DOLL

In an exclusive interview, **Sir Ian Wilmut** discusses genetic engineering, his recent Parkinson's diagnosis and, of course, the **most famous sheep** in the **world**.

THE MANMADE SHEEP

It's important to "be ambitious!" On the eve of the IBMS Congress 2019, this is the advice to biomedical scientists considering a career in research from the pioneering lead scientist behind Dolly the sheep, Professor Sir Ian Wilmut. "There is almost no value in repeating work that has already been done, provided that the design and technical procedures were appropriate," he continues. "So, consider carefully what ideas arise from recent work and develop new hypotheses."

This approach has borne scientific fruit for embryologist Sir Ian, who was described by US senator Tom Harkin as "one of the true trailblazers in human history". For example, in 1998 (then) Dr Wilmut was awarded the Lord Lloyd of Kilgerran Prize "for developing and using embryo manipulation techniques in farm animals, leading to many potential uses in bio-medicine and livestock breeding". While Sir Ian's 2008 knighthood recognised his contribution to "revolutionising biology through the cloning technique which underpins the science of stem cell technology" and for his "outstanding" commitment to bringing the benefits of basic science to medical treatment, he "stressed that it was a recognition to be shared by his research colleagues."

Although forever associated with Dolly the sheep, Sir Ian's achievements also include bovine and ovine firsts with Frostie and Tracy, respectively, just a few of the highlights of a distinguished scientific career which he reflects on in this exclusive interview for *The Biomedical Scientist*.

Early days

"I was born at Hampton Lucy, near Warwick, in 1944 and raised in Coventry – my parents were teachers – until I was six, when we moved to Shipley, Yorkshire. Aged 10, my first career choice, inspired by a

merchant navy captain friend of my grandfather, was to be a seafarer, but that was thwarted with the discovery that I'm colour blind."

Wilmut's love of the outdoors led him to study agriculture at the University of Nottingham, "which is where, my interest in embryology began", he recalls. "After graduating, I went to the University of Cambridge to undertake my doctoral thesis at Darwin College, researching the freezing of boar semen. I worked at the Agricultural Research Council's Unit of Reproductive Physiology and Biochemistry, Cambridge, alongside a pioneer of animal reproductive science Professor Chris Polge, who had shown in the 1950s how glycerol can protect frozen cells."

Addressing the challenge of freezing semen that would retain viability upon thawing demanded a systematic approach that accommodated many variables associated with the process. Adopting a similar stepwise strategy later paid dividends for Wilmut and colleagues in relation to cloning.

In 1970 Wilmut reported that frozen and thawed boar semen in a yolk-glucose-glycerol diluent achieved an 83% fertilisation rate when deposited directly into the oviducts.

Frostie

"By 1971 I had completed my doctorate but continued working with Chris Polge, this time on cattle, trying to prevent embryo-damaging ice crystals forming during cooling, and before embryo transfer," says Wilmut. "I established the optimal cooling rate that prevented ice crystals forming, and we were the first to successfully freeze a calf embryo, thaw it and transfer it to a surrogate mother."

The world's first "frozen calf" – named Frostie – was a Hereford-Friesian cross. Wilmut says: "Frostie's birth in 1973 was filmed by a Dutch television crew. This also led to my first experience of dealing with the media, when I had to give a live television interview on the BBC news."

The prevalent view of scientific research favoured a utilitarian approach over fundamental, 'blue sky' thinking

Roslin

In 1973 Wilmut moved north with his family, having been appointed to the Animal Breeding Research Organisation (ABRO), located on the southern outskirts of Edinburgh, and which in 1993 became the Roslin Institute, now part of the University of Edinburgh.

"At that time the prevalent view of scientific research favoured a utilitarian approach over a fundamental, 'blue sky' thinking one," says Wilmut. "This was, in my view, a misguided attitude because fundamental research tends to make bigger advances, even if they are applied less effectively. It is critical to have both fundamental understanding and efficient use of the new understanding if we are to make the best possible progress."

