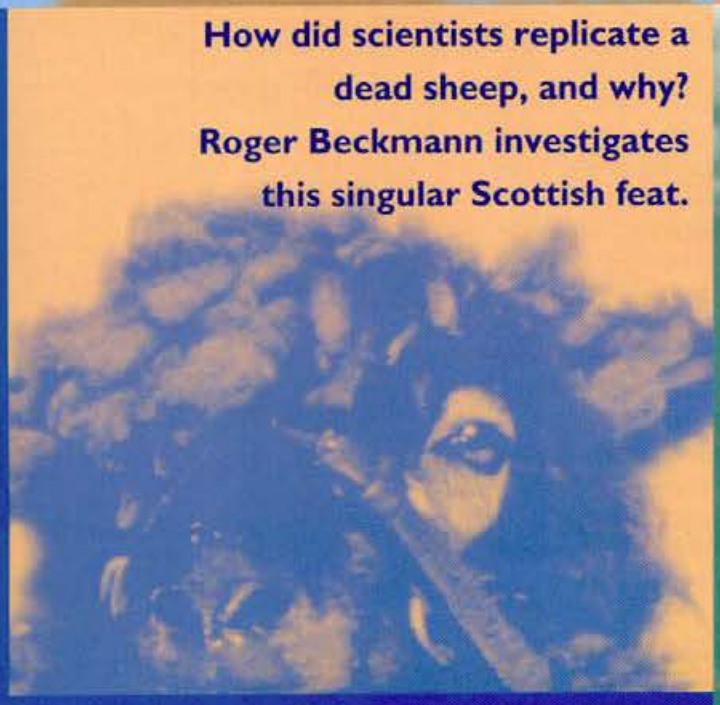
How did scientists replicate a dead sheep, and why? Roger Beckmann investigates this singular Scottish feat.



Copys

News of a major biological breakthrough hit the media earlier this year in the form of Dolly the sheep. Dolly is a case of 'immaculate conception'. No act of mating occurred to produce her, and her real parents were dead before she was born. She is in fact a clone – an identical copy – of an adult sheep.

In nature, the joining of sperm and egg produces a unique combination of genes. Each sperm cell and each egg are themselves unique, because the genes of the male and female are shuffled around in creating them. That means the same two parents produce offspring which are not all the same, although they may resemble each other. Sex is designed to provide diversity of genetic make-up in a population.

Genetically identical organisms are called clones. Clones occur naturally, especially in the plant world, where reproduction often takes place asexually. Examples are when grass sends out runners, or when a fragment of cactus drops off and grows into a new plant.

Animals have less ability to reproduce in this way. About the only example is when a simple animal such as a flatworm is cut in half; it's possible for each half to grow into a completed worm again. Unfortunately for

A case for variation

A POTENTIAL drawback of multiple cloning for agriculture is one that has faced plant-breeders. The farmer finishes up with a collection of nearly 'perfect' plants or animals, all of which are all identical: in short, a monoculture.

When circumstances change – for example, with the arrival of a disease-causing organism or poorer quality food – all the animals will react in the same way. If one is genetically susceptible to a certain pathogen, they all will be. You won't find a 'rogue' animal that, although not especially desirable under normal conditions, harbours an ability to fight off a particular infection or survive by eating a plant that the others won't touch.

It would be dangerous if all the world's sheep or cows were exactly the same. Animal geneticists, forewarned of these dangers, are likely to distribute not one elite clone line, but several. Nevertheless, cloning will never completely replace other methods of animal breeding.

animal breeders, that trick doesn't work with more complex animals. But clones not derived from adults can still occur naturally, even in humans, by the process of early embryo splitting.

We all start from a single cell: the fertilised egg cell or zygote. When this cell divides in two, the long process of growing up begins. After the first few divisions, the cells start to differentiate; some will become nerve cells, others muscle cells, others skins cell and so on. All these cells have the same genetic information, but during the process of differentiation, certain genes are activated while others are switched off.

Although a cell from your liver is genetically identical with one from your brain, these specialised cells can't actually revert to the state of the fertilised egg. Most of their genes have been permanently switched off. For example, a brain cell doesn't need the genes for making fingernails, so those genes are 'de-activated'.

But back at the early stages of development, (the two or four-cell stage of the embryo), the cells have not yet specialised. And if they are pulled apart, each cell will grow into a complete organism, rather like the cut flatworm. Occasionally, for unknown reasons, the cells of the early embryo do get separated and the result is identical offspring: natural clones. Identical twins in humans (not the same as fraternal or dizygotic twins) are natural clones. They come about when an early embryo is split at the two or four-cell stage, rather like the unfortunate flatworm, and each half grows up into a complete organism.

