

# Cloning: Science Ahead of its Time?

By Prof Ng Soon Chye, Dr Liow Swee Lian, Dr Chen Naiqing & Mr Ng Yifan

**D**olly the sheep burst into the limelight when Dr Ian Wilmut and his co-workers in Scotland published her existence in the journal *Nature* in February 1997, even though she was born in 1996. Before her arrival were Megan and Morag, delivered in the summer of 1995, from cultured cells originally derived from a nine-day-old embryo. One may ask what all the fuss was about? Dolly was a miracle, created by coaxing an adult differentiated cell in a petri dish to go all the way back into an embryo, and then develop once more into a lamb, and then finally into a fully grown sheep. This was done with an egg, which had its nucleus removed before the donor cell was introduced. Before this feat, it was thought that such nuclear "re-programming" was impossible with adult differentiated cells. Clones produced before Dolly have been from embryos, whose nuclei were not differentiated, and hence it was easy to take one or two steps back in development into an early embryo.

When the scientific fact was finally accepted, there were worries of early death and inability to reproduce in such cloned animals. All these came to pass, with Dolly giving birth to Bonnie on Easter Monday in 1998. She was finally put to sleep on 14 February 2003, suffering from a virus that caused a tumour in her lung, at the age of 6+ years old. Her lifespan is arguably normal for a Finn Dorset sheep, though the tumour is uncommon. With her passing, was the passing of an era, an era of biological marvels. The egg was designed by nature, evolved through the centuries, to shape and modify the genes of the sperm, a highly differentiated cell, to prepare for the continuation of life, down the passage of time, till eternity. The egg is not designed to re-program a fully differentiated somatic cell, though many scientists have utilized the ability to modify the sperm to do likewise for the fully differentiated somatic cell.

Dolly left many legacies, the most prominent being Polly, the first transgenic sheep produced through cloning technology. Polly was born in the summer of 1997, with the gene for human factor IX inserted in her cells such that she secreted the human protein in her milk.

Because of Dolly, there has been a great deal of attention in the media, both good and bad, on cloning. Science fiction scenarios have raised public expectations of the promise of therapeutic cloning to cure diseases and suffering such as paralysis, to rescue highly endangered or even extinct animals, such as the Tasmanian Tiger and the mammoth (which is theoretically possible now, with the recent discovery of a mammoth frozen in the Ice Age 10,000 years ago in Russia's far-northern Yakutia region); and the production of cattle and goats that are designed to produce special proteins, such as Factor VIII. Some of these scenarios are now fact rather than fiction, such as the cloning of the Banteng, the Gaur and the Mouflon, and transgenic cloned cows and goats that produce milk with human proteins that cannot be produced by cells (both animal and bacterial).

However, the ability to form cloned human tissues to repair diseased and dysfunctional organs is still not attainable, not

because it is not technically possible potentially, but because research using human cells to clone tissues is currently not allowed. However, there was a very recent study in the 11 April 2003 issue of *Science* by Simerly et al that suggested that molecular requirements for nuclear re-programming may be stricter in non-human primates than in other species. More work needs to be done to confirm their findings and to determine the basic mechanisms of nuclear re-programming.

The positive and negative views on the production of human tissues through nuclear re-programming are many. On one extreme, all transplantation procedures can be done with one's own tissues, and hence there is hypothetically no immune rejection. The other extreme scenario, painted at the same time, are Frankenstein-like situations, such as headless clones produced solely for the purpose of tissues for transplantation. Therefore, an alternative title for this article may well be: "Is cloning all hype?"

For the purpose of this discussion, I would like to put aside science fiction and ethical considerations, and cut directly to the meat of the matter, which is, "Is the science beneficial?"

Therapeutic cloning, preferably called "nuclear transplantation to produce embryonic stem cells", has many potential applications. They can be classified into:

- 1) Production of autologous spare tissues and/or organs to replace diseased organs in the body. There will not be tissue rejection because these tissues will be the patient's own genetic make-up.
- 2) Correction of diseased genomes genetically before production of tissues as in (1). This may be useful in certain genetic disorders such as thalassemia major where the abnormal haemoglobin genes may be corrected in fibroblasts taken from the patient, then developed into haematopoietic stem cells through therapeutic cloning, and then transplanted back into the patient's bone marrow.
- 3) Genetic enhancement of "normal" genomes to improve function, viz
  - a. to enhance immune surveillance, e.g. to prevent cancers or relapse of cancers; this has been reported recently with adult neural stem cells in the mouse to target disseminated glioma islands intracranially by delivering therapeutic cytokine IL-4 *in vivo*; and
  - b. (conversely) to decrease genomic activity in "diseased" genome, e.g. gene therapy in cancer therapy.
- 4) As a research tool to understand early human development (normal and abnormal), such as early pregnancy loss.
- 5) As a research tool to understand cellular and molecular basis of diseases, by producing embryonic stem cells with defective genomes, to study these diseases.
- 6) To develop novel predictive tests, based on molecular or metabolic profiles early in development.

