Cloning by Nuclear Transfer in Farm Animals

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Genetic modification (or engineering) of farm animals has been inhibited by the inefficiency and lack of precision of the techniques. Because it is time-consuming and expensive it has also been mainly used by the medical biotechnology rather than the animal breeding industry; this restriction has been exacerbated by our almost complete lack of knowledge of the genes controlling most agriculturally important traits. These limitations to the use of transgenic technology are being overcome by new advances in research in embryo manipulation and genome analysis of agricultural traits. I shall detail the former in this paper and try and fit the advances into a wider ethical context.

Transgenics

The genetic modification of the germline in the mouse was first achieved in 1982 and of all farm animals by about 1985. This involves microinjecting a gene into a single-celled embryo under a microscope; the embryo is then re-implanted into a surrogate mother and the injected gene gets incorporated into its DNA and thence into every cell. The modification is permanent and once made is passed on from generation to generation. The success rate of this technique is low: around 1% of all embryos injected become transgenics. Over the years there have also been considerable difficulties in regulating the expression (i.e. the activity) of the injected transgene.

Embryo Manipulation

To try and overcome the poor efficiency and lack of precision of the current transgenic techniques there has been a great effort to transfer the technique to cells grown in culture. This was first achieved some years ago with the development of embryo stem (ES) cells in mice. These cells grow well in the laboratory in culture. Techniques have been developed to target genes

into them; the inserted genes, in this case, line up against the equivalent endogenous gene and replace it. This allows not just the addition of genes, but also more subtle alterations in them and the ability to silence them (knock them out; extremely important for creating animal models of human inherited disease). The modified ES cells are then injected into an embryo, placed in a surrogate mother and after one generation of breeding, a transgenic is produced. In principle, this technique produces 100% transgenics with precisely engineered modifications. Unfortunately, despite many years of trying, scientists have been unable to produce ES cells in farm animals.

To overcome this problem Ian Wilmut, Keith Campbell and colleagues at Roslin, used sheep cells that were at later stages of development: embryo, fetal and adult cells. By controlling the conditions of growth they found they were able to reprogramme the nuclei of these more developed cells so they would function against an early embryo stage. The nuclei could be transferred to an unfertilised oocyte and produce live lambs. The fact that this could be done with adult cells (Dolly) as well as embryo cells caused all the recent excitement and concern about «cloning». More recently embryo cells have been genetically modified in culture, nuclear transferred and produced live lambs (Polly). We can therefore now carry out the equivalent of ES cell technology in farm animals (Campbell *et al*, 1996; Wilmut *et al*, 1997; Schnieke *et al*, 1997).

Uses

The ability to make precise genetic changes using the nuclear transfer (Polly) technology allows better and more efficient production of pharmaceutically active proteins in transgenic animals. These would be proteins that are difficult for technical reasons to make in sufficient quantities in other ways and will be used to treat human disease. The first proteins from the original microinjection technology are now in clinical trials.

Genetic modification of pigs either by adding human genes or masking or knocking-out pig genes opens up the possibility of producing organs suitable for human transplantation (xenografting). As has already been noted, knocking-out of genes also allows the production of animal models of human disease (such as cystic fibrosis) that cannot be obtained in other ways and permits the development of novel approaches to therapy.

In animal breeding, a combination of genome analysis and cell culture-based transgenesis would permit a more controlled approach to animal breeding especially for currently intractable traits like fertility and disease resistance. In addition cloning from adult cells (as with Dolly) would permit the replication of (for example) a proven high yielding and productive dairy cow.

Ethics and Welfare

The issues surrounding these novel technologies are not new. Many were raised after the development of artificial insemination (AI) of dairy cattle 50 years ago and others with existing

approaches to animal breeding and production. It is the pace of new developments that causes both public interest and concern and for this reason Mr John Gummer, when he was Minister of Agriculture, established a Committee under the Chairmanship of the Reverend Professor Michael Banner to investigate and report on the «Ethical Implications of Emerging Technologies in the Breeding of Farm Animals»; the report of the Banner Committee (of which I was a member) was published in 1995.

As I believe the Banner Committee Report (1995) is comprehensive and deals with the technologies described above I will not cover the area in detail; I would however like to deal with a number of often raised issues.

«Playing God». Each higher organism (including humans) contains around 70,000 genes; these genes produce the primary proteins that catalyse or control developmental and physiological processes. The genes and their DNA sequences are not stable: they are mutating at a regular but low rate (about 1/10,000 times). At the current stage of evolution after many millions of years most new mutations have little effect (are «neutral») or are deleterious (for example causing human inherited diseases). The variation that mutations give genes is however the stuff of evolution: without variation evolution could not occur and each of us contains many (albeit benign) inherited mutations and 2-3 new ones. Mutational variation is also the raw material for animal breeding by artificial selection. Humans first selected (or domesticated) feral animals for their use between 10,000 years (dogs) and 4,000 years (farm animals) ago. Selective breeding, especially in the last 200 years, has changed the genetic constitution of farm animals beyond all recognition altering hundreds of genes: such that the modern Holstein-Friesian looks nothing like the European Bison nor the Large White pig like the wild boar. Classical domestication or animal breeding has therefore altered the frequency of many genes in an often uncontrollable way. On the other hand, transgenesis (for example) only manipulates a single gene whose function is often well understood and can be assessed under controlled conditions.