Until a few months ago, the only way to clone an animal was to separate the cells of the early embryo artificially. In some species, they can be separated up to the 16 or 32 cell stage and each cell can still yield a complete organism. All that is required is enough wombs to put them in.

Udderly new

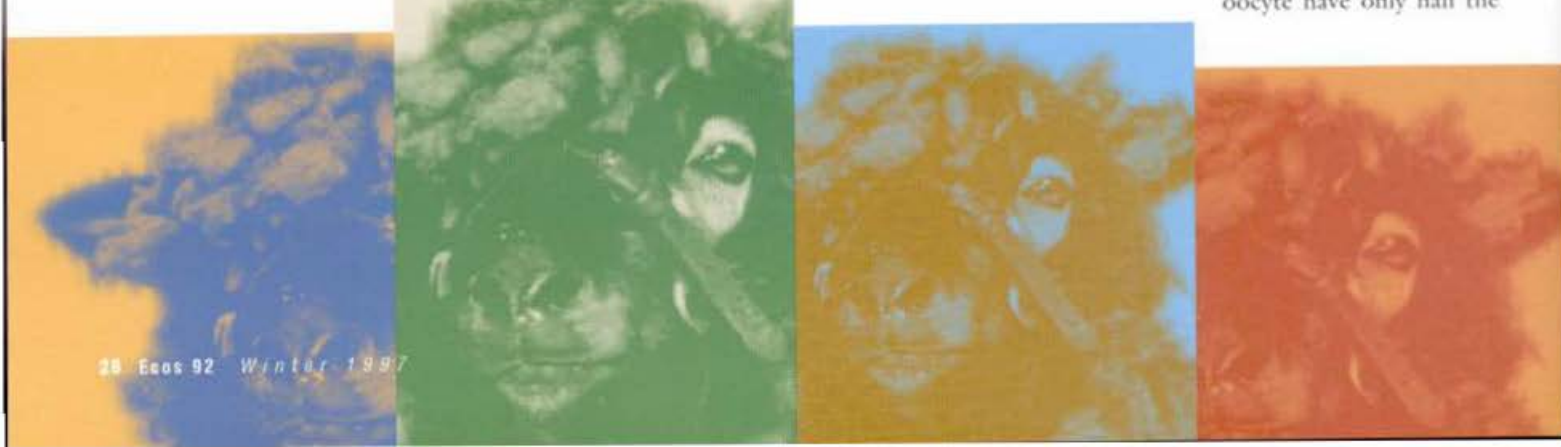
Dolly represents the breaking of a major barrier. She grew from a differentiated cell taken from an adult, something that was considered impossible. Scientists led by Dr Ian Wilmut at the Roslin Institute in Scotland removed a small piece of tissue from the udder of a six-year old pregnant ewe and grew the cells within it. They then removed the nucleus, which contains the genetic information, from a sheep oocyte (egg cell) taken from a different ewe's ovary. Using an electric current, they fused the enucleated egg cell with a cell from the udder tissue. (This cell was many times smaller than the egg cell.)

All that remained was to implant this newly created cell into the womb of a ewe hormonally prepared for conception and let nature take its course. And that's what happened. The nucleus from the udder tissue cell was 'accepted' by the egg cell, and the genes in that nucleus directed the development of the egg cell into an embryo and ultimately, an adult sheep. That adult was a direct genetic copy of the ewe from whose udder the original cells had been taken.

The idea of cloning from an adult cell is not new. It was tried many times decades ago, but never worked. It seemed the genes in the differentiated and specialised cells of mature organisms were too firmly set in their ways. Speculation hinged on the possibility of de-repressing every single gene, but no-one knew whether that was truly feasible and, if so, how to do it. It was considered a problem that might be resolved only in the long-term.

But Wilmut flicked all the gene switches back on by slowly starving the adult cells over a few days. The cells stopped their usual activity and became, as Wilmut put it, 'quiescent'. Presumably, all the genes in their nuclei returned to an embryonic state. As a result, when these genes were put into a well-nourished egg cell, they were 're-awakened' without any of their previous controls in place. This was unprecedented, and it wasn't easy. Wilmut and his colleagues fused no less than 277 adult udder cells with enucleated oocytes. Only 29 of these reached the early stage of a foetus, and only one continued development right up to full term.

If Dolly's origin sounds bizarre or undignified, we should think about our own strange beginnings. We too come from the process of cell fusion, where a small swimming cell in the culture fluid known as semen fuses with a large oocyte. But in this case, the sperm cell and the oocyte have only half the



number of chromosomes for a human. When they fuse, the result is a zygote with the normal number of chromosomes.