Progress in molecular biology led Wilmut to consider how genetic engineering could be applied to improve the performance of farm animals as the era of so-called "pharming" unfolded. The idea was to give farmyard animals, such as cows and sheep, as Wilmut later wrote, "an entirely novel role and convert them into living drug factories to make the human proteins factor VIII and factor IX used to treat haemophilia, and the enzyme AAT (alpha-1 antitrypsin) used to treat lung disorders, such as cystic fibrosis."

Tracy

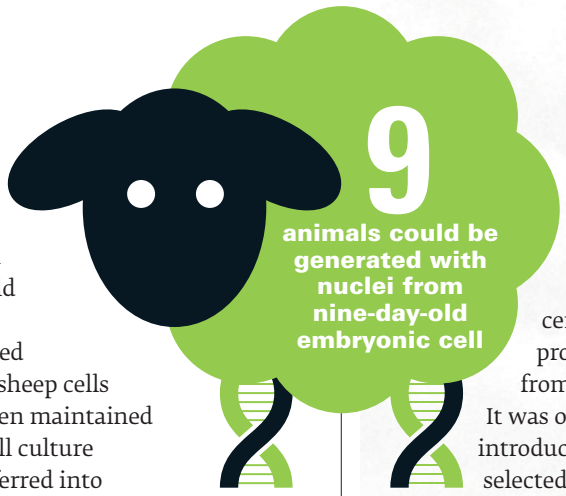
The basis of "pharming" was the introduction of a human gene into an animal embryo, which could be transferred to a surrogate host that would give birth to a transgenic animal; a process, which Wilmut recalls was "hopelessly and maddeningly inefficient." But nonetheless the feasibility of the technology was established at Roslin, when six transgenic sheep were produced by microinjection of DNA into early embryos. A Scottish blackface transgenic sheep named Tracy was born in 1990: "Tracy's milk "contained 35 grams of AAT per litre," Wilmut explains, "and she was probably the world's most famous sheep before Dolly arrived."

Megan, Morag and Dolly

The creation of Dolly was not the primary objective of Wilmut's team. Rather, it stemmed from an idea that occurred to him while addressing the inefficiencies of transgenic technology.

As he wrote: "First take stem cells from an early sheep embryo, multiply them to form millions of cells, then add DNA to those cells and select only the ones in which the process had succeeded for the use in cloning – nuclear transfer – to make animals."

With the birth of the Welsh Mountain sheep Megan and Morag in June 1995, Wilmut's team had shown that viable animals could be



generated when nuclei from nine-day-old partly differentiated embryonic sheep cells that had been maintained by *in vitro* cell culture were transferred into enucleated oocytes.

"Significantly we had established the important role of the cell cycle – more specifically, the induction of quiescence – in enabling partly differentiated cells to be reprogrammed," Wilmut points out. Following Megan and Morag's births, in which embryonic cells had been used for nuclear transfer, we had confidence in the possibility of cloning an adult."

Lamb $6LL_3$, better known as Dolly – whose embryo was derived from the mammary gland of a Finn Dorset ewe and transferred to a Scottish Blackface ewe recipient – was born on 5 July 1996, the first time that a mammal had been cloned from an adult cell.

Dolly's birth succeeded after 276 previous attempts had failed and was both a tribute to the high degree of technical expertise needed – including the demanding hand-eye coordination of microscopic surgery – and to patient teamwork, sometimes of military precision; for instance, when it came to recovering ewes' eggs at various times of the day.

The demonstration by Wilmut's team that differentiated adult cells contain all the genetic information needed to form a whole organism and that oocytes can potentially be re-programmed somatic cell nuclei has revolutionised biology and its applications to the treatment of disease.

