloned cattle often show developmental problems such as the large offspring syndrome. Cloned pigs show some increased early mortality, and in the few animals studied, the life expectancy was reduced. When sheep clones were produced, only 42% of pregnancies were maintained and only 50% of live-born lambs survived to weaning. For goat clones, 31% of pregnancies were maintained but 80% of live-born kids survived to weaning. In three studies of horses, 2%, 3% and 26%, respectively, resulted in a birth (Campbell, 2016).

Many of these problems are a result of epigenetic abnormalities. If the clones survive the juvenile period there are usually no further welfare problems. For example, Sinclair *et al*. (2016) found that the osteoarthritis described in the SCNT-produced, cloned sheep Dolly was not present in 12 other similar cloned sheep. Neither were there metabolic or other joint disorders. The severe welfare problems are during the production of the cloned animals rather than in those that survive.

In general, mortality is too high and problems are too frequent with SCNT. Chavatte-Palmer *et al*. (2012) report that after cloning using SCNT, placentomegaly and foetal overgrowth are commonly observed; placental vascularization is modified; steroidogenesis is perturbed so there is lack of preparation for parturition; production of glycoproteins in the mother and production and transport of sugars in the young animals is modified. Gene expression analyses of the SCNT placenta show that multiple pathways and functions are affected. There have been some attempts to improve methodology but Rodrigues Sangalli *et al*. (2014) found that treatment of cloned cattle cells with valproic acid did not improve survival. For farm animals, cloning procedures are very negative for welfare and are not likely to be acceptable to the public or legislators, or to be commercially viable except in experimental situations.

Effects of Genetic Modification Procedures on Animal Welfare

The following are some effects of genetic modification procedures on animal welfare. Most GM work is part of biomedical research with a small amount of work involving farm animals. However, most of the examples given here are for farm animals:

1. The production of the DNA often involves no animal welfare considerations because the source is tissue culture, human cells or animals that are killed humanely.

However, if embryos or tissues must be removed from living animals in order to obtain the DNA, effects on welfare must be considered.

2. The production of an embryo for the insertion of DNA involves procedures used in producing lines of GM animals that may have negative welfare consequences for the donor animals: (a) The donor female may be injected with hormones to produce large numbers of oocytes; (b) In large animals, artificial insemination may be used, sometimes using laparoscopy or laparotomy to fertilize the oocytes; (c) Embryo collection may involve killing the female or procedures such as oviduct flushing during laparotomic surgery.

3. During microinjection of DNA into the embryo, there is evidence that microinjection of the transgene itself can lead to increased foetal loss. Many embryos injected with DNA die. However, this occurs at an early stage of development so is not a significant welfare problem for the foetus.

4. When there is production of GM offspring, the insertion of the DNA construct within the genome can cause disruption of genes at that site, or there may be effects of the inserted gene. These effects may be apparent at birth, or may only become apparent at a later point in the animal's development, or when it is put under some kind of stress or put into a particular type of environment. The survival of transgenic, cloned offspring after SCNT in cattle is similar to or better than that of non-GM cloned animals. However, in pigs, survival is somewhat worse. There are alternatives to SCNT and these can result in fewer problems.

5. When GM animals are fostered onto normal females, the welfare of the fostered pups may be poor. Also, normal pups of the foster mother may have been killed to allow fostering of GM pups and the method of killing could affect welfare.

6. The consequence of the genetic change for the GM animals is a major animal welfare issue for laboratory animals because many of the GM animals are produced in order that they will be susceptible to developing pathological conditions. For example, GM mice are produced that are likely to develop a tumour in order that anti-cancer treatments can be tested on them. Most people would say either that this should never be done or that the tumour development should never be allowed to reach the point where the animal would suffer. Some genetic modifications lead to an unexpected malfunction.

It is clearly necessary to use good quality animal welfare science measures to check each GM line that might be continued in order that they will not be continued if problems exist. No problems were revealed in a study of the behaviour of sheep genetically modified to produce human alpha-l-antitrypsin, which is used for treatment of human emphysema, in their milk (Hughes *et al*., 1996). However, the sheep did not live long. Salmon and other fish transgenic for a growth hormone gene have been produced. Many of these have an enlarged head and a bulging operculum. The problems become worse with increasing age.

Other positive and negative examples of genetic change effects are that GM catfish with a gene for cecropin are more resistant to enteric septicaemia. GM grass carp transgenic for human lactoferrin are resistant to haemorrhagic virus and *Aeromonas hydrophila* infection. Transgenic chickens that can synthesize RNA that interferes with influenza virus replication and packaging are less likely to suffer from or transmit the disease (Lyall *et al*., 2011). Some other GM chickens had positive and negative

anti-disease consequences. As mentioned above, pigs transgenic for human growth hormone have many negative effects. However, Huber *et al*. (2012) assessed the welfare of a large number of pigs transgenic for the green fluorescent protein gene and found no deleterious effects.

