The Economist

## Clones

# Hello, again, Dolly

**Twenty years ago the world met the first adult clone, a sheep called Dolly. Her legacy lives on.**

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IN THE summer of 1996 Karen Mycock, a cell biologist, was attending a wedding in the Scottish highlands. Returning to her hotel to change her hat, she found a fax pushed under her door. It said: "She's been born and she has a white face and furry legs." An unusual birth announcement; an unusual birth.

In February Ms Mycock (now Mrs Walker), who worked at the Roslin Institute, an animal-research centre near Edinburgh, had passed a tiny jolt of electricity through two sheep cells in a dish. One was an egg cell which had had its nucleus, the bit of the cell which contains almost all its genes, removed. The other, its gene-bearing nucleus intact, was from the udder of another ewe. The electric jolt had caused the two cells to fuse, forming an embryo.

The egg donor was a Scottish Blackface sheep; so was the surrogate mother that took the embryo to term. The udder cell came from a white-faced Finn Dorset. And that, the fax told Mrs Walker, was what the newborn lamb looked like, too. The "nuclear transfer" she had overseen had worked. An adult sheep had been cloned.

Instantly understandable to an excited Mrs Walker—"I knew we had done what we had thought we had done"—the fax had been kept terse and cryptic because the breakthrough was, at the time, hush-hush. The existence of Dolly the sheep would not be revealed to the world at large until the following February, when a scientific paper was published in *Nature*—at which point a furore broke out that went far beyond the scientific world.

The fuss among scientists was due to the fact that many believed cloning animals was impossible. John Gurdon of Oxford University had cloned frogs by nuclear transfer in 1958—but his creations never developed beyond the tadpole stage. All efforts to do the same in mammals had failed. These failures had led biologists to believe that, although all cells in a body shared the same genetic material, they were not equally capable of the same reproductive feats. "Stem cells", such as those found in early embryos, could develop into the various sorts of specialist cells found in skin, muscle or nerves. But those "differentiated" cells could not change back into stem cells. Development was a one-way street.

The research at the Roslin Institute showed that this need not be the case. The key advance was made by Keith Campbell, who realised the importance of synchronised "cell cycles"—the rhythms according to which cells grow and divide. By starving the donor cells in a way that forced them to stop dividing, Campbell matched them to the eggs' cycle.

By showing that the DNA in a differentiated cell could be repurposed through nuclear transfer, Dolly opened up two new possibilities. One, which came to be known as "reproductive cloning", was the copying of individual animals. The other was the creation of embryonic stem cells (ES cells) capable of being turned into all sorts of other cells. Various ailments are caused by a lack of specific types of differentiated cell: insulin-secreting beta cells in the case of diabetes, for example, or myelin-forming cells in multiple sclerosis. Making embryos through nuclear transfer seemed likely to provide copious ES cells with which to research and treat such conditions—something which came to be known as "therapeutic cloning".

The udder mother

The Roslin Institute's main concern was reproductive cloning. Its researchers were interested in improving the "transgenic" animal business, in which genes are added to an animal so that it secretes some protein of particular value. The ability to produce multiple copies of the most productive such animals would be a great boon.

The Roslin scientists knew that nuclear transfer would have other uses. Mrs Walker recalls that when the sheep was still a secret, the team would talk among themselves about the therapies she might lead to. What they did not appreciate was that, once Dolly was unveiled, the public would pretty much want to talk about one thing only: making copies of people.

Dolly was supposed to be announced at a press conference timed to the *Nature* paper. But the news broke a few days early when the *Observer*, a British newspaper, scooped it. The story's second paragraph predicted that: "It is the prospect of cloning people, creating armies of dictators, that will attract most attention." It duly did. "Dreaded Possibilities Are Raised" one headline declared; "Cloned Sheep in Nazi Storm" shouted another. *Der Spiegel* put a regiment of Hitlers and Einsteins

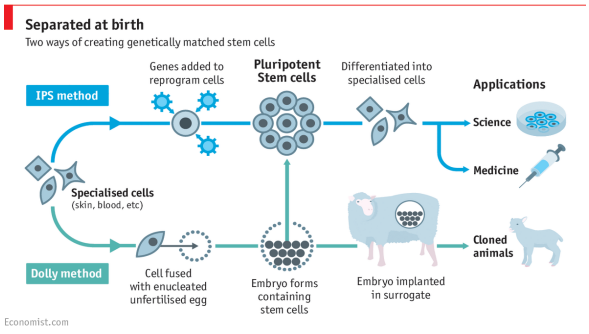
on its cover. The media and public became obsessed with the idea that human clones were just around the corner.

