The Rocky Road from Dolly to Human Embryonic Stem Cells: Has it Been a Worthwhile and Justifiable Scientific Pursuit?†

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Abstract

The announcement of the birth of Dolly the sheep, the world's first adult cell somatic mammalian clone, in February 1997, caused excitement and concern in equal measure. Since then, the technique has been extended to 7 further species and has been refined to allow the introduction of new genes into clones as well as modification of existing ones. Health problems continue to be an issue of concern and the technique remains highly inefficient. This inefficiency is due to reprogramming difficulties in the donor nuclei, a problem that confounds immediate solution but one that is fuelling a lot of interesting basic research. Cloning could also be used to make embryonic stem (ES) cell lines from healthy cells taken from sick patients and after further manipulation, tissue made from these ES cells could be used to replace damaged tissue. Proof of principle of this concept, otherwise known as therapeutic cloning, has been obtained in mice, but its implementation in humans is a long way off.

Ann Acad Med Singapore 2004;33:121-7

Key words: Animal cloning, Dolly, Embryonic stem cells

Introduction

The first mammal cloned from an adult cell was born in July 1996.[1] The delivery of Dolly, a Finn Dorset sheep, was attended, unusually for a sheep, by a retinue of concerned animal handlers, technicians and vets. In April 2003, Dolly was euthanised after contracting an incurable and debilitating viral disease. In those near 7 years of life, Dolly had become the most widely known individual non-human mammal probably since "Lassie" the TV dog and "Willy"(real name, Keiko) the killer whale. What made Dolly's life so notable were the reverberations it caused in human society. The choice of her name probably completed the anthropomorphic transition between a cute, woolly lamb and a human, so Dolly rapidly became an honorary person and a measure of things to come. For many people, Dolly's birth signalled too great a departure from the mores governing developments in assisted reproduction, an area of activity already fraught because of recent technical advances; to these same people it indicated the failure of science to police itself and led to a worldwide flurry of legislative activity and philosophical debate. The situation became further inflamed when cloning became linked with another controversial scientific development, the production of human embryonic stem cells. Although, as this paper will point out, there is overlap between the 2 technologies, much of the future biomedical potential in human embryonic stem cell research owes nothing to cloning technology.

In this paper, I would first like to focus on the science that led to Dolly and the scientific achievements and problems surrounding the technology that made her. I will conclude that in large part, the genome reprogramming needed to make cloning successful, is incomplete. Unfortunately, whilst we have a good idea of where the problems lie, a solution seems some way off. Nevertheless, the technique does work, if very inefficiently, and can be used to answer major questions in basic biological research as well as in novel biomedical applications.

A second focus of this paper covers the overlap between cloning and human embryonic stem cell technology. Here I will conclude that therapeutic applications of hES cells arising from cloned embryos are a long way away and that application of this technique in humans is premature. However, use of the technique for the production of hES lines from individuals suffering from specific, early-acting congenital lesions, might provide a valuable resource for garnering insight into the developmental consequences of these mutations.

Cloning of Mammals

Fertile adult frogs were first obtained by nuclear transplantation of differentiated somatic cell nuclei in 1966.[2] The principal objective of this work was to prove that during cell differentiation, inactive nuclear genes were not lost or permanently inactivated; in other words, the nuclei retained

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† *Presented at the 22nd Annual Scientific Meeting of the Chapter of Physicians, Academy of Medicine, Singapore on 16 August 2003.*