Hence it can be seen that there are many possible therapeutic applications of cloning. Why then is it not allowed in almost

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◀ Page 11 – Cloning: Science Ahead of its Time?

all countries? The answer is ethics and religion. These topics need to be discussed carefully and dispassionately. Many arguments put forth so far cannot be shaken because of specific convictions or opinions.

Another possible uncertainty lies in the therapeutic pitfalls. The most obvious is that the cells produced are incompletely re-programmed and hence unable to produce the tissues required, or even to function properly. A possible target tissue are insulin-producing cells. Inadequate re-programming may result in suboptimal production of insulin when required.

Another argument is that there is lack of evidence that therapeutic cloning is necessary or useful for medical treatments. Our counter argument is that more research is needed. The cloning technology is very recent, and research in the field of therapeutic cloning is in its infancy. This situation is very different from adult stem cell research which has been ongoing for several decades.

Hence, we feel that there is an urgent need to start on such research. However, given the current ethical and political climate, it will not be possible to research on human cells with cloning technology. Thus, there is a need for an animal model which can be directly translated into human trials – such as non-human primates.

Once it has been shown to be safe, using non-human primates first, researchers should be allowed to move cautiously forward with human therapeutic cloning, within a legal framework. The proposed legislation and controls by the BioEthics Advisory Committee (BAC), Singapore, as well as the similar United Kingdom framework, seem to be the best under the present circumstances. In Malaysia and Indonesia, there have been discussions between the relevant authorities and their Ministry of Health on this matter. In both societies, human reproductive cloning is not permitted, whilst the status on human therapeutic cloning is still being discussed.

This issue is highly charged in the United States, mainly due to the stance of the pro-life groups. Because of that, the debate has taken a political turn. The latest outcome of the debate in the US on human cloning, in no small way helped by claims by the Raelians and also fueled by Antinori's claims, is the total

ban on all human cloning, both reproductive and therapeutic (27 February 2003). The Bill, if passed through the Senate and enacted into law, would impose penalties of up to ten years in jail and fines of at least US\$1 million on researchers who violate these bans. The bill also prohibits treatments and cures developed in other countries using SCNT from being imported into the United States for use by patients. However, it is likely that US citizens will go to these countries that offer viable treatment options for their illnesses. It is similar to patients having abortions in countries that allow the procedures to be done, or terminally ill patients seeking euthanasia.

As Professor Lim Pin, Chairman, BAC, said, "Once the issue of access is satisfactorily settled, the research is expected to proceed apace and the enormous potential of cell therapy may be realised for the benefit of patients." (February 2003).

History is rife with examples of advances thought to go against the thinking of the times. Galileo Galilei, the Italian physicist and astronomer, was summoned to Rome by the Inquisition to stand trial for "grave suspicion of heresy", and was sentenced to life imprisonment in 1633 for his belief that the Earth revolved around the sun. This is recorded in his book "Dialogue on the Two Chief World Systems" which was published at Florence in 1632. The Dialogue was ordered to be burned, and the sentence against him was to be read publicly in every university. Likewise, the life-saving procedure of blood transfusion was initially forbidden by the Paris medical faculty, following a death shortly after transfusion by Jean-Baptiste Denis (France) with sheep's blood, though his first attempt in 1667 was with no apparent ill effects. This ban on blood transfusion throughout France and Italy lasted until the 1940s, when techniques of crossmatching, anticoagulation, and storage of blood and the establishment of blood banks made routine blood transfusion possible. And the birth of Louise Brown, the first "test-tube" baby, in 1978 following the pioneering efforts of RG Edwards and P Steptoe in the UK led to a storm of protest on the possible manipulation of embryos and "designer babies". Now, IVF and ART are accepted treatment modalities in infertility.

So, is cloning science ahead of its time? We will leave the answer to the reader to form his or her own opinion. ■