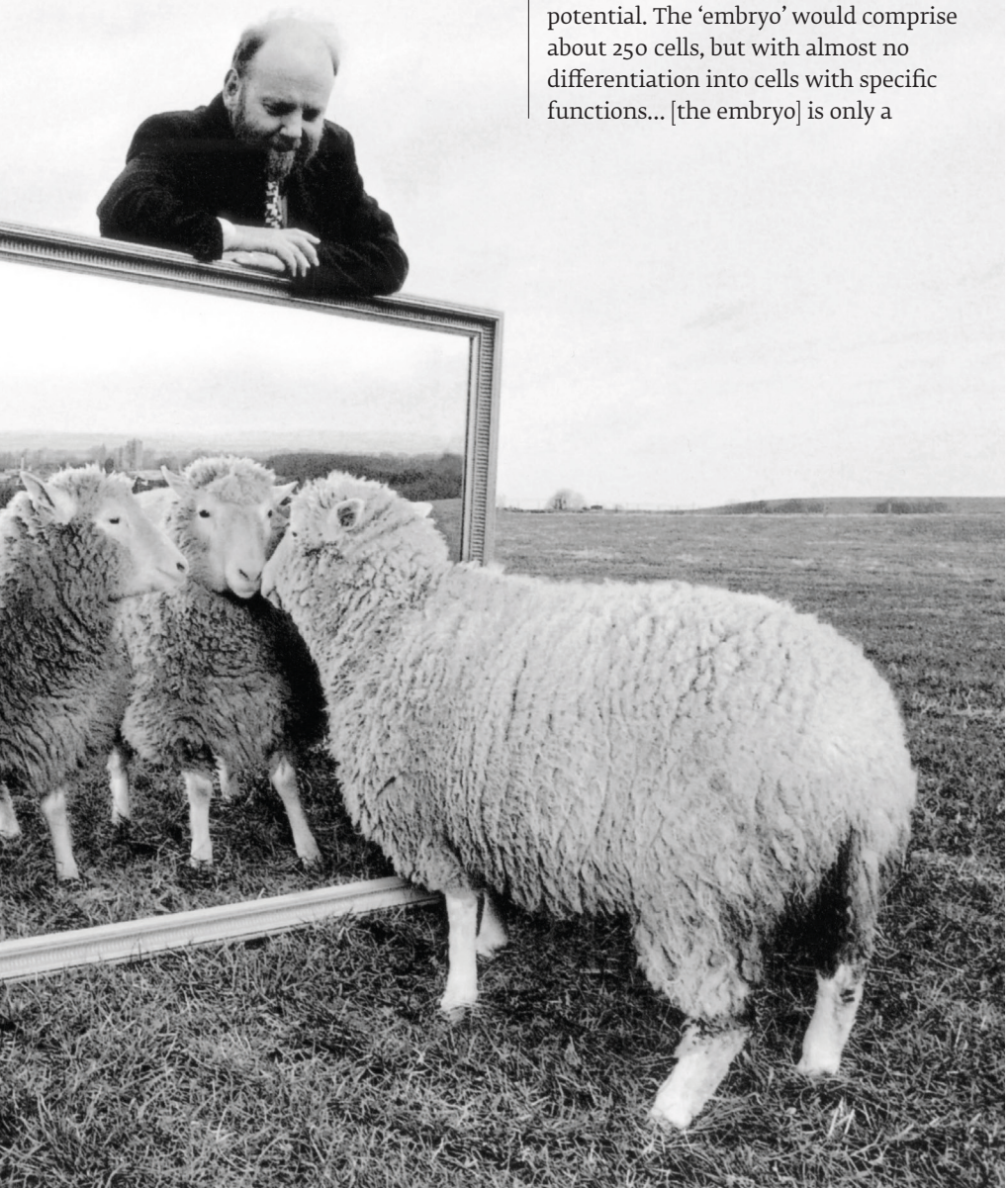
"The most important effect of the Dolly experiment," observes Wilmut, "was to make biologists think differently. Physiologists had developed the view that once a cell had acquired a particular tissue type it could not be changed, certainly not by a single protein, nor to a cell type from a different germ layer. It was only following the introduction of several carefully selected transcription factors that precise change occurred in some cells. In some cases, from one germ layer to another."

The ethics of cloning

The global impact of Dolly's birth changed the scientific landscape, but also raised ethical issues. Was this an unexpected challenge? "In my experience there have always been some people who protested about research with animals," Wilmut says. "But it was certainly true that there was far more interest following the birth of Dolly. Anticipating that this would be the situation, Roslin Institute and our collaborators PPL Therapeutics arranged for a key member of the team Keith Campbell and I to receive training from two former BBC employees. There was no doubt that this helped us cope with different styles of interview."

