What Good Is Cloning, Anyway?

At the 2000 SSR meeting in Madison, the above question was posed at the Minisymposium on animal use in research. The ACEC is in the process of writing a FAQ list to assist SSR members who give public talks about research with animals. The following is an extended answer to the above question.

Let’s start with a sub-question: what is cloning?

Cloning is essentially making a duplicate copy of an entity, traditionally used in immunology (poly- and monoclonal antibodies) and molecular biology (cloning a gene). Nature has been cloning for millennia, with the production of identical twins. In this type of cloning, a fertilized egg divides into 2 genetically identical embryos. This can also be performed in the laboratory, and have been produced in farm and laboratory animals by splitting a multiple-cell blastocyst and permitting each to develop into offspring.

An important technique in modern cloning is nuclear transfer. This involves the use of two cells. The recipient cell is an unfertilized egg (oocyte) taken from an animal soon after ovulation, and the donor cell is the one to be copied. The recipient cell’s nucleus is removed with an extremely fine micropipette, and this cell is then fused with the donor cell, complete with its nucleus. Some fused cells start to develop like a normal embryo, and will produce offspring if implanted in the uterus of a surrogate mother. The advance that made Dolly possible is the use of cells from adult animals as donor cells. Donor cells were fused with a recipient oocyte, and a few developed into embryos and a live lamb was born. This alteration of the destiny of a differentiated cell is ‘nuclear reprogramming’, and represents the major breakthrough associated with cloning.

An important use of cloning will be to take an adult cell, to reprogram it in vitro, and then to transform it into another cell type. This has obvious implications for medicine. Skin cells could easily be taken from a patient, and then transformed into heart cells, pancreatic islet cells, neurons etc., to repair damaged tissue. The advantages are many: there would be no rejection problems, as the new tissue is genetically identical to the patient, and there are no ethical problems involving the use of human embryos. However, we are a long way from this noble objective, as there are many technical obstacles to be overcome. Much research is required yet, particularly concerning the reprogramming of cells, of which we know very little. Current cloning technology is insufficiently developed to achieve this ultimate goal, but this is the only tool we have to investigate the mechanism of nuclear reprogramming.

An excellent source of cloning and related information, aimed at kids, can be found at: http://animalsciences.missouri.edu/biotech/.

The following points summarize some of the proposed or actual uses of current cloning technology:

1. **Therapeutic cloning—tissue/organ transplanation.** There is uniform opposition to the use of somatic cell cloning to reproduce humans, based on ethical concerns and the unacceptably high risk to the unborn. However, a process referred to as therapeutic cloning may eventually revolutionize the practice of western medicine. In this scenario, people who need cell, tissue or organ transplants could first generate a cloned embryo by nuclear transfer that would provide embryonic stem (ES) cells from which a cell line could be derived. ES cells can be stored frozen and, when needed, thawed and used directly or after they have been induced to differentiate into the desired cell type, for instance hepatocytes, neurons, cardiomyocytes, hematopoietic or pancreatic islet cells. These cells would be recognized as self and therefore immunologically compatible with the host and not rejected after transplant. In the future we may even be able to derive or create tissues or whole organs comprised of different cell types from ES cells but this is not possible yet. Further, this approach is not likely to be efficient enough for this purpose; other techniques, such as bone marrow transplants or reprogramming adult cells, are likely to
supersede embryo cloning.

2. **Therapeutic cloning—pharmaceuticals and antibiotics.** Current production of antibiotics and drugs is laborious and expensive, often requiring bioreactors and related facilities. An alternative is transgenic production, where a gene of interest is inserted into an embryo and then the protein expressed in adult animals. Currently most effort is focussed on producing drugs in the milk of cattle and sheep, but alternatives being actively pursued include pig seminal fluid and chicken eggs. The production of transgenic animals is slow, expensive and very hit-and-miss. Traditionally, a gene of interest is microinjected into many fertilized eggs, a few of which will incorporate this DNA into their genome and even fewer express the inserted gene in the desired tissue. In contrast, cultured cells can be made to incorporate DNA constructs much more readily than fertilized eggs, and screening for expression of the inserted gene is considerably easier and more rapid. These transgenic cells can then be reprogrammed, and used to make transgenic clones more efficiently than microinjection. A number of pharmaceuticals are being evaluated for production by this technique, including human factor IX (for hemophilia), alpha-1-antitrypsin (for cystic fibrosis) and interferons (for cancer).

3. **Animals essential for biomedical research.** Many human genetic diseases are also shared with macaques. Propagation of these very valuable animals by cloning would have enormous utility for biomedical research in the development of nonhuman primate models for human disease. Genetically identical monkeys would also be particularly valuable for immunological studies, neuroscience studies, and vaccine trials. Production of genetically identical macaques with specific MHC class I and II alleles would represent an unequalled opportunity to characterize the entire immune response to SIV in the setting of vaccination and infection and should be an invaluable resource for understanding pathogenesis and vaccine-induced immune responses. Genetically identical animals would also enable us to study the relative contributions of environmental versus genetic factors in the etiology of various diseases.