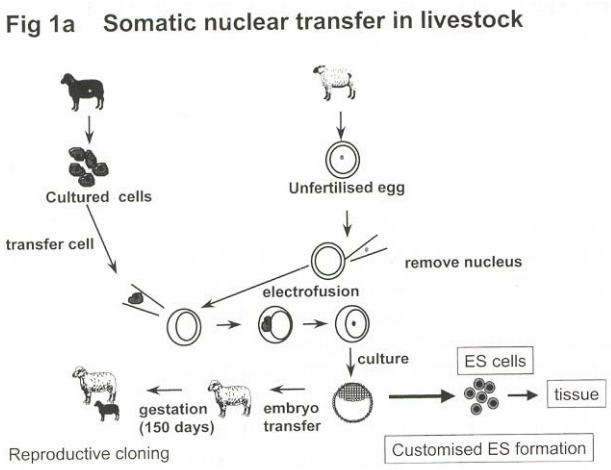

Figure 1a shows the standard method of somatic nuclear transfer that has been used in all mammals except the mouse. Cells are cultured from disaggregated foetal or adult tissues and cultured in the laboratory. Individual cells are then fused using an electrofusion chamber, with matured oocytes whose genetic material was previously removed by suction into a glass micropipette. The resulting "reconstructed" embryo is then cultured in vitro until the blastocyst stage (in sheep this culture is performed *in vivo* in a temporary recipient). For reproductive cloning, the blastocyst is then transferred to a hormonally prepared recipient and taken to term. Alternatively, the embryo is immediately returned to a final recipient and taken to term. For the generation of embryonic stem cells, the blastocyst is disaggregate and ES cells generated *in vitro*.

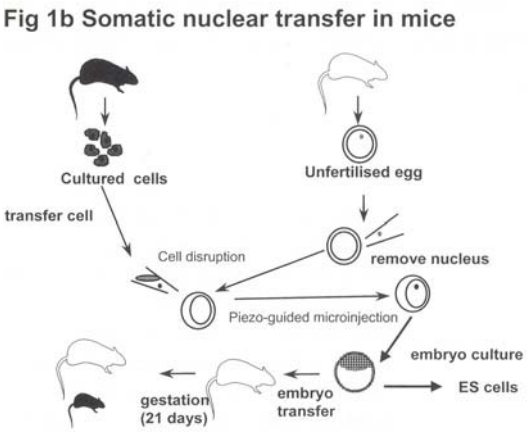

Figure 1b shows the procedure in the mouse. The major difference is that the donor cell is disrupted by suction into a glass micropipette and then the liberated nucleus is deposited into the enucleated oocyte. The use of a piezo-impact pipette drive unit causes the glass needle to vibrate very rapidly and ensures minimal damage to the oocyte.

totipotency. Although this seminal conclusion was criticised on technical grounds relating to the cellular identity of successful donor cell nuclei, later data confirmed its validity. Understandably, the success obtained with frogs encouraged attempts at nuclear transfer in many other species including insects, ascidians, fish and mammals. The first report of live births in mammals (mice) appeared in 1981.[3] This involved the injection of inner cell mass cell nuclei into zygotes but the results were never confirmed. Reproducible success was obtained using 4- and 8-cell blastomere nuclei but only where the recipient cytoplasm was a 2-cell embryo.[4] However, even in mice and ruminants where nuclei from more advanced embryo stages have been successfully used,[5] live births were only obtained from embryonic cell donors. Nevertheless, from the large body of mammalian research, 3 major biological factors emerged which were determined to influence the success of nuclear transfer studies:[6] these were the biological state of the recipient cytoplasm, the developmental status of the donor cell, and the relative cell cycle stage of donor nucleus and recipient cytoplasm. The births of Megan and Morag in 1995[7] and of Dolly in 1996[1] exploited these observations and were a watershed in mammalian cloning for 2 reasons: First, in the absence of any proven embryonic stem cell lines, the use of primary cell cultures which were competent for nuclear transfer, opened up the possibilities of applying more sophisticated gene manipulation and targeting strategies to livestock. Second, the successful use of an adult cell donor provides final proof of the totipotency of the nucleus in an adult somatic cell. Given the early pre-eminence of frog studies, it is ironic that this final demonstration was made in a mammal. In a sense, the frog was a poor model. The extremely short frog embryonic cell cycle masked the importance of synchronising donor and recipient cell cycles and may have induced the frequently observed chromosomal abnormalities which occurred in the progeny of the transplanted nuclei. In addition, the microinjection technique that worked so well in amphibia,[8] proved physically damaging (mice), very difficult (cow) and until recently,[9] relatively ineffective in mammals.

