THE POWER OF THREE

Techniques that transfer DNA from diseased human eggs to healthy ones — creating offspring with three biological parents — are on the verge of clinical use.

BY EWEN CALLAWAY

ILLUSTRATION BY VASAVA

Douglass Turnbull spends much of his time seeing patients who have untreatable, often fatal, diseases. But the neurologist has rarely felt more helpless than when he met Sharon Bernardi and her young son Edward.

Bernardi had lost three children within hours of birth, owing to a mysterious build-up of acid in their blood. So it was a huge relief when Edward seemed to develop normally. "He did all his milestones: he sat up, he crawled and started to walk at 14 months," Bernardi recalls. But when he was about two years old, he began to fall over after taking a few steps; he eventually started having seizures. In 1994, when Edward was four, he was diagnosed

Leigh's disease, a condition that affects the central nervous system. Doctors told Sharon that her son would be lucky to reach his fifth birthday.

Turnbull, who works at Newcastle University, UK, remembers despairing that "whatever we do, we're never going to be able to help families like that". His frustration sparked a quest to develop assisted-reproduction techniques to prevent disorders such as Leigh's disease, which are caused when children inherit devastating mutations in their mitochondria, the cell's energy-making structures.

The procedures — sometimes called three-person *in vitro* fertilization (IVF) — involve transferring nuclear genetic material from the egg of a woman with mutant mitochondria into another woman's healthy egg. Turnbull and others have tested the techniques in mice, monkeys and human egg cells in culture; now, they say, it is time to try them in people. The UK Parliament is set to vote on the issue later this year; if legislation passes, the country would be the first to allow this kind of genetic modification of unborn children.

But some scientists have raised concerns over the safety of the procedures, and an increasingly vocal coalition of activists, ethicists and politicians argues that a 'yes' vote will lead down a slippery slope to designer babies. US regulators and scientists are closely watching the debate as they consider allowing similar procedures. "I admire what they've done in Britain," says Dieter Egli, a stem-cell scientist at the New York Stem Cell Foundation, a non-profit research institute. "I think they are far ahead in discussion of this, compared to the US."

A FATAL INHERITANCE

The mitochondrion, according to one popular theory, was once a free-living bacterium that became trapped in a host cell, where it boosted the cell's capacity to generate the energy-carrying molecule ATP. As a result, each mitochondrion has its own genome — but it no longer has all the genes it needs to function independently (the human mitochondrial genome, for example, has a paltry 37 genes).

Unlike the genome in the cell nucleus, which includes chromosomes from both parents, all of a person's mitochondria derive from the thousands contained in the mother's egg. For reasons still being studied, the mitochondrial genome is much less stable than the nuclear genome, accruing random DNA mutations about 1,000 times faster. As many as 1 in 5,000 children are born with diseases caused by these mutations, which affect power-hungry cells such as those in the brain and muscles. The severity of the conditions depends on the proportion of diseased mitochondria a mother passes on to her children.

Turnbull first got interested in mitochondrial disease and energy metabolism in the late 1970s, when he was working as a junior doctor on a neurology ward. A member of the Royal Air Force arrived at his clinic with a mysterious ailment: whenever he went on training runs, his muscles would suddenly give out and force him to stop. Turnbull at first suspected that the airman had a mitochondrial disease — and although he turned out to be wrong, his curiosity was piqued.

Turnbull found that the treatment options for mitochondrial diseases were limited to managing symptoms, for example by prescribing anticonvulsant drugs to ward off seizures, rather than addressing the underlying biological problem. "You see them develop a mitochondrial disease and there's bugger all you can do about it," he says. The young neurologist went on to do a PhD on the inner workings of mitochondria, and has devoted his career to understanding how they malfunction.