The training paid off, because Wilmut's contributions have enhanced the cloning debate while avoiding hyperbole. For example, Philip Kitcher reviewing Gina Kolata's Clone: The Road to Dolly and the Path Ahead (1997) for the London Review of Books lists the "excellent reasons to celebrate Dolly's birth as an event of scientific importance. They do not

Using that embryo as the basis... is no different than transplanting the organs of a dead person into a living one.



underwrite Kolata's claim that it changes our understanding of what it is to be human. Overstatement of this kind contrasts sharply with the modesty and good sense with which Ian Wilmut has spoken about his achievement."

These were apparent when Wilmut, contemplating cloning technology, wrote that the idea of producing human cells to treat diseases like Parkinson's, type 1 diabetes or AIDS was controversial because of the need to produce a human embryo from which you could then derive "stem cells" in vitro: "The controversial point is whether that embryo is a human being, or only a ball of cells with human potential. The 'embryo' would comprise about 250 cells, but with almost no differentiation into cells with specific functions... [the embryo] is only a potential person. To me, using that embryo as the basis for developing differentiated cells in a tissue culture dish is no different than transplanting the organs of a dead person into a living one. Most people are now comfortable with that." Wilmut is clear, however, in his opposition to human cloning.

Dolly's legacy

Social anthropologist Professor Sarah Franklin has noted: "Dolly is the viable offspring of a complex scientific lineage in which agriculture, medicine, embryology, and reproductive physiology [has] long been combined in the service of both extending basic science and producing new clinical applications."

By 2002, however, despite Wilmut's team demonstrating the developmental plasticity conferred by adult somatic cell nuclear transfer, they had acknowledged that relatively little was known about the molecular events occurring within hours of nuclear transfer, but predicted that "[a] new era of developmental biology and regenerative medicine awaits".

This was heralded by a development descended from the pioneering work which led to Dolly's creation: in 2006 a major breakthrough in stem cell research occurred when Takahashi and Yamanaka reported the generation of induced pluripotent stem cells (iPSCs) by using a retrovirus to deliver four reprogramming transcription factors into adult mouse fibroblasts.

Embryonic stem cells (ESCs) and iPSCs are pluripotent – capable of differentiating into cell types from all three germ cell layers (ectoderm, mesoderm, and endoderm) – so what advantages are conferred by using iPSCs over ESCs? Ethical reservations over ESCs, and their limited supply have constrained clinical application, and these, combined with safety concerns in relation to immune rejection of ESCs, mean that Takahashi and Yamanaka's innovation represents a

change of research emphasis.

With iPSC technology successfully translated from murine to human fibroblasts, researchers can "generate disease-specific and patient-specific iPSCs for modelling human diseases, drug development and screening, and individualized regenerative cell therapy". Thus genetically matched iPSC lines can be generated from patient cells *in vitro* and differentiated into affected cell types, effectively providing a "disease in a Petri dish" model.

Such models allow researchers to gain unique insights into the disease, for which new disease-specific drugs could be devised. For instance, drugs to prevent the death of medium spiny neurons in patients suffering from Huntington's disease.

