INVITED ESSAY

GENETICALLY MODIFIED ANIMALS, WELFARE AND UK LEGISLATION

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Introduction

The first experiments in genetic engineering took place in the late sixties and early seventies, but animal transgenics (the insertion of DNA into the genome of an animal) really came of age with the production of mice whose genetic sequences contained a gene for human growth hormone (Palmiter *et al* 1982). Since then there has been a dramatic increase in the quantity of research into genetic modification, using various techniques. This has been largely driven by the potential medical and financial benefits of being able to modify animal and plant genomes (Carver *et al* 1993; Smith 1994; Stewart *et al* 1993).

The laboratory mouse has been the subject of most of the research on genetically modified animals, but other species such as rats, chickens and other farm animals have also been used. Much of the research is directed at understanding how genes act and how to use this information to control disease in humans and domesticated animals. Another area of interest is the potential of transgenics to modify the genome of farm animals so that they produce large amounts of valuable pharmaceuticals (Romagnolo & Diaugustine 1994; Wilmut & Whitelaw 1994). One recent example of this technique is the production of human ∝-1-antitrypsin, a protein that can be used to treat hereditary emphysema and cystic fibrosis. This protein is expensive to make, but transgenic sheep have now been produced which incorporate the human gene for it and which express it in their milk (Carver *et al* 1993). More controversially, genetic modification might be used to create farm animals that could be used as the source of transplant organs. Another possibility is to 'improve' farm animals so that they grow faster, larger, leaner, or are more productive or more resistant to disease.

The use of transgenics and other genetic modification techniques has raised controversial issues of patentability and safety. However, animal welfare has not been prominent in the public debate, probably because there is a general lack of understanding both of the genetic techniques used and the welfare issues involved. Given the relative novelty of the techniques and the rapid increase in genetic modification research, it now seems appropriate to consider the welfare implications of the various stages of the process and the degree to which these are addressed by current UK legislation and/or self-imposed controls.

Genetic modification, the techniques

It is only relatively recently that the principles of genetic inheritance have been understood. None the less, for thousands of years man has modified the genome of animals and plants by artificial selection to produce organisms more suited to his needs. However, man's artificial selection of animals has not always improved the animal's welfare. For example dog fanciers have bred brachycephalic breeds that have difficulty in breathing, while some other breeds suffer from skeletal abnormalities. In agriculture double-muscled cattle have been produced and these often suffer birthing difficulties. In the laboratory, both naturally arising and artificially induced mutations have been used to produce new animal strains for

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medical and physiological research. Examples include various immuno-deficient strains and strains with a predisposition to develop tumours or other diseases.

The aim of genetic modification research, as with artificial selection, is usually to alter the genome so as to produce a desired phenotype. However, genetic engineering is not a simple extension of previously existing techniques. Modern procedures allow the experimenter to 'knockout' or turn off targeted genes, or to insert into the genome new or extra copies of genes which may be derived from a different species. These methods give researchers very precise tools to investigate the function of particular portions of DNA, but they also allow fundamental changes to the genome of animals that could not, or could not readily, arise in nature. The changes to the animal may be beneficial, neutral or deleterious, but they can also be large and are generally expressed in the first generation rather than gradually, as usually happens with artificial selection. There is, therefore, less time to assess the implications of the changes.

Two methods of genetic modification are in common use. In the first, pronuclear microinjection, portions of DNA, either from another member of the same species or from a different species, are injected into the pronuclei[1] of oocytes[2] immediately following fertilization using a micro-pipette. Liposomes[3], or recombinant viral vectors[4] have also been used to insert foreign DNA into cells.

Any embryos that develop and appear to be undamaged by the procedure can then be inserted into a surrogate mother. If the injection is successful and the DNA insertion took place before the first cell division, all the cells of the resulting embryo will contain the new DNA. The animal can then be used to found a transgenic lineage.