The Nuclear Transfer Technique

The nuclear transfer procedure is shown schematically in Figures 1a and 1b in its current 2 main variations. In each case, a diploid nucleus is introduced into an enucleated metaphase II (MII) oocyte to generate (after successful activation-see below) a reconstructed embryo. In both cases, enucleation is performed by effectively pinching off a region of the oocyte containing the maternal chromosomes without puncturing the oocyte plasma membrane, an innovation first introduced by McGrath and Solter.[10] In nearly all successful examples of mammalian cloning, the incoming nucleus has been delivered by virus-induced fusion or electrofusion of the donor cell with the oocyte (Fig. 1a). In the recent cloning of mice from adult donors,[9] the donor cell was disrupted by suction into a glass microneedle which was then inserted, and the nucleus delivered, into the oocyte using a piezo-electrically controlled pipette holder (Fig. 1b). In ruminants, most success has been obtained where the recipient cytoplast is a MII oocyte, although 2-cell embryos have also been used in mice. Surprisingly, the zygote has proved a very poor recipient cell, with development to term of a reconstructed zygote only occurring when donor pronuclei were used. This might reflect the sequestration of important

nuclear factors (e.g. transcription factors, histones, embryonic lamins) within the large, host pronuclei prior to their removal. Certainly, developmentally important components are stored in the oocyte nucleus (germinal vesicle)[6] and might partition into the zygote pronuclei during their formation.

Activation of the oocyte can accompany the fusion event or can be delayed for several hours. Activation stimuli include electrofusion, ethanol, ionomycin, dimethylaminopurine and cold temperature, and activation requires the presence of calcium ions, although strontium ions have been used in mice. Activated, reconstructed embryos are then returned immediately to surrogate recipients (mice and rabbits), or cultured for several days *in vitro* (cows, goats and sheep) or in the ligated oviduct of a temporary recipient (sheep) before final transfer to a suitably prepared recipient (reproductive cloning). With cows and sheep, there are confirmed examples of high levels of pre- and postnatal mortality[11,12] and large birth weights in offspring resulting from manipulated and/or cultured embryos, both in the presence or absence of nuclear transfer. Problems in imprinting (see below) in the donor nucleus prior to transfer,[13] or developing during *in vitro* manipulation could be contributory factors in all these complications of assisted methods of reproduction. Thankfully, similar problems have not been reported in human *in vitro* reproduction programmes.

Eight Mammalian Species Have Now Been Cloned From Somatic Cell Nuclei

The publication announcing the birth of Dolly did not convince all scientists that Dolly was the product of an adult somatic cell. Although the molecular phenotyping reported by Wilmut et al[1] was thorough, the fact that the success was limited to one single animal led some scientists to demand further corroborative evidence, or better, more "Dollys". More evidence was soon forthcoming with the publication of the first DNA fingerprinting of sheep[14] as well as more microsatellite DNA testing.[15] However, more relevant were the further reports of sheep cloning and the successful extension of the technique to many more mammalian species. Cows, mice, goats, cats, horses, pigs and rabbits have now been cloned.[16-20] Considerable work has been performed with rats, dogs and monkeys, but with no success reported as yet. In fact, a recent report from Simerly et al[21] that embryo reconstructions led to no pregnancies, led the authors to conclude that it might never be possible to clone any primate, including humans.