An alternative to transgenesis is the direct administration of transgenes to the tissues of adult animals, resulting in a transient transgene expression in these tissues. Han *et al*. (2007) infused a vector carrying the bovine lactoferrin gene into the mammary glands of goats via the teat canal. Lactoferrin was expressed in the milk for up to about a week, with the potential to protect against mastitis. As in most GM studies, the consequences have not been evaluated using a range of welfare indicators.

Genetic Modification, Cloning, Public Attitudes and Laws

Biotechnology can lead to much faster and greater change in the animals than can conventional breeding, so needs special legislation. The production of GM or cloned animals is allowed only in specified circumstances by the law in the UK and several other countries. The creation or duplication of favourite pets, or of animals intended as toys or fashion accessories would not be permitted. Box 12.3 summarizes the views of government committees, such as the UK Animal Procedures Committee, and of the public in the EU about what is not acceptable in GM animal production.

Use of GM Products and Animal Welfare

Whilst the majority of this section refers to genetic modification and cloning of animals, it is also necessary to consider carefully any proposed use of genetically modified materials that would change an animal in some way. An example is the injection of a form of the hormone bovine somatotrophin (BST) produced by genetically modified bacteria. Although BST is a naturally occurring hormone, the GM form is slightly different,

Box 12.3. What are publicly unacceptable consequences of genetic modification?

1. Animals should not be produced if they would be subject to harm of a degree and kind that ought not, under any circumstances, to be inflicted upon an animal; for example, GM animals that would suffer severe or lasting distress, including animals to be created as disease models, unless there is clear evidence that the problems could be handled humanely.
2. The production of GM animals should not occur if such work is likely to strip animals of their biological integrity or render them incurably insentient.
3. There should not be production of chimaeras, especially human–animal chimaeras, or of hybrids that involve a significant degree of hybridization between animals of very dissimilar kinds.

(after Broom, 2014)

chemically, and the amounts that can be given to cows to increase milk production are much greater than those that would normally be present in the animals. The question considered by two EU scientific committees was whether or not there was scientific information about the consequences of the use of BST that would allow a decision about permission for its use in the EU. One report concerned animal welfare, the other human consumer health. A later report concerned dairy cow welfare in general.

An assessment of the risk to consumers if dairy cows are regularly injected with recombinant BST was conducted (European Union Scientific Committee on Public Health, 1999). This identified a very small increased risk because there is increased concentration of insulin-like growth factor (IGF-1) in milk and IGF-1 can make existing tumours grow faster. However, a much bigger effect on animal welfare was found (European Union Scientific Committee on Animal Health and Animal Welfare, 1999). There was an increase of about 35% in the risk of clinical mastitis above the risk in non-treated cows, as demonstrated using meta-analyses or large datasets. BST increased the incidence of foot disorders by 2.2 times, with 2.1 times more days affected. The pregnancy rate dropped from 82% to 73% in multiparous cows and from 90% to 63% in primiparous cows, and multiple births were substantially increased. There were severe reactions at the injection site in at least 4% of cows. These comparisons were made between untreated cows and BST-treated cows with a much greater milk yield. The extra milk yield is a key factor as the highest-producing cows have greater incidences of lameness, mastitis and reproductive disorders, whatever the means of pushing the cows metabolically to high production (EFSA, 2009; Oltenacu and Broom, 2010). A recent paper by American animal scientists using data on use of a dose of 500 mg for 14 days found no adverse effects of BST usage on some reproductive problems, lameness or mastitis (St Pierre *et al*., 2014). However, the quality of some of the measures of welfare was not clear, and any comparison of BST-treated and other high-producing cows is subject to the problem described above. As a result of the publications and the reports summarizing this information, the use of bovine somatotrophin was banned in the EU and in most other countries. Although low-producing cows, caused to produce at a somewhat higher rate by BST, may not have worse welfare as a consequence, many producers use BST to make fairly high-producing cows very high-producing. This will always cause poor welfare, and, if BST use is legal, some producers will use it to push cows to very high levels of milk production. In order to prevent poor welfare of cows, EFSA recommended that producers who have more than 10% of their cows lame should be penalized and that the ban on BST should not be continued. Both of these measures would improve welfare, and the number of cows dying or culled early would be reduced, so there is also an economic advantage.

Conclusions

Some conclusions can be drawn as a result of information like that described above.

1. Moral issues associated with biotechnology will be considered by the public and should be taken into account at an early stage in any biotechnology research.
2. One of the possible consequences of the use of GM products or GM animals is on animal welfare. Many different systems for coping with the environment should be considered when assessing welfare. These systems interact, and health is an important part of welfare in such assessments.

3. A checklist for animal welfare should take account of current scientific knowledge about assessing animal welfare and should be further developed for general cage-side use in the case of GM animals.
4. When sold, every GM product for use with animals should have details of properly tested effects on animal welfare.

Acknowledgements

I thank Dr Richard Kirkden and Dr Toni Oltenacu for collection of data and helpful discussion.

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