In the second method the DNA of totipotent embryonic stem cells[5] (ES cells) is subjected to selective mutations or gene targeting using homologous recombination[6], before allowing the cells to recolonize a blastocyst. This technique is commonly known as a 'knockout' procedure as it allows the experimenter to knockout or modify a targeted gene with the aim of elaborating its function, or to generate a 'pseudo-model' that mimics some of the symptoms of a human disease (Evans *et al* 1994). Where recolonization is successful, the resultant embryos are chimeras and those cells that contain the modified gene may be able to pass on the mutation to succeeding generations if they become part of the germ line[7]. The advantage of this technique is that cells can be selected *in vitro* not only for incorporation of the transgene, but also for incorporation at the desired point in the chromosome.

[1] The nucleus of a germ cell after the maturation divisions. Immediately after the oocyte's fusion with a spermatozoon both male and female pronuclei exist within the oocyte. The male pronucleus is usually used for pronuclear injection because it is larger.

[2] A female gametocyte.

[3] A spherical shell made up of one or more concentric phospholipid bilayers, within which other molecules can be incorporated.

[4] A virus that has been modified to prevent further replications, and that has had a gene spliced into its genetic material. When it infects the target cell, the transgene is inserted into the host's DNA.

[5] Cells within the blastocyst that have not yet differentiated into different cell types.

[6] A technique called electroporation may be used to insert the transgene into the ES cell. Marker sequences included in the transgene specify where in the host's DNA the insertion takes place.

[7] Stem cells that lead to the production of male or female gametes.

Legal constraints on research on genetically modified animals

The *Animals (Scientific Procedures) Act 1986* controls all regulated procedures (experimental and other scientific interferences) carried out in the UK on any living vertebrate other than man, and now also includes *Octopus vulgaris*. Section 1.2 of the Act gives coverage to foetal, larval and embryonic forms from halfway through the gestation or incubation periods. However, the Act has a broader scope as any procedure that results in the birth or the hatching of a protected animal and that may have the effect of causing pain, suffering, distress or lasting harm to that animal is a regulated procedure (Section 2.3). As it is impossible to predict whether a novel, genetically modified animal will undergo pain, suffering, distress or lasting harm, and because the procedures carried out on the gamete donors are regulated, it follows that the production of a protected, genetically modified animal always falls under the 1986 Act. It is possible that animals proven to be free from welfare deficits could be discharged from the 1986 Act's controls for breeding or further research. There are, however, a number of European and British pieces of environmental protection legislation, beyond the scope of this article, that cover the release of genetically modified organisms.

The *Animals (Scientific Procedures) Act 1986,* has numerous provisions that have to be complied with for regulated studies, but one of particular importance for this paper is that prospective licensees are required to submit their estimate of the suffering caused by a project against the likely benefits of the research. Licensees are also required to file annual returns of the numbers of animals that they have used in regulated procedures.

Welfare points

1) Effects on sources of gametes (embryo collection)

In a typical study using pronuclear injection, 25 to 40 females are superovulated to provide up to 500 eggs, and are then mated. F1 cross females, 3–4 weeks old, are usually used as their hybrid vigour produces a better response to the superovulation and their youth helps to ensure effectiveness of the superovulation hormones. The donor females are then euthanased, the oviducts removed and the fertilized eggs flushed or dissected out.

A 3 to 4 week-old mouse is quite young for first mating, and the procedure may involve some stress. Superovulation may also carry some welfare penalties but does mean that fewer mice are required in a study. The superovulation procedure is covered by the 1986 Act, and so would be taken into account in assessing the severity of the project. The numbers of mice used would also be listed in the returns. However, the deaths of animals euthanased using Schedule 1 methods, which are not regulated procedures, are not taken into account when assessing the severity of the project.

In ES cell studies, the technique is slightly different as inbred strains of mice are usually preferred to the F1 cross females. Also, in these studies many laboratories do not, for technical reasons, superovulate the females. This avoids any stress associated with the superovulation or early mating.

2) Effects on foster parents

Females

Once pronuclear microinjection of DNA, or injection of recombinant ES cells into the blastocyst has been carried out, the embryos that survive the gene transfer procedures are reimplanted into a female host.