Hank Greely, a law professor at Stanford University who specialises in issues surrounding reproductive technology, points out that the alarm at such a prospect was hardly surprising. People are often disconcerted and disgusted by changes in human reproduction. In vitro fertilisation (IVF) and surrogacy were worried about, debated and staunchly opposed in some quarters. “People were used to babies coming out the old fashioned way,” says Dr Greely. The way that cloning could conceivably render men unnecessary added to the concerns. Much was made of the fact that Dolly was cloned from an udder and named after a singer noted for her ample bosom as well as her talent.

Baaad news

And cloning tapped into deeper concerns. From the Frankenstein-y frisson of Mrs Walker’s vital spark of electricity to the fact that the most famous fatherless human in history is known to believers as the “lamb of God”, it would have been hard to craft a scientific advance with a richer and more treacherous cultural context. Blasphemy, “Brave New World” and “The Boys from Brazil”, a story about efforts to clone Adolf Hitler, all added to the brew—and the backlash. There were nightmares of reproductive cloning and therapeutic cloning becoming the same thing, with sentient clones harvested for spare parts, as in Michael Marshall Smith’s novel “Spares”—published shortly before Dolly’s unveiling—or, later, Kazuo Ishiguro’s “Never Let Me Go”. It did not help that a previous unnatural intervention into British agriculture—the addition of cows’ brains to cattle food—had earlier in the 1990s led to the scandal of “mad cow” disease and the culling of 4.4m animals.

Zanussi, a washing-machine-maker known in Britain for its slogan “the appliance of science”, captured the mood with an advertisement that branded Dolly the “the misappliance of science.” President Bill Clinton instructed America’s National Bioethics Advisory Commission to report on human cloning within 90 days; similar instructions were issued by the French president, the president of the European Commission and the director of UNESCO. The Biotechnology Industry Organisation, a pro-technology lobby group in America, called for an outright ban. The Vatican also wanted a ban, saying that humans had a right to be born in a “human way and not in a laboratory.”




Many argued that human reproductive cloning was contrary to nature and undermined human dignity. For those who did not feel this, the obstacles, both practical and ethical, seemed enormous. In the case of Dolly, 277 successful nuclear transfers had produced just 29 normal-looking embryos, which were implanted into 13 surrogate mothers. Only one survived. It was hard to see an ethical defence of applying such a wasteful process to potential people, even if the end was, in itself, not offensive. A further concern was the health of the offspring. Dolly developed osteoarthritis and a lung infection at an early age, prompting an unresolved debate about whether she died prematurely; experience

with clones in other species has shown a tendency to various other anomalies. That said, four clones of Dolly herself are currently enjoying a healthy old age at the University of Nottingham.

The fact that most researchers considered human reproductive cloning a quagmire did not stop some attention-seekers from stepping forward to claim they were going to clone humans—or, later, that they had. First came Richard Seed, a Chicago physicist. Then there was a Swiss sect called the Raëlians, who claimed success in 2002. An Italian gynaecologist, Severino Antinori, also said he had succeeded in 2009. Experts remain highly sceptical about these claims, which have not been backed up by scientific evidence.

The bleat goes on

Yet moves in the late 1990s towards an outright ban on human cloning hit a snag: the apparently impressive potential of therapeutic cloning. This could not be realised if scientists were not allowed to develop nuclear-transfer techniques for humans. No embryos, no ES cells. Some opposed therapeutic-cloning research as another form of embryo research, a practice to which many were already opposed; in 2001 the American government banned the use of federal funds to produce new embryonic cell lines through nuclear transfer. But some countries, including Britain, already had a more liberal attitude to the use in scientific research of "spare" embryos originally created for the purpose of IVF, and sought a regulatory distinction between admissible applications of nuclear transfer for therapeutic research and prohibited reproductive applications.