Many Cell Types can be Used as Nuclear Donors

Mammalian somatic cell cloning is an arduous procedure and most of the reconstructed embryos never make it through development. To date, all the somatic cell populations used for cloning are primary cultures and none of the cultures is homogeneous for cell type. Dolly was cloned from an adult mammary gland cell, but the exact identity of the cell could and will never be established. Likewise, although fibroblast, Sertoli, cumulus, muscle, lymphocyte and other specialised cell types have been cited as capable of generating normal animals,[16,17] the combination of heterogeneous culture and low efficiency make all such claims tenuous at best. The best evidence that the nuclei of at least some terminally differentiated cells can support full development comes from the work of Jaenisch and co-workers.[22] Using mouse B and T lymphocytes, these workers used the nuclear transfer procedure to prepare several embryonic stem (ES) cell lines. [Terminally differentiated B and T lymphocytes contain unique DNA rearrangements in their immunoglobulin and T cell receptor loci, respectively.] In each ES line, every cell contained the same genome rearrangement as the original cell donor. These ES cells were then used to make mice using a technique known as tetraploid embryo complementation. This involves the injection of about 20 ES cells into the lumen of tetraploid mouse blastocysts, which are then implanted back into the uteri of foster mice. During the ensuing development, the tetraploid cells only contribute to the placenta so that the emerging newborn animal is composed entirely from derivatives of the injected stem cells. In the case of the B lymphocytes, 16 identical live mice were made from the cells of one ES cell line. All the tissues examined in these animals contained rearranged DNA matching exactly that of the donor cell line. Results with the T cell-derived ES lines were less successful with only one animal that died shortly after birth. Nevertheless, all examined tissues contained the same T cell receptor gene rearrangement. The inescapable conclusion is that some terminally differentiated nuclei are capable of programming full development. Whether the reprogramming required for this pluripotentiality was only acquired gradually during the ES cell culture does not detract from the fact that this could be achieved.

Cloning is Highly Inefficient and Leads to Considerable Pre- and Post-natal Mortality and Morbidity

It is now over 7 years since Dolly was cloned. Yet during this time, the technique has not improved markedly in efficiency. Much thought and experimentation has been put into discovering the reasons for this. One convenient reference point for comparative work has been the gene activity that occurs in the blastocyst. The blastocyst is the latest stage where the product of nuclear transfer can be examined in a detailed way prior to birth or, more likely, natural abortion. It has been known for a long time that the transcriptional profile of an early mammalian embryo is very different from those of specialised cell types. It is also axiomatic that for developmental success, the transcriptional profile in a reconstructed embryo would need to approach that seen in a normal embryo of the same age. Several comparisons have been performed, examining in individual embryos the transcripts of genes known to be transcribed at the blastocyst stage. In one such study Bortvin et al[23] reported that only ~60% reconstructed embryos, reaching the blastocyst stage, expressed a set of 11 Oct 4-related genes at levels similar to control blastocysts. Oct 4 is a particularly relevant choice because it is known that this gene is crucial for post blastocyst development and that in ES cells, the level of

Oct 4 transcripts can dictate the pathway of ES cell differentiation. However, Boiani et al[24] found that even if the Oct 4 gene is activated at the correct developmental time, inappropriate spatial expression can occur and they speculate that this too could disrupt subsequent development.

These and other studies attest to faulty transcription of specific genes during early development. Sometimes the aberrations can be more subtle. There is a class of 50 to 100 genes – the "parentally imprinted" genes – where the developmentally regulated transcription of the genes is allelle-specific, with the paternal versus maternal origin of the gene being important. Although the parental effect can be maintained into adult tissues, it is generally believed that mis-expression of most imprinted genes in adult tissues is irrelevant to cell function. This might explain why nearly, if not all, seemingly healthy cloned individuals that have been so examined display imprinting abnormalities.[25] In contrast, imprinting disparities have been blamed for the phenomenon of "large offspring syndrome" which in part arises from a large and dysfunctional placenta.[26] It is highly possible that many of the specific problems associated with the early development of clones are in turn due to faulty placental development. Of course, the placenta itself develops from the trophectoderm, the first differentiated lineage to occur during development and reprogramming failures, rather than faulty imprinting, may be an additional cause of placental abnormality. Irrespective of exact cause, it is reasonable to conclude that many of the developmental difficulties seen during cloning may be ascribed to faulty gene transcription of one form or other. Unfortunately, whilst we now have a more profound understanding of the causes, this knowledge has not immediately suggested a remedy to problems in cloning. Clearly, the deployment of a non-invasive procedure that allowed real time monitoring of early gene expression, could result in a dramatic improvement in the numbers of implanted embryos developing normally to term.