After Turnbull met the Bernardis in the mid-1990s, a muscle biopsy confirmed that Sharon carried mutant mitochondria. "He couldn't believe I looked so well," she says. The diagnosis helped Sharon to understand some of her health problems — and her family's. Her mother, it turned out, had lost several children, and was experiencing heart difficulties in her fifties; a cousin and other family members had also lost children. "It's been a family wiped out," says Bernardi, who lost three more babies after Edward was born. Her tragedy spurred Turnbull to seek ways to keep children from inheriting their mothers' mutant mitochondria.

Others had been thinking along similar lines. In the 1980s, embryologists working with mice had begun using 'pronuclear transfer' techniques to investigate the developmental role of egg cells' cytoplasm. The procedures involve moving nuclear DNA from one fertilized egg to another, leaving in place most of the other contents, including the mitochondria. In 1995, researchers raised the idea that similar procedures could interrupt the transmission of mitochondrial diseases in human eggs[1].

Turnbull's laboratory began replicating the mouse research in the early 2000s, aiming to move quickly to human eggs. Working with Mary Herbert and Alison Murdoch, reproductive biologists at Newcastle University and an affiliated fertility clinic that provides IVF, they planned to start with eggs that had not been fertilized correctly and had no hope of generating a fetus.

It took 18 months to convince regulators to allow the first experiments.

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The UK Human Fertilisation and Embryology Authority (HFEA) twice denied the team's application, on the grounds that the procedures would alter the "genetic structure" of the egg — illegal under the 1990 Human Fertilisation and Embryology Act, which had established the agency. In response, the researchers argued that the term was vague and did not apply to pronuclear transfers. They filed a third appeal, this time with lawyers to argue their case, and won approval in 2005.

Around the same time, the UK Parliament began updating the 1990 law. Revised legislation came into force in 2009 and prohibited clinical application of pronuclear transfers — but it allowed for the topic to be revisited by Parliament without passing entirely new laws, pending a full airing of the scientific, regulatory and ethical issues. The law change gave the Newcastle team hope that its experiments, if successful, could one day be translated to the clinic.

A STEADY HAND

Human egg cells are one-tenth of a millimetre wide, and pronuclear transfers must be done under a microscope, in a specially designed chamber that controls temperature and air flow. It takes an expert embryologist with a steady hand: "People don't breathe when they're doing this," says Herbert.

First, a fertilized egg cell is zapped with a laser, making a hole in its membrane. Then the embryologist eases a pipette into the hole and plucks out the pronuclei, twin genetic structures that result from fertilization. Next, the researcher empties a fertilized donor egg of its genetic material and squirts the pronuclei into the hollow egg. The feat takes several minutes (see video at go.nature.com/ufatcq). If the United Kingdom approves clinical use of the procedure, the egg would then be incubated for a few days until it develops into a blastocyst of between 50 and 200 cells, which would then be transplanted into a woman's uterus.

In a paper published in May 2010, the Newcastle researchers showed[2] that the abnormally fertilized eggs they had been using could undergo pronuclear transfer and then develop in culture almost as well as untouched egg cells. Crucially, the transferred pronuclei brought few mitochondria with them, suggesting that a resulting embryo would largely be free of any disease-causing mutant mitochondria.

But many questions remained. Could the transfers be done efficiently enough that a woman could hope to become pregnant? Did they cause subtle molecular or genetic changes that might hinder further development or cause health problems after birth? And would the UK government ever allow them to reach the clinic?

The Newcastle team has spent the past few years looking for answers, optimizing its technique in healthy human eggs. "We're reasonably comfortable there's a chance of pregnancy with this," says Herbert. The

AN UNPLANNED EXPERIMENT

Mitochondrial-transfer pioneers

It began as a way to help a handful of patients to have babies. But fertility specialist Jacques Cohen, then at Saint Barnabas Medical Center in Livingston, New Jersey, inadvertently launched an experiment that could reveal whether mitochondrial-replacement therapies are safe to try in humans.