But regulatory approval or no, producing human ES cells through nuclear transfer turned out to be a tall order. In 2004 Hwang Woo-souk, a South Korean researcher, announced that he had successfully created a new line of ES cells from a cloned human embryo. The following year he said he had created 11 more such cell lines. His results, published in eminent journals, were far more credible than those of the Raëlians or Dr Antinori. But by 2006 an investigation had concluded that almost all his research was fraudulent—though he had cloned a dog.

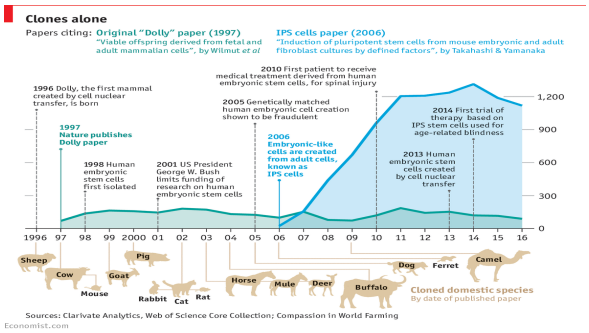
By the time Dolly would have been celebrating her tenth birthday, in 2006, nuclear transfer had still not produced human ES-cell lines. Different species and groups of animals take to nuclear transfer in different ways. Cats and mice, it now turns out, are quite easy: dogs and rats hard. In primates, according to Ian Wilmut, who led the Roslin team, the technique proved persistently disappointing, with "very limited development and no offspring". But an alternative technique that Dolly inspired had produced something almost as good—and much less morally problematic.

Shinya Yamanaka, a Japanese scientist, says that when he first read of Dolly as a post-doctoral researcher he had become "almost depressed" over wondering what to do. Dolly excited him and gave him a goal. Her creation showed that chemical factors in the egg had been able to force adult DNA to rejuvenate itself. Dr Yamanaka set about looking for them. He started by putting into mouse cells the genes for 24 factors known to have a role in keeping stem cells from differentiating. The results looked quite similar to ES cells. Assuming not all the factors were essential he repeated the work with fewer of them. By 2006 he had narrowed the field to four factors which, administered together, could convert differentiated tissues back into stem cells. It was a way of turning back the biological clock without the fiddly business of nuclear transfer.

Pluripotent possibilities

Dr Yamanaka called his cells “induced pluripotent stem cells”. These IPS cells garnered a huge amount of attention, funding and effort (see timeline). Not only could they be made without the ethically troubling intermediary of an embryo. They could also be made from cells donated by a potential patient. This meant that if they were then used for therapy, the patient’s immune system would raise no objections—something which was not necessarily the case for ES cells. Many labs trying to make human ES cells from cloned embryos stopped when IPS cells came out, says Robin Lovell-Badge, a stem-cell expert at the new Francis Crick Institute in London.

In 2012 Dr Yamanaka received a Nobel prize for this work. The IPS cells he invented have become a scientific workhorse, providing limitless supplies of differentiated cells and tissue for use in the lab. They are an invaluable tool for modelling human diseases and screening drugs. New techniques such as genome editing are extending their uses. But they have yet to prove their therapeutic mettle.




Dr Yamanaka now runs an institute in Kyoto where hundreds of researchers are pushing forward on IPS cells. There have been advances. Scientists at the New York Stem Cell Foundation have turned skin samples from patients with progressive multiple sclerosis into IPS cells and then into myelin-forming cells. Yet turning such achievements into treatments has proved challenging. The only clinical trial of IPS cells to date, conducted by the Riken Centre for Developmental Biology in Kobe, was stopped abruptly in 2015. The idea was to take stem cells made from skin cells and turn them into retinal cells which could be used to reverse macular degeneration, which leads to blindness. After just one patient had been treated, the trial was halted because mutations were found in the cells. It may well be possible to overcome such problems, but any adult cell that is turned back into a younger state through genetic engineering is likely to have its genome scarred in some way.

And IPS cells are no longer the only game in town. In 2013 Shoukhrat Mitalipov, a reproductive biologist at Oregon Health and Science University, finally cracked the tricky problem of how to create human ES cell lines. The timely addition of a little caffeine to stop the egg developing too fast turns out to be crucial.