Cloning is a Tool for Basic Biomedical Research

Despite the inherent problems of the cloning technique, its success has presented new opportunities to address fundamental research issues. A major focus discussed above concerns the nuclear reprogramming which occurs during the cloning process. How does it occur, can it be improved, could it be effected without disrupting the donor cell, etc? Other applications include, for example, ageing in mammals. This has been attributed to the loss of telomere repeat motifs at the end of chromosomes. These DNA elements are thought to prevent erosion at the ends of chromosomes and their loss during cell replication has been suggested to trigger cellular replicative senescence in the cell. Although the phenomenon of telomere shortening is not seen in all cells and varies from species to species,[16] it is quite clear that cell culture has a dramatic reductive effect on telomere repeat numbers. Since cell culture is a standard part of the cloning procedure, the nuclei used in the cloning procedure suffer unusually severe telomere shortening. Indeed, comparison of Dolly and other cloned sheep's telomeres with those from age-matched controls indicated a major reduction due to cloning.[27] Interestingly this was also seen in some but not all cloned cows and was not seen at all in cloned mice. Where it did occur (e.g. Dolly), there was no evidence of premature ageing.

Cancer is generally a disease of old age and this is a consequence of the mutational burden on a cell increasing with each cell cycle, as well as exposure to environmental mutagens. An inevitable question regarding the use of adult somatic cells in the procedure is whether a greater susceptibility to cancer is demonstrated in clones from older somatic cells. It is not really clear whether this is a legitimate question since cancer often is caused by the selective, clonal expansion of a rare cell in which particular mutations have occurred. The likelihood during cloning of such a cell being selected from a healthy animal must be very low although again, the *in vitro* pre-cloning culture of cells may bias the population towards cells which have lost the normal degree of cell cycle control.

We may conclude that the accumulation of cancerous mutations in cell populations may not impact on the long-term health of cloned animals. In fact, it may be that cloning can assist in discriminating to what extent mutations causing cancer are epigenetic in nature. To this end, Jaenisch and colleagues, using the combination of cloning and tetraploid embryo complementation described earlier, were able to provide preliminary data that animals made from nuclei taken from inducible melanoma cells still displayed a tumour phenotype, demonstrating that the cause of cancer was due to an irreversible genetic change in the tumour cell [Jaenisch – personal communication].

A final example concerns how genetically identical, clones are to each other and the nuclear donor. It has been shown in sheep clones that the majority of mitochondria come from the donor oocyte, not the somatic cell.[28] Since it is rare for a group of cloned animals to share the same oocyte donor, sibling clones may have the same nuclear DNA but different mitochondrial DNA. What impact this could have is unknown, although some minor histocompatibility antigens have been linked to the mitochondrial genome. As I have suggested before,[16] one way of testing for immuno-incompatibilities would be to perform skin transplants between otherwise identical clones.

Cloning and Biomedical Applications

Although, in principle, cloning offered the obvious agricultural benefits of being able to replicate elite animals, the continued inefficiencies of the technique, together with the unknown long-term consequences on the individual clones, have impeded serious applications of this sort. More success has been achieved in biomedical areas where as a new method for genetic manipulation, the cloning technique offered major benefits over existing technology. Before the birth of Dolly, transgenesis in all mammals, except mice, relied mainly on the DNA microinjection of zygote pronuclei. Biomedical uses of transgenic animals made in this way suffered from a number of

disadvantages:

a) Genes could be added to the host but host genes could not be removed or modified.
b) Transgenic animals were often mosaic and this made breeding particularly difficult for large transgenics like cows. Mosaicism also complicates experimental interpretation.
c) The method allowed no control over the site of chromosomal integration of the injected DNA; the exact location of integrated DNA impacts heavily on its expression.
d) The majority of DNA-injected animals which developed to term were non-transgenic; the technique was therefore wasteful and expensive, particularly for ruminants where only 1 or 2 offspring are the norm.

One of the early steps in cloning is cell culture and this lends itself wonderfully to techniques of gene manipulation. With the subsequent demonstration that genetically-modified somatic cells could be successfully cloned,[29] it was clear that the combination of the 2 techniques had the potential to rectify the disadvantages of DNA microinjection by:

a) ensuring that all animals were transgenic;
b) ensuring that no animals were mosaic;
c) allowing pre-screening of manipulated cells in order to obtain some information regarding integration sites;
d) allowing the removal or modification of host genes.