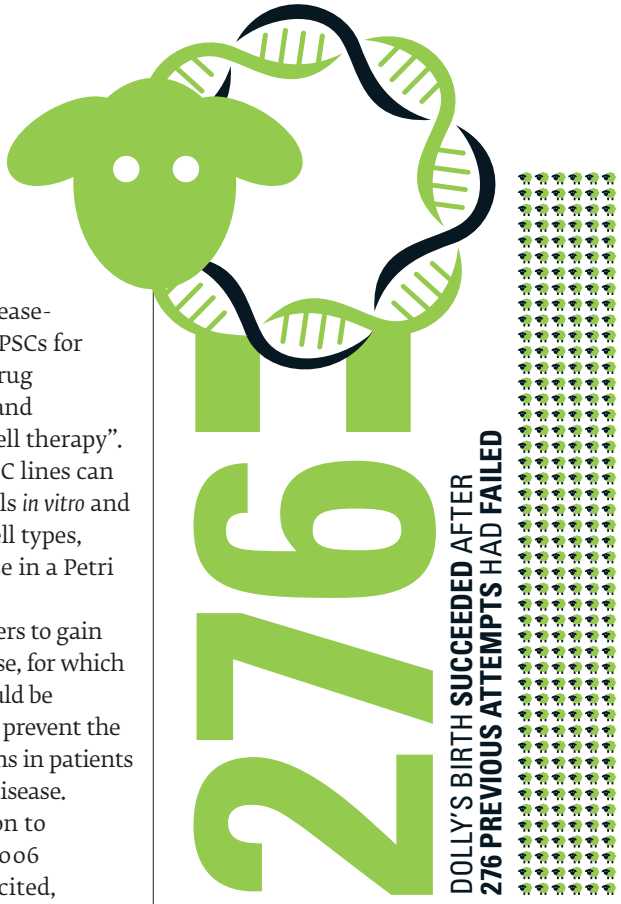
What was Wilmut's reaction to Takahashi and Yamanaka's 2006 breakthrough? "I was very excited, and still am. I believe that therapies derived from stem cells will provide revolutionary, new treatments to degenerative diseases, which, at present, have no treatment.

"On first consideration, many people judge that this will be through the provision of cells to replace those that have died or ceased to function normally because of the disease. However, to many of us this seems unlikely because cell therapy depends upon overcoming so many challenges."

These challenges include those of further defining the immunogenicity of iPSCs to enable better evaluation of innate and host immune responses and the establishment of standardised protocols for iPSC generation and differentiation.

New challenges

From 2000 to 2005 Wilmut was the Roslin Institute's Head of Department, Gene Expression and Development; from 2005 to 2006, Professor of Reproductive Science, University of Edinburgh; and in 2006 he became Founding Director, MRC Centre for Regenerative Medicine (CRM), University of Edinburgh until his retirement in 2012. However, he retains an active interest in ongoing research at the CRM.



On World Parkinson's Day, 11 April 2018, Sir Ian disclosed that he had been diagnosed with Parkinson's disease (PD). The announcement coincided with the launch of the Dundee-Edinburgh Parkinson's Research Initiative to investigate causes of the disease and translate discoveries into new therapies. Supporting the initiative, Wilmut said he would be "happy to act as a guinea pig and either donate tissue or try new treatments", adding: "Initiatives of this kind are very effective not only because they bring more people together, but because they will include people with different experience and expertise. It was from such a rich seedbed that Dolly developed, and we can hope for similar benefits in this project."

Meanwhile, progress continues apace. For example, in December last year a CRM team led by Dr Tilo Kunath developed PD-resistant dopamine neurones which could improve cell therapies for the disease. Cell replacement therapy is an emerging treatment for PD, and laboratory-generated midbrain dopaminergic neuronal precursors can mature into functional dopaminergic neurons when transplanted into pre-clinical models of PD. But trials have shown that transplanted grafts in PD are prone to toxic Lewy body formation, typical of PD brain cells. Kunath's team used gene editing technology to delete the SNCA gene – associated with Lewy body formation – and found that this conferred a measure of resistance to Lewy pathology: cells that had been gene-edited did not form the toxic clumps, compared with unedited cells, which did.

Commenting on his team's findings, Dr Tilo Kunath said: "We know that Parkinson's disease spreads from neuron-neuron, invading healthy cells. This could essentially put a shelf life on the potential of cell replacement therapy. Our exciting discovery has the potential to considerably improve these emerging treatments."

Stepping forwards

Prof Sir Ian Wilmut's *curriculum vitae*, he reflects, "illustrates the equally profound effects of happenstance and serendipity ..." But notwithstanding its serendipitous nature, Frostie, Tracy, Morag, Megan and, of course, Dolly, have etched Wilmut's name in the annals of biology, and current progress in stem cell research shows that his influential contributions continue to make an impact.

It is perhaps fitting to quote from the final paragraph of his *After Dolly: The Uses and Misuses of Human Cloning* (2006). "I want to be able to change my destiny rather than be condemned to a particular fate... For me, the widespread use of genetic and reproductive technologies is not a step backwards into darkness, but a step forwards into the light." BMS