Dr Mitalipov has compared his nuclear-transfer ES cells to IPS cells and ES cells taken from embryos created by IVF; the sort of cells which provide the gold standard in such matters, according to Dr Lovell-Badge. The nuclear-transfer ES cells look more like the gold standard than the IPS cells, perhaps because the IPS cells retain “epigenetic” memories of their differentiated past —chemical modifications to their DNA that influence their genes’ expression.

So, 16 years after the world was wowed by Dolly, a technique for cloning embryos had finally been demonstrated in the laboratory. But nuclear transfer remains difficult and the creation of cloned embryos for research or therapy remains ethically fraught. It is banned in some countries, including

France, Germany and Russia; in other places, such as America, there is no overarching regulation, which brings its own problems. And even in places like Britain and Japan, where it is allowed, getting permission takes time and effort.

What is more, cell lines made this way might not match a patient's immune system in the way an IPS-cell therapy produced from the patient's own cells can. Researchers at ViaCyte in San Diego, California, have used IVF-derived ES cells to create insulin-producing beta cells with which to treat type 1 diabetes. They anticipate that the cells will, when placed in patients' bodies, need to be encapsulated in a plastic mesh to protect them from the immune system. That may work for some conditions; it won't work for all of them.

That is why many feel that, whatever flaws IPS cells have, they are the most promising option for future therapies. More than half a century after creating the first cloned tadpoles, Dr Gurdon is now one of those searching for factors beyond those identified by Dr Yamanaka that will take the technology further, bringing IPS cells closer to the gold standard.

Copy cats and dogs

After 20 years of work on such possibilities (more, in Dr Gurdon's case) some see the Petri dish as half-full, some as half-empty. A couple of decades seem to some a reasonable timeline for such technically demanding and fiddly work; run-of-the-mill drugs can often take a decade to develop, and this sort of thing is far less well understood and more demanding. What's more, regulations have slowed things down; Dr Mitalipov says much of the time between his successful cloning of monkey cell lines in 2007 and his production of cloned human ES cells in 2013 was "navigating US regulations on embryo research". The fact that progress has been slower than once hoped has costs. One of the members of the team that created Dolly, Marjorie Ritchie, died in 2015 after suffering with multiple sclerosis—a disease that many hoped would benefit from advances in stem-cell medicine. But that is not to say there is no progress.

Others, more sceptical, see the 20 years as evidence that even if such therapies can eventually be produced they will always be complicated affairs, and therapies "matched" to the immune system will of their very nature have to be handcrafted. Even if they can be made to work they will be very costly. A guide to quite how expensive these might be came last year when GSK, a drug giant, unveiled the pricing for a personalised, stem-cell therapy for severe combined immunodeficiency. The therapy extracts adult stem cells from bone marrow, introduces a missing gene and then uses the corrected cells to cure the patient. It costs $665,000.

Beyond the clinic, and beyond the human, cloning has made slow but steady progress; it has now been successfully used on more than 20 species. The original idea of applying it to transgenic animals has not amounted to much, but the technique has proven useful in cattle and dairy farming, allowing multiple copies of elite animals. In New Zealand and America it is regarded as a normal animal-breeding procedure and clones are part of the pedigree market. Meat and milk from cloned animals is routinely farmed and sold in America, Argentina and Brazil. In Europe, though, it is banned on grounds of animal wellbeing. A study by the European Food Safety Authority in

2008 said that developmental abnormalities in clones and unusually large offspring resulted in difficult births and excessive neonatal deaths.

As well as cloning thousands of farm animals ViaGen, a small firm based in Cedar Park, Texas, has cloned many horses and pets; there are people happy to spend lavishly in the hope that they can get a genetic copy of a lost companion. According to the firm's website, a cloned horse will set you back $85,000. The disgraced Dr Hwang has also started a firm that seems to have cloned more than 400 dogs for customers willing to pay about $100,000 a pup. In Tianjin, China, an outfit called Boyalife has been building an enormous new facility, capable of producing 1m calves a year as well as dogs and horses. But its clone factory seems to be well behind schedule.