In a typical transgenic project (microinjection technique) the aim is to produce 5 to 6 lines, each of which may show a different expression of the transgene as there may be multiple insertions and differences in location of insertion of the transgene. This would require at least 5–6 transgenic founder mice. Given the normal success rate, these would be found from some 40 to 75 mice born, ie the offspring of approximately 10 recipient (foster) mothers. Not all of the recipients will become pregnant or successfully give birth to a litter and so to provide these 10 litters, a pool of usually about 12 but up to 40 recipients might be used.

In ES cell studies there is no need to generate multiple lines of offspring because before insertion, the researcher selects *in vitro* for the ES cells with the desired modification. Moreover, because the gene is targeted, the expression of the gene is standardized. Both of these considerations means that fewer offspring and hence fewer recipient females are required in ES cell studies.

In mice the insertion of embryos into the foster mother is always carried out under full anaesthesia, as the peritoneum has to be opened to place the embryos within the infundibulum of the oviduct (or in-utero for ES cell work). In large farm animals such as cattle, it is possible to implant via the vagina.

Males

Prior to transfer the pseudopregnant surrogate mothers are produced by mating with a sterile, usually vasectomized, male. The vasectomy is carried out under anaesthesia, and this procedure would be included in the licence for the project.

3) Effects on offspring

Not all embryos survive implantation, and in studies using microinjection only a proportion of those born (in the examples above 5–15%) will be positive for the transgene. This is normally determined in mice by snipping a portion (approximately 5mm) of the tail (a licensed procedure) for analysis. Alternatively, the disc of tissue obtained from an ear punch used for identification purposes may be analysed. Non-positive offspring would normally be euthanased or could be used in other studies, for example as surrogate dams. It can take some time to obtain the screening results and this can put pressure on space in the animal house. Selected animals that express the transgene are used to found transgenic lineages through cross-breeding the offspring and back crossing. Subsequently each generation has to be screened for the transgene unless a line is bred to homozygosity.

In studies with ES cells the percentages of chimeric offspring would be higher than the percentages positive for the transgene in microinjection studies (being of the order of 28–50 per cent). Moreover, if the mouse strains used as sources of the ES cells and blastocysts have different coat colours, it is possible to detect chimerism and germ line transmission by coat colour. Screening at this stage is then unnecessary. If subsequent generations are required, only those offspring that show the appropriate colour need to be screened. Once

germ line transmission has been achieved, half of the offspring showing the coat colour marker should contain the modified gene.

In transgenic studies using microinjection techniques it is not currently possible to control the number of copies of the transgene that are incorporated into the host's DNA or their location in the chromosomes. This lack of specificity and regulation of the fusion genes can have unexpected negative side effects (Berlanga *et al* 1993; Bird *et al* 1994). Some offspring show marked abnormalities, and depending on the experiment and the degree of suffering involved, these would normally be euthanased. If the licensee suspects that a particular construct is likely to cause suffering, he is required to include this in the severity rating of the project. Although the Act protects embryos from halfway through their development, in practice it would usually be impossible to assess embryo suffering while they are in the uterus.

Some genetically modified animals are produced with the aim of introducing deliberate defects as potential models of human diseases, and Petters (1994) discusses the use of pigs for this purpose. Clearly these defects can cause suffering and it is important to ensure that the transgene is an appropriate model (Poole 1995). In all of these types of research, the 1986 Act requires justification and assessment of suffering for the severity banding, and consideration of appropriate end-points beyond which animals would be euthanased. As in all studies which involve the use of animals, it is important to ensure that there is a thorough ethical assessment of the experimental proposals.

It has been suggested that transgenics could be used to increase the disease resistance of farm livestock. This is an approach that could improve welfare but could also result in the adoption of farming systems that are even more intensive than at present, and this may not be beneficial for the animals. However, attempts to use transgenics to increase the health, product output and quality of farm animals (Robinson & McEvoy 1993; Müller & Brem 1994) have not so far been successful due to: frequent unwanted side effects (Ebert & Schindler 1993); difficulties in obtaining viable zygotes (around 1200 *in vivo* derived zygotes may be required to produce one transgenic calf); gene expression (Powell *et al* 1994); and detection of positive embryos (Eyestone 1994). The use of large numbers of oocytes obtained from slaughterhouses can also produce unpredictable results and welfare consequences (van Reenen & Blokhuis 1993). Moreover, at least 30 pregnant cows must be carried to term for every transgenic calf born (Eyestone 1994). Even if output could reliably be increased, the extra burden of production could stress the animal. Furthermore, it is not clear that there are sufficient controls for assessing the welfare of these animals, as such stress may be insidious and difficult to detect.