In the mid-1990s, Cohen was struggling to help a small number of women who were unable to conceive, although they could make enough eggs for *in vitro* fertilization. The women were not old, but their eggs were a mess — the cytoplasm around the nucleus was fragmented and littered with debris.

Cohen wondered what would happen if he added a little cytoplasm from another woman's healthy egg. He tried it in mice and it worked; so in 1997, he and his team began testing the cytoplasm-transfer technique in humans. They painstakingly 'normalized' the eggs of 33 infertile women with less than a picolitre (10^{-12} litres) of another woman's egg cytoplasm. Seventeen babies were born as a result of the procedure[7].

Cohen knew that the transplanted cytoplasm probably contained the cellular battery packs known as mitochondria, which he had hoped would enhance embryo development. Tests reported[8] in 2001 confirmed that at least two babies had mitochondria (which each carry 37 genes) from both their mother and the cytoplasm donor. The team was the first in the world to alter a human's genetic inheritance in this way.

The health implications for the children are unclear: studies suggest that mice with such mixed mitochondria develop hypertension and obesity in middle age[9], and have impaired cognition — they escape from mazes more slowly than normal mice, for example[10]. One of the babies born as a result of the procedure was diagnosed with an autism spectrum disorder, and two further fetuses had a genetic defect known as Turner syndrome (one was miscarried, the other aborted).

The team stopped performing the procedure in 2001, when the US Food and Drug Administration said that more research was required before it could be used in humans. But nobody followed up on the 17 children, who are now teenagers.

Cohen, now lab director of Reprogenetics, a pre-implantation genetic-diagnostics company in Livingston, wants to change that. He has teamed up with researchers at Saint Barnabas for a two-phase follow-up study, including phone surveys with the families and saliva tests of the teenagers, if they are willing. The saliva will show whether the teenagers' mitochondrial genes come from both their mothers and the cytoplasm donors.

Serena Chen, a reproductive endocrinologist who joined Cohen's team in 1999 and is the project's principal investigator at Saint Barnabas, says that the timing is right for the study. Its results could make a crucial contribution to US and UK debates over related techniques aimed at helping women with mitochondrial disease to give birth to healthy babies.

"We feel like this is something that would be helpful for the other researchers in this area looking at mitochondrial disease, to provide some reassuring data that human research into this area is not unreasonable to consider," she says. **Karen Weintraub**

still-unpublished experiments have proceeded slowly, partly because healthy human eggs for experimentation are hard to come by. But Herbert says that the group has already performed more than 100 pronuclear transfers on such eggs. It also hopes to conduct safety studies to assess whether the procedures alter the transferred genome or epigenome. But such checks cannot provide complete reassurance before the leap into humans, the researchers acknowledge. "We can never say for sure that it's 100% safe," says Herbert. "It has to, at some point, go to treatment."

BATTLE LINES

In 2010, the Newcastle researchers asked the UK government to consider changing the law that prohibits them from conducting their mitochondrial-replacement procedure in humans. The request prompted a flurry of hearings, consultations and reports, involving independent scientists, bioethicists, regulators, the general public and others; another scientific review is expected in the next few weeks. But the protracted process has thrown up no major roadblocks.

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Nancy Lee, a senior policy adviser at the Wellcome Trust, the United Kingdom's largest biomedical-research charity, praises the review as "a good example of evidence-based policy-making and informing the public as much as is possible". The London-based charity has funded Turnbull's team to the tune of £4.4 million (US$7.4 million), and has thrown its considerable political clout behind changing the law.

Yet some scientists argue that the procedures have not been vetted rigorously enough. Klaus Reinhardt, an evolutionary biologist at the University of Tübingen in Germany, worries about incompatibilities between the nuclear and mitochondrial genomes in individuals conceived using the procedures. Both nuclear and mitochondrial genes are needed for mitochondria to function, and it is likely that gene variants in both structures have evolved together, he says. Mitochondrial replacement in mice, fruit flies and other organisms has occasionally resulted in problems with respiration, cognition and fertility, several studies have found[3]. Reinhardt, who has expressed his concerns to the panel in charge of reviewing the science, questions whether there are enough safety data to go forward with clinical trials. "I don't really know how robust everything is," he says. In response, Turnbull's team casts doubt on the relevance of mitochondrial-replacement experiments that use inbred lab animals, and points out that other studies of mitochondrial replacement in mice failed to find health problems[4].