One lucrative niche unanticipated by science-fiction writers is polo. Crestview Genetics of Buenos Aires, owned by Adolfo Cambiaso, the world's best polo player, and two partners, has cloned more than 45 steeds including over 25 copies of Mr Cambiaso's polo ponies—one sold at auction for $800,000. One of the ponies he cloned was a much-loved chestnut stallion called Aiken Cura which he had to have put down more than a decade ago, after it broke its leg in a match. Last December his team, La Dolfina, rode six clones of the same mare to victory in a prestigious match in Buenos Aires.

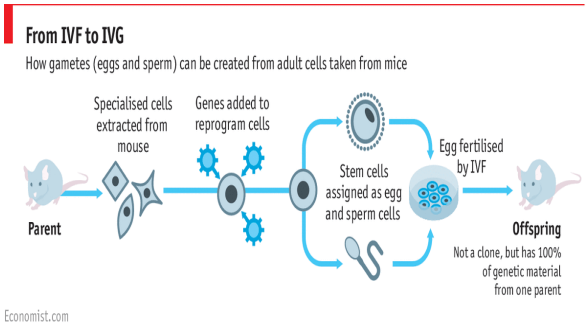

One of Crestview's founders, Alan Meeker, says that "rich individuals" have from time to time asked about cloning humans. He refused. Yet there can be little doubt that there is at least some demand for human cloning—and it doesn't come from Nazis. After Dolly's existence was announced the Roslin Institute received agonising requests from parents whose children had died; researchers at fertility clinics also suddenly found themselves asked about the possibility. It is likely that they still are.

The thrust in reproductive technology remains a desire to allow people who could not otherwise be able to do so to have any child at all, rather than to make specific people. That does not mean the field does not still throw up ethical and legal issues. Its most recent *cause célèbre* is the development of "three-parent babies", in which faulty mitochondria—power stations that drive a cell's metabolism—in an egg are replaced by healthy mitochondria from a donor before IVF. And it does not mean, in time, that the issue of reproductive cloning, or something similar, might not re-emerge.

Parents: three, two or one?

One odd possibility comes from work on IPS cells that might provide a new alternative for the infertile. In mice it is now possible to turn IPS cells derived from skin cells into sperm and eggs. If this technique—known as in vitro gametogenesis or IVG—can be perfected and adapted to humans (still, at this stage, an imposing if) it could allow people afflicted by various disorders that stop their bodies from producing eggs and sperm to have children. It would also allow same-sex couples to have biological children of their own, with sperm derived from one woman fertilising another's egg,

or an egg derived from one man's cells being fertilised by his partner's sperm (though that would also require a surrogate mother).




And it would also, in principle, allow one parent to provide both the sperm and the egg. Because people have two copies of every gene, but eggs and sperm get only one, the resulting child would not be genetically quite identical to its parent—but it would be far closer than any natural relative. Such creations would have to be screened carefully for genetic disorders and perhaps even gene edited. Reproducing this way would be, in effect, the closest sort of inbreeding imaginable. And it is not clear what might lead someone to want such a child.

But if IVG becomes a part of the toolkit for reproductive biology such possibilities will open up. And Dr Greely thinks that IVG could eventually become a big thing. As the possibilities of genetic screening—and in time, perhaps, genome editing—become clearer, people may see having embryos made carefully outside the body as a much safer bet than letting them haphazardly assemble themselves within it. And if that is the case, a plentiful supply of eggs derived from skin cells would suit many women much better than the difficult procedures needed to dig eggs out of ovaries. Some specific applications of IVG—including, most definitely, any attempts to produce "one parent" children—would undoubtedly trigger the "yuck factor" that has always greeted developments in reproductive technology. But, if the technology can be made safe, it may well become accepted. As it did with IVF, the sight of grateful parents with beloved children will prove a powerful argument.

This may not be the way things work out. It may be that IVG proves impossibly hard to apply to primates. There may turn out to be no demand for what it offers, or at least not enough to encourage clinics or companies to involve themselves in developing it; the commercial obstacles seem high. And there may be a public outcry. But the prospect of children created in this way is probably a lot closer today than human clones were 20 years ago. And so far the world has made barely a bleat of protest.

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