Embryos, cloning and transgenics

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The news about Dolly has had a substantial impact on the way we think about ourselves and out identity as individuals. However to put this into context with respect to New Zealand animal industries it is necessary to look at the technological progress in embryology over the last few years and discuss the possibilities for the future.

Embryonic clones

Identical twins are part of our experience and, scientifically, identical twin cows were used in numerous experiments in New Zealand more than 30 years ago. Such identical twins arise from the natural splitting of a zygote in the early stages of embryogenesis. Several years ago, embryo splitting became an integral part of many embryo transfer protocols where embryos were split after recovery and then transferred into surrogate dams. Many of these went to term producing identical twins. In some cases, embryos were split further but the success rate was poor.

A variation on this then was the recovery of an individual cell (or perhaps nucleus) of the early embryo and the transfer of this into an enucleated ovum. Naturally the nucleus, or cell had to come from the early embryo before the cells had acquired positional information or alternatively it had to be taken from the inner cell mass of the blastocyst, (that is, that part of the blastocyst where the cells are still totipotent or undifferentiated). Thus the offspring of such manipulations were identical or were clones, albeit, embryonic clones.

However, there is also a subtlety here when a cell is transferred to an enucleated nucleus. While the vast majority of the DNA in a cell is found in the nucleus, there are some genes coded for in mitochondrial DNA, which is maternally inherited (i.e. passed down via the ovum). Consequently the mitochondrial DNA is not removed when the egg is enucleated before the two "cells" are fused. As a result the resultant embryo actually has two sources of mitochondrial DNA - the term for this is heteroplasmy. The resulting embryo will likely be heteroplasmic but it is possible that one source of mitochondrial DNA will be predominant.

Successful systems for the culture of embryonic stem cells from mice were developed many years ago. However, this has proved somewhat more difficult to achieve in other species, although both Scottish and New Zealand scientists have been successful recently and produced cloned sheep using cultured embryonic cells. This was the forerunner to the cloning of Dolly.

Dolly

Dolly is not an embryonic clone. She is cloned from an adult and is thus 'identical' to her mother. To achieve this, Wilmut and his colleagues induced an adult mammary gland cell to de-differentiate; that is, they induced it to revert to the embryonic state. It was then transferred to an enucleated ovum and the rest is history. In fact, they attempted 277 transfers and one lamb went to term. There are numerous questions about adult cloning. For example how old is Dolly - is she the age of her mother at the time the cell was recovered or is she really only as old as her birthday indicates?

Embryos *in vitro*

In vitro production (IVP) of embryos is another area where there has been considerable recent progress. This involves recovery of immature oocytes from the ovaries of females (either by transvaginal oocyte recovery from live animals or by recovery from ovaries of slaughtered females). The oocytes are then cultured *in vitro*, fertilised and then cultured to the stage of transfer to a surrogate dam. This technology has been used successfully in sheep, cattle and deer. AgResearch at Ruakura have a major involvement in the development of this technology in New Zealand.

However, it is the combination of this IVP embryo technology with embryonic cloning which could well have a major impact over the next few years. In this respect, Australian researchers have recently taken embryonic cloning to a new level. They cultured cattle embryos *in vitro* to the blastocyst stage, recovered up to 30 cells from the blastocyst and then fused individual cells with enucleated ova. The resultant embryos were then cultured and grown, separated and fused repeatedly. Their record to date is 470 healthy cloned embryos, although only a very few cloned embryos have been implanted into surrogate dams.

Transgenesis

Fifteen years ago, the front cover of Nature showed a normal mouse alongside its transgenic littermate which exhibited over-expression of a growth hormone gene. This successful transgenic experiment initiated a range of studies in farm animals. Although transgenic animals were produced, the technique was difficult and the success rate poor, and in some cases the health of the transgenic animals was compromised. However some groups persevered and developed the technique to successfully produce transgenic animals with foreign genes expressed in their mammary glands. These genes produced proteins which could be recovered from milk.

Probably the most advanced of these programmes internationally is that of PPL, who have sheep producing human α1-antitrypsin in their milk. PPL have now established a New Zealand base with semen from this line of sheep being used on ewes in this country under strict quarantine. New Zealand is attractive because of our freedom from scrapie. The intention is to evaluate the α1-antitrypsin as a treatment for emphysema in humans.

Implications for domestic animals

Transgenics will likely have little impact on farm animal production in the next 20 years. However, they could well find specialist application in the production of pharmaceuticals

for human use, using the transgenic mammary gland technology. Other possibilities include transgenic eggs produced by chickens whose egg cell line has been rendered transgenic by the incorporation of a foreign gene into the germ line.

While I do not see transgenic animals having a major impact, the prospects for embryonic cloning are a different matter altogether. Clearly there are numerous difficulties to be overcome in developing a commercially applicable cloning technology. However, the international research activity is at a high level and considerable progress is being made as evidenced by the success of the Australian group combining IVP of embryos and cloning.

Consequently I believe that IVP of embryos is likely to have a significant impact when combined with embryonic cloning and embryo sexing, where the prospects are extraordinary. Much research is now concentrating on the factors involved in ensuring normal development of the embryo and foetus *in utero*. This system also lends itself to economic screening of the embryo clone using genetic markers. Obvious immediate applications of markers include sexing and screening for disease susceptibility but markers for productive traits will be important in the longer term.

Practically there are various scenarios where such cloning could be advantageous. An example would be evaluation of a subsample of clones in terms of productivity before mass producing them to order. However, there are a number of ethical issues to be considered, not the least being the impact on the genetic diversity of the species. This could be particularly so with Holstein-Friesian cattle. Consequently the uptake of such technology may well be influenced greatly by the reaction of markets to the products of such animals.

Further reading

Westhusin, M.E. and De Azambuja 1996 Development of *in vitro* derived bovine embryos following pronuclear transplantation and *in vitro* culture. *Animals Reproduction Science 45*: 29-35.

Anderson, I 1997. Will many clones make light work? *New Scientist* (15 March) p 4.