Some critics use more emotive language. In a March Parliamentary debate and a column in *The Daily Telegraph*, Conservative Member of Parliament Jacob Rees-Mogg equated mitochondrial replacement with cloning, and said that the techniques would promote eugenics. "In a country nervous about genetically modified crops, we are making the foolhardy move to genetically modified babies," he said in the debate.

An international coalition of several dozen scholars and bioethicists, many at religious institutions, expressed similar sentiments in March 2013 in a letter to *The Times* newspaper, arguing that mitochondrial replacement "would open the door to further genetic alterations of human beings with unforeseeable consequences".

To counter this opposition, Turnbull and other supporters point out that the techniques will be used only to prevent serious mitochondrial diseases. The researchers have made patients' stories, such as the plight of the Bernardi family, central to

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their appeals. They have compared mitochondrial replacement to changing the batteries in a camera (a poor analogy, some other scientists say), and they argue that mitochondrial DNA makes up a tiny fraction of the overall genome, with little influence over a person's defining traits. "This is not a slippery slope, in my view," Turnbull says. "This isn't 'designer babies'. This is about preventing serious, life-threatening, disabling diseases."

MONKEY TRIAL

A similar debate is shaping up across the Atlantic. While Turnbull and his team were developing their pronuclear-transfer technique in human egg cells, a US team was testing a related method in monkeys. In 2009, reproductive biologist Shoukhrat Mitalipov at the Oregon Health and Science University in Beaverton and his colleagues reported the birth of two healthy rhesus macaques whose mitochondria and nuclei had come from different egg cells[5]. The monkey twins — named Mito and Tracker, after a reagent used to make mitochondria glow — were conceived through a method called maternal spindle transfer (see 'Genome transplant'). This involves shuttling an egg's nuclear genetic material to an empty donor egg before fertilization, rather than after as in pronuclear transfer. There have not yet been any side-by-side experiments to compare the merits of the two techniques, although both teams are keen to try.

Mitalipov's team has used maternal spindle transfer to conceive five monkeys, including one from a previously frozen egg (to mimic a likely clinical situation). Mito, Tracker and two others born in 2009 have celebrated their fifth birthdays, and are still healthy. Mitalipov plans to breed them soon to determine their fertility. His team has also proved its technique in human eggs: the embryos formed blastocysts, albeit at a low rate, and produced embryonic stem cells with the potential to give rise to all the body's different tissues[6]. In unpublished work, the researchers have since drastically improved the efficiency of the procedure, he says. "Now we want to transplant these embryos."

First, he will need approval from the US Food and Drug Administration (FDA). The agency has required researchers to seek permission for mitochondrial transfers since 2001, after a New Jersey fertility clinic carried out dozens of procedures that involved moving small amounts of cytoplasm — including some mitochondria — between human eggs to improve conception rates (see 'An unplanned experiment').