To date, all these hypothetical advantages have been brought to fruition but there is only sufficient space to cover one in any detail.

There is currently a worldwide shortage of organs for transplantation. This deficit is likely to increase rather than decrease. The only credible alternative realistically conceivable at present (but see below) is the use of animal organs, specifically those from the pig. There are many logistical, regulatory and safety reasons why even this solution may be unacceptable. However, all these are moot if the technical challenges, posed by the need for a xenotransplant to be immunologically and physiologically compatible with the human recipient, prove unassailable. Transplants of normal pig organs into primates are rejected within 20 to 30 minutes.[30] This hostile, "hyper rejection" response is triggered by the binding of naturally occurring, circulating recipient antibodies to the sugar residue, alpha 1-3 galactose. This is a component of many surface glycoproteins in pig and all other mammalian tissues, excepting those of old world primates and man; in these latter species, the enzyme responsible for catalysing the galactose addition, alpha 1-3 galactosyl transferase (GT), is mutated and inactive. Numerous attempts have been made to down-regulate the activity of this enzyme and whilst some attempts resulted in a 90% decrease of surface galactose, this did not prevent hyperacute rejection. It was recognition that all such non-genetic approaches were doomed to failure that drove our attempts to generate pigs in which the gene had been removed or mutated. It was the development of somatic cell cloning that made this objective a near-term possibility.

Pigs proved initially difficult to clone; however, this step was achieved in early 2000.[31] Subsequently, we perfected the use of homologous recombination to target and modify specific genes in sheep fibroblasts which were then used for nuclear transfer. Similar techniques were then applied to pig fibroblasts with the result that in 2002, pigs were born in which 1 allele of the GT gene was inactivated.[32] It seemed only a matter of time before backcrossing heterozygous animals of different sex (a procedure estimated to take at least 15 months) would lead to the birth of homozygous GT-/- animals. However, using a novel procedure to modify the second allele in GT+/- fibroblasts, Phelps et al[33] were able to produce GT-/- animals within 7 months of the birth of the first GT+/- animals. Cells from these animals had no detectable alpha 1-3 galactose on their surface and were not lysed by human serum. Pig-to-primate organ transplantations are in progress and preliminary results indicate extended survival of the transplanted organs.

Cloning and Stem Cells

The report in 1998 about the first established lines of human ES cells[34] led to parallel outcries about the damage to human dignity to those seen on the publication of Dolly's birth. Unfortunately, the nature of the publicity led many to believe that the hES line generation inevitably involved the process of cloning. Whilst cloning can be a part of the process (see below), the immediate therapeutic benefits of hES cell use will not involve this process and it is unfortunate that the 2 techniques have been so strongly linked.

A stem cell can be defined as a cell which on cell division faces 3 developmental options: The daughter cells can be identical to the original cell (self renewal); they can be the progenitors of more specialised cell types (differentiated) or finally, 1 of each cell type can be formed. Adult stem cells comprise a wide variety of types including neuronal, skin and the blood forming stem cells which are the active component in bone marrow transplantation. These latter stem cell types are also the principal feature of umbilical cord-derived stem cells. Adult stem cells can mature both in the laboratory and in the body into functional, more specialised cell types although the exact number of cell types is limited by the type of stem cell chosen. Adult stem cell-derived therapy has been touted by some as *the* way forward because of the clinical accessibilities of some stem cell types and a purported plasticity which allows facile interconversion between different stem cells. However, this latter claim has been seriously challenged recently and adult stem cells have 1 major disadvantage – they do not divide well in culture thus limiting their potential numbers and consequently, use in cell therapy. In contrast, human embryonic stem cells (hES cells), appear to have unlimited proliferative capacity. These cells, which are derived from the inner cell mass of a 5-day human blastocyst, are believed capable of differentiating into all the different cell types present in the adult human and are thought to do so by recapitulating early and late developmental events. This pluripotentiality, combined with their expansion capabilities, has made hES cells attractive

starting material for research into cell therapy. All lines presently established have been made from supernumerary embryos left over from *in vitro* fertility programmes. There has been much debate about the ethics of using "spare" embryos in this way and, in several countries, generation of hES and/or their use has been banned. However, some countries, for example, the United Kingdom, Sweden, Holland and the USA, sanction their derivation and use (although in the USA, **new** hES derivation has to be privately funded), whilst permissive legislation is pending in other countries (e.g. Singapore).