A transgenic or genetically modified animal remains under the 1986 Act until it can be demonstrated that the modification will not result in pain, suffering, distress or lasting harm to the animal. An animal that does suffer such adverse effects must be killed immediately the procedure has been completed, or when it reaches an endpoint of suffering as set out in the project licence. Compromised individuals, therefore, cannot be discharged from the controls of the 1986 Act for farm production. If no problems are seen after breeding two complete generations under farm conditions (Broom 1993; MAFF 1995) and after studying homozygous as well as heterozygous individuals, the animal could be treated as a normal animal, although it would still be subject to various restrictions under other legislation concerning its release into the environment (Advisory Committee on Genetic Modification

1989). One way of achieving the appropriate controls over this process might be to carry it out under a licence issued by MAFF under Section 1(2) of the Agricultural (Miscellaneous Provisions) Act 1968, and by regular inspection by experienced members of the State Veterinary Service.

Number of animals used in genetic modification studies

As in all experiments which use animals, it is important to ensure that the number used is kept to a minimum. However, because of the rapid growth of genetic modification research, the number of animals used in this area is likely to increase in the foreseeable future. As techniques improve it may be possible to improve the success rate of transgenic animal production. This would reduce the number of donor females, surrogate mothers and wasted transgene negative offspring. However, a much greater number of mice are used in the breeding programmes required to establish a genetically modified line than in the process of producing the genetically modified animal *per se*. If screening is not carried out efficiently, an unnecessarily large number of transgene negative animals can be produced in a very short space of time, particularly where heterozygous lines are concerned.

Conclusion and recommendations

The welfare considerations for the genetic modification of animals do not appear to differ greatly in type from any other sort of animal research, and the *Animals (Scientific Procedures) Act 1986* comprehensively covers the procedures required to produce a transgenic animal. None the less, there are a number of welfare and ethical considerations not necessarily exclusive to genetic modification studies which should be taken into account. To address these, I suggest the following recommendations:

1 It is a project licence requirement that the severity of the project has to be balanced against any likely benefits. To aid this assessment, institutions carrying out genetically modified animal research should organize a peer group reviewing system to examine the value and ethical implications of project proposals.

2 Investigators should enter new genetically modified animals into the database available on the internet (TBASE at the Welch Medical Library, Woychik *et al* 1993). This currently includes a section on phenotype, but there is no specific section on welfare implications. Authors should consider providing such an assessment. As the database grows, it should allow a more accurate prediction of the likely consequences of particular types of genetic modification. The desire to maintain industrial property will be a problem in some cases, but the potential welfare benefits of using this database are great.

3 The Animals (Scientific Procedures) Act 1986 does not require that euthanasia carried out using Schedule 1 techniques should be included in the project's severity rating. However, the number of animals used is clearly an ethical issue. Users should predict and be aware of the total numbers of animals used in a study (including the breeding programme). There should be a record of the numbers of animals killed in a laboratory per annum. This proposal applies to all studies, not just transgenic ones.

4 Superovulation: F1 cross mice are usually used because of their hybrid vigour. When inbred strains are used, superovulation may not be so successful. Therefore preliminary

research should be performed to ensure that superovulation is a useful procedure in these cases.

5 The mating of young donor females may be stressful. This should be taken into account in the project licence application.

6 It is important to maintain a tight control over the breeding of both stock and genetically modified lines, to avoid wasting animals and to ensure that space allowances required by the Home Office Codes of Practice for breeders or for users are not compromised.

7 To achieve this control, there should be good liaison between separate users, and users and animal house staff. Space requirements should be kept in mind when planning experiments. If problems are experienced during the course of a study (such as delays in screening for transgenic status, or in microinjection), the non-transgenic animals should be considered for use in other studies rather than wasted.

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