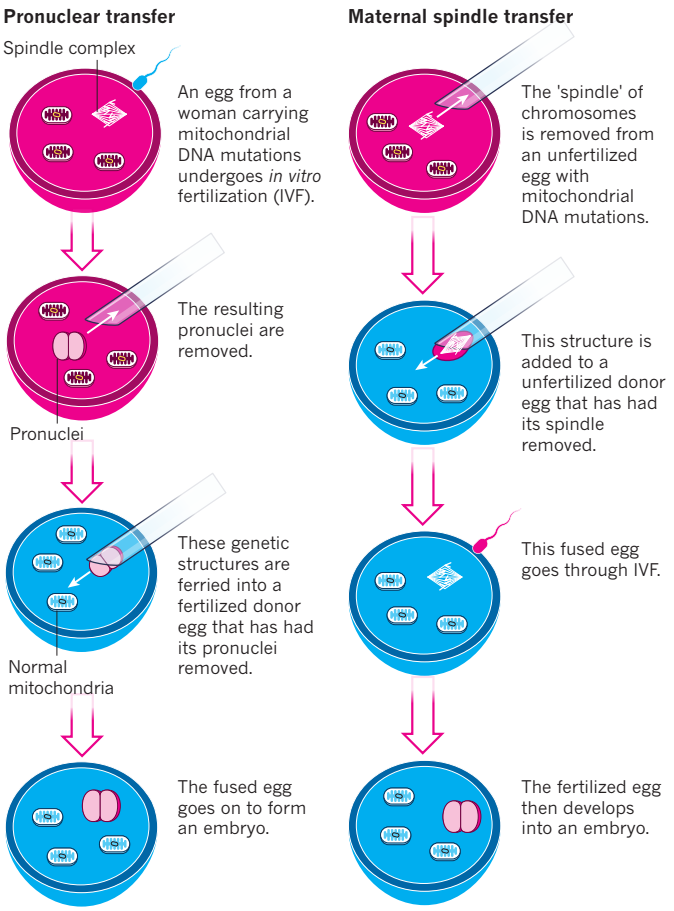
Mitalipov last year put in a proposal to carry out clinical trials in humans, and an FDA advisory panel met in February to discuss the issue. The committee spent two days chewing over the same questions that the United Kingdom has been grappling with, such as how to establish the safety and effectiveness of the procedures in cells and animal models, and what the first patient trials might look like. Committee chair Evan Snyder, a stem-cell biologist at Sanford-Burnham Medical Research Institute in La Jolla, California, says that most of his colleagues are disposed to take cellular therapies to patients, and that they recognize the potential to unshackle families from the consequences of mitochondrial mutations. But, he says, "I think what everybody was a little bit uncomfortable with was just how much is not known". Some panel members wanted to see multiple generations of monkeys born healthy using the procedures, as well as more safety work on human eggs.

Mitalipov found the meeting frustrating: "I don't want to go back and do another decade or two decades of research, which we can do. But meanwhile, there will be thousands and thousands of children born every year that will suffer." He says he would consider moving his lab to Britain to help bring his research to patients more quickly. Snyder, however, senses that his committee is not far from green-lighting clinical trials, and that safety hurdles could be surmounted in two or three years.

Back in the United Kingdom, the legislation to allow mitochondrial replacement is still being hammered out — a consultation of the draft law finishes on 21 May — but proponents are quietly confident that Parliament will say yes. The move has support across the political spectrum, and most of the scientific and ethical advice given to the government has been encouraging. However, a law change would merely give the HFEA the power to allow the procedures, and the agency would probably want more safety and effectiveness data before it approved any trials.

GENOME TRANSPLANT

Two different techniques could be used to prevent children from inheriting their mothers' mutant mitochondria.



Bernardi hopes that clinical trials will eventually go ahead. But "I think it would be bittersweet if somebody had a baby" conceived with the procedures, she adds. Her son Edward lived well beyond the expectations, although he was eventually confined to a wheelchair and his health worsened in waves as doctors struggled to find medications to quell symptoms including spasms that rendered his arms stiff and immobile. Bernardi strove to give him a normal life: he attended school, went on class trips and developed crushes. "He liked his girls, he did," she says. Bernardi resisted feeding him through a tube until he was unable to eat normally, at the age of 20. "Up until the last ten weeks, I would say he had a very good quality of life," she says.

Edward Bernardi died in March 2011 after a 21-year struggle with Leigh's disease. "I don't think this would benefit me," says Bernardi of the procedures that may be on the cusp of helping other women with mitochondrial disease. "But this keeps Edward's legacy." ■

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