One problem that besets most clinical transplantation is that of immunohistocompatibilty. Most transplantation involves allografts (i.e. from 1 individual to another) and the genetic mismatch can trigger transplant rejection or even graft-versus-host disease if concomitant immunosuppression is not used. Unfortunately, long-term immunosuppression has associated side effects. In the context of hES-mediated cell therapy, this problem could be avoided if the hES-derived therapeutic tissue were genetically identical to the patient. This scenario could be realised if the nucleus from a patient's healthy tissue were used in the cloning procedure shown in Figure 1a, with the resulting blastocyst being used to make a patient-customised hES line which could then be used after appropriate differentiation, to replace the damaged or diseased tissue. There should be no immunological consequences to this strategy. Although there is no doubt that this rather fanciful method (which has the misleading name of "therapeutic cloning") could work in the long term – indeed, a partially successful proof of principle study using "customised" mouse ES cells has been published[35] – issues concerning product safety and purity, provision of human oocytes and controlling differentiation, convince this author that attempts at therapeutic cloning in humans would be premature and should not be pursued for the present time. However, this does not mean that the technique itself has no immediate value. In fact, it should be possible, using cells from patients suffering specific congenital diseases (e.g. those cases of motor neurone disease which have a genetic component), to use the technique to derive disease-specific hES lines which could service *in vitro* studies to understand the aetiology of the disease during early human development.

Conclusion

Somatic cell cloning in mammals has proved to be a reproducible if inefficient technique, which has now been successfully used in 8 different species. The general inefficiency of the technique is most likely caused by the challenges posed by the requirement for the donor nucleus to be adequately reprogrammed within the short developmental window available between reconstruction of the nuclear transfer embryo and the start of cell division and development. It is not clear at the present time how these reprogramming inefficiencies can be rectified, although it should be possible in the future to monitor aberrant reprogramming and only transfer "good" embryos back to foster mothers. This at least might ensure a better health profile in neonatal clones. Even so, the long-term effects of cloning on health remain unknown for large mammals, although there is evidence that longevity is curtailed in some cloned mice. It is probable, however, that any detrimental effects on health will affect only the cloned animals themselves and the naturally conceived offspring of such animals should be healthy. This opens the way for the generation, through cloning, of new strains of specific mammalian species, e.g. the knock out, GT pig referred to earlier.

The advent of human embryonic stem cells has, in conjunction with cloning, raised hopes about the provision of patient-matched human embryonic stem cell lines that could be manipulated to provide patient-specific repair tissue. Such developments are a long way off, and it would be better to concentrate first on controlling the differentiation of human embryonic stem cell lines which do not come from cloned embryos. Such work would also provide a handy reference point against which deviations in differentiation of future, congenitally defective hES lines could be compared.

All the work reviewed in this paper has been controversial. Over the last half-century, there have been many developments in biomedical science which have raised concerns amongst the public. For example, the first successful kidney and heart transplants were met with a high degree of opprobrium, as was the birth of the world's first test tube baby, Louise Brown, in 1978. All of these advances are now generally welcomed by a majority of people. The public and legislators worry about human reproductive cloning. I agree that this application of cloning should be illegal. The fact that a technique could be practised illegally does not constitute good ground for preventing its emergence. This logic would lead to the banning of kidney transplantation on the grounds that impoverished parents in some Third World countries sell the kidneys of their healthy children in order to feed their families.

All 3 animal celebrities featured in the introduction have died (Dolly, Lassie and Keiko). All in their ways have made their contributions to human entertainment. Only Dolly has left an enduring legacy to medical science.

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