Cloning can also be beneficial to the welfare of animals. The use of genetically identical animals would eliminate genetic variation and markedly reduce the inter-animal variability in experiments, increasing the power of the experimental design, and thus substantially reduce the numbers of animals required for generating statistically valid data.

4. **Multiply animals with desirable genetic traits.** Selective breeding has been used for thousands of years to produce animals with desirable genetic traits. Cloning will allow animals (or their clones) with desirable genetic traits to be used (mated) more efficiently for animal production to propagate their desirable traits. Some of these animals may be unable to breed (for non-genetic reasons i.e., death, disease or accidents) and cloning maintains a gene pool that would otherwise have been lost. The cloning of Starbuck at the University of Montreal Faculty of Veterinary Medicine is a good example. Starbuck was a genetically superior bull whose semen was widely sought-after. He stopped producing sperm, first due to age and eventually death; however his unique genetic contribution will be available again from his clone. When Starbuck II starts producing sperm it will enable farmers to again have access to the desired genotype. Another good example is 862, the first calf to be cloned specifically for disease resistance, born at the College of Veterinary Medicine at Texas A&M University. The genetic donor for the clone, Bull 86 was a particularly special animal since he was found to be naturally disease-resistant to brucellosis, and under laboratory conditions resistant to tuberculosis and salmonellosis.

One limitation to the production of large numbers of cloned animals is that a similar susceptibility to a virus or bacteria could wipe out an entire herd. There is also the risk of inbreeding: there are industry standards which apply to all bulls used for artificial insemination, clones or otherwise (maximum level of inbreeding allowed is 6%).

5. **Preserve endangered species.** Rare or endangered species may be cloned using the oocytes from a closely related and abundant domesticated species. Attempts have been made to use oocytes of cattle (*Bos taurus*) to clone the Gaur (*Bos gaurus*). A few pregnancies have been
produced, however to date the only live offspring died at 2 days of age of clostridial enteritis, a bacterial infection that is almost universally fatal in newborn animals. Also on the list of endangered species is the bucardo, a newly extinct Spanish mountain goat. The last known remaining animal was killed by a falling tree in January 2000, and tissue samples were taken for potential cloning. Others include the cheetah, ocelot, giant panda and there is even talk of restoring the woolly mammoth using cloning technology.

6. Mitochondrial genes. Cloning involving nuclear transfer does not produce true clones in the strictest sense. Offspring produced this way will have identical nuclear DNA to that of the nuclear donor, however, there is another pool of DNA within a cell: mitochondria. Mitochondria are organelles that contain their own DNA. In nuclear transfer, there is no transfer of mitochondrial DNA to the recipient cell, therefore, animals derived from them contain mitochondrial DNA from the recipient cell (oocyte), not from the nucleus donor. This technical fact may be advantageous in domestic animal production. Even within species and breeds, it is known that mitochondrial DNA can be polymorphic and that some polymorphisms present in specific maternal lineages (mitochondrial DNA is inherited exclusively through the mother) correlate with dairy and growth performance traits. Cloning by nuclear transfer could enable the matching of the best mitochondrial DNA to the best nuclear DNA. This may be applicable also to human medicine. There are many pathologies that originate from mitochondrial gene mutations. Women carrying these mutations have a high risk of transmitting them to their offspring. As no treatment is currently available to remove the unwanted mitochondrial DNA from their germ cells, the oocytes of an unaffected donor might be used to clone a couple’s healthy nuclear genes, though much research needs yet to be done before this can be accomplished.

Animal Welfare Issues Involved With Cloning

There is concern that cloned animals may be predisposed to disease, or may suffer defects causing stress. This issue is worth considering seriously, as USDA guidelines and the US Animal Welfare Act are currently under review, and it has been suggested that transgenic animals may be automatically placed in the highest pain category, irrespective of the gene inserted. It must be understood that cloning from adult cells is in its infancy, and like experimental surgery, early failures are to be expected. Most losses of cloned animals occur in utero, thus no pain or distress is suffered. Those that reach term are so few that no conclusions can yet be drawn. However, it should be acknowledged that the incidence of physical defects (heart and lung problems in particular) is high. Some animals have died post-natally, of natural infectious causes or from congenital defects. Many of those that reach term appear to be healthy, and show no signs of pain or distress. There appears to be no inherent infertility problems (Dolly has produced several ‘natural’ offspring), and fears of premature aging may be unfounded.

References:


FASEB has a very nice booklet on cloning available at their website: http://opa.faseb.org/pdf/cloning.pdf.