Dolly has the normal number of sheep chromosomes because the egg cell from which she came had its nucleus (and hence chromosomes) removed, and the adult udder cell that fused with it had the full complement of chromosomes. This avoided doubling the chromosomes. The egg cell therefore contributed nothing in the way of Dolly's genetics, apart from what lay in its cytoplasm (the part of a cell that is not the nucleus).

Bring on the clones

Dolly doesn't just represent an interesting piece of theoretical biology. She was created to improve animal breeding. Breeding from animals known to have desirable qualities is still a hit-and-miss affair. This is because every egg or sperm cell carries a slightly different assortment of genes, and offspring from an elite male and female will not inherit the same characteristics from their parents.

Embryo cloning didn't help much either. It gave plenty of offspring from the same two parents, but still couldn't guarantee that the cells of the embryo would carry the right characteristics when grown up into an adult animal. Achieving significant improvements in herd genetics still relied on several years of performance-testing to select the best clone lines.

But with cloning from adult tissue, it's possible to choose your animal on the basis of its characteristics when mature. And you don't have to dilute those genes with another animal's genes, or have them shuffled around during the creation of egg and sperm cells. A cell from a selected adult, rather than growing up fully as Dolly did, could be allowed to become an early embryo and used to make multiple copies at that easier stage.

Cloning cattle from embryonic cells is well established in Australia. In Melbourne, biologists from Genetics Australia and Monash University have produced several hundred embryos from a single cattle embryo. If these were put into surrogate mothers, a successful calving rate of 20-30% would be expected.

Dr Sandy McClintock, a consulting geneticist to Genetics Australia, says cloning of adult cells is far too inefficient at present to be attractive to animal breeders. But it does hold immediate interest for pharmaceutical manufacturers.

McClintock says many pharmaceuticals are proteins that are difficult to produce using bacteria. The milk of a farm animal naturally contains a mixture of proteins, so with a little genetic modification, could become a source of proteins with pharmaceutical properties. For example, blood-clotting factors that are missing in the blood of haemophiliacs are extracted from normal donated blood. But they pose potential disease risks and are expensive.

Dairy cows could be genetically modified to produce one or more of these factors in their milk, McClintock says. After daily milking, the valuable clotting factors could be extracted and purified. Only a small number of animals would be required to produce the entire world requirements of some of the more valuable proteins, so large-scale cloning is not vital for pharmaceutical applications.

The breakthrough in Scotland raises the inevitable question of human cloning. Would it be possible to take a sample of cells from someone's skin or saliva, for example, and copy that person? At the moment the answer is no, but soon may be yes, at least, in theory.

The practicalities of having a surrogate mother for the embryo to develop in, and a donor for the oocytes whose nucleus would be removed, mean that it would certainly involve more than one individual, not to mention a team of biologists. The ethical implications are another matter entirely! (See boxed story at right.)

Significant differences exist between sheep and human embryos, however, which will probably make the technique harder in humans and may delay the day when we can clone ourselves. In sheep, the DNA in a normal fertilised egg does not start actively controlling the cells until after the third or fourth round of cell division (when there are eight or 16 cells in the embryo). Thus, an added nucleus has plenty of time to 'settle in' and reactivate its genes.

In humans, the genes start working after the second division (at the four-cell stage). An adult nucleus (from a starved cell) put into an enucleated egg cell would have little time to recover before its genes had to be active. This could make a difference.

Me and my shadow

ALTHOUGH there are no specific laws about human cloning in Australia, existing legislation in Victoria would prevent human cloning there. It would probably be classed as illegal in the rest of the country, because it contravenes guidelines on introducing DNA into human reproductive cells. (Although is an enucleated oocyte still classed as a reproductive cell?)

Scientists and ethicists have nearly all condemned the idea of human cloning, although some circumstances have been suggested where it might be warranted. For example, if a couple lost a young child and were no longer able to have any more children of their own, one could envisage a scenario where they would feel justified in asking for cells to be taken from their recently dead child for fusion with an enucleated oocyte of the mother to produce their baby again. But this would require an improvement in the present technology, given the high number of embryos (277) needed at present to ensure procedure's success.

The idea of cloning organs, but not complete individuals, has also been suggested to provide the best options for rejection-free transplant surgery.

Despite this, cloning won't be able to produce a 'double' or instant identical twin, or 'bring back to life' a favourite dead relative as they were, because any clone would be much younger than the individual who provided the genes and will inevitably experience a different environment from that person, causing it to develop a different personality.

Other potential problems surround cloning adults of mammals. Cells seemed to be programmed to divide only a certain number of times. Dolly's genes came from a cell that, obviously, had already used up some of its allowance. How will this affect the aging process in Dolly herself? We don't know yet, but some scientists are speculating that Dolly may have problems later on in life. We shall have to wait and see.