«The species barrier: the new technology crosses the species barrier which does not occur in nature». Genes do in fact cross the species barrier in nature all the time. In addition, at a chemical level, the species barrier is a difficult concept. Take for example a protein (such as insulin) of 150 amino acids long, 5 of which differ between humans and pigs. If, in the laboratory, we change the DNA sequence of the pig gene so that it makes the human protein (i.e. those 5 different amino acids) ... is it now a pig or a human gene? In fact it is just a piece of DNA that makes a protein ... nothing more or less.

«Animal Welfare: the new technologies compromise animal welfare in new ways». I consider that animal welfare is about the physical or psychological state of the animal rather than the way it is bred. For example, if an animal is seriously lame either because of poor treatment housing or diet, poor breeding techniques or, transgenesis ... then it is not acceptable. It is not the technique but the resultant effect on the animal. In fact a detailed study of the behaviour of transgenic sheep at Roslin could find no differences to control animals in eight measurements in three husbandry situations (Hughes *et al*, 1996).

«Classical». Animal breeding is not itself without welfare problems. Some modern strains of farm animals have been selectively bred for single and rather simplistic traits such as: growth-rate. After many (30+) generations other problems can occur such as: obesity, lameness and poor fertility. The modern animal breeding scientist now uses more sophisticated selection criteria involving the health and integrity of the whole animal to avoid these side-effects. The other major problem with «classical» animal breeding is that it is in a black box situation: you can only select rather simple traits, with no knowledge of the genes involved and also only where genetic variation exists. Traits associated with fertility and viability are difficult to improve by this approach as they have little genetic variation in normal populations.

«The technology is not regulated». All experiments on animals in the UK are controlled by the Animals (Scientific Procedures) Act 1986: all scientists and projects require licences and all animals are inspected randomly and without notice by Home Office Vets. For example, the double muscling gene in Belgium Blue Cattle occurs naturally but would not be permitted if produced by transgenesis. All experiments involving genetic modification are regulated by the HSE's Advisory Committee on Genetic Modification and the DoE's Advisory Committee on Release into the Environment. Any food from transgenic animals would have to be approved by MAFF's Advisory Committee on Novel Foods and Processes. It is in fact a testament to the foresight and lobbying by the scientific community that many of these regulations (e.g. ACGM) were put in place.

When it comes to applying the technology to humans, we have the «belt and braces» approach of the Human Fertilisation and Embryology Act which not only prevents many of the techniques (e.g. germline transgenesis) being applied to humans but all experiments on human embryos need the prior authorisation of the Human Fertilisation and Embryology Authority. A similar authority was proposed for farm animals in one of the seventeen recommendations of the Banner Committee; unfortunately it was the only recommendation that was not accepted by the Government. The position we have taken on cloning of humans from adult cells (like Dolly) is as follows:

- We don't know whether it is possible; the embryology of humans is different from sheep.
- We can see no clinical reason for it.
- We don't intend to do it or licence our patents to anyone who wishes to do it.

«The public should be consulted first». This is a difficult issue. Quite often scientists are not sure until they achieve it whether an advance is possible. Science is also international and moving forward on a broad front with one lab usually only a few months ahead of others; moratoriums would not therefore be of much help. I believe it is better not to regulate science but rather its uses; almost invariably the uses are both beneficial and capable of abuse. The responsibility of scientists is therefore to keep Government and the public aware of scientific advances and stimulate public debate on their uses; regulations have to be international.

«Patenting». There is a belief that patenting prevents academic research and restricts the use of new technology. In fact a patent does not prevent academic use of the research findings (our patented work has been taken up in several other laboratories) and does not give a right to exploit the invention commercially but only to prevent others from doing so. Use of an invention is still regulated by national and international law and without patents there would be little investment by industry in much valuable biomedical research.

«It will reduce biodiversity». In principle, all artificial breeding could reduce biodiversity. AI itself has restricted (especially with dairy cattle) the male side of breeding to a few outstanding individuals. New technologies will be used in a statistical framework that has already been well worked out for AI to prevent dangerous levels of inbreeding. In fact the new technologies could have a positive improvement on biodiversity allowing cells from rare breeds or species to be cryopreserved and recovered by nuclear transfer; FAO is already studying the feasibility of this approach.

«Socioeconomic impact». The argument has been put forward that new technologies will only profit the large farmer or breeding companies and damage the small farmer. The commercialisation of agriculture and the increase in size of holdings has increased throughout most of this century. Although the driving force is economics of scale, this has been facilitated by mechanisation, the availability of new crops and animals and the use of fertilisers and vaccines. The new technologies are only one part of this overall change in agricultural practice and with the various international agreements (such as GATT) we have to continue to improve our efficiency to compete on a world stage.

«Overproduction». It is also often asserted that with overproduction in Europe no more new technologies are required. Unfortunately it is not the level of production that is important but the efficiency; if we allow other countries access to technology which we ignore we will soon lose our market position.

Conclusions

We live in a time of unprecedented changes and advances in the biological sciences and their commercialisation. The new technologies present enormous benefits to us but also some dangers from misuse. I believe, in the main, in the UK, the regulatory framework is adequate for guarding against abuse of ethical or animal welfare standards. It is, however, important that the scientific community continues to publicise scientific advances in ways that the public and governments can understand, so that the uses can continue to be regulated in an informed manner.

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