WIRED MAGAZINE

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Seven Days of Creation

The inside story of a human cloning experiment.

By Wendy Goldman Rohm

DAY ONE 5:10 pm
It's late on a Sunday afternoon and nearly dark inside the tiny, windowless lab; fluorescent light is said to be bad for human embryos. I'm sitting beside Robert Lanza, medical director at Advanced Cell Technology. He's breathing softly, hands folded neatly in his lap, his head bowed as if in meditation. For years he's been preparing for this day - making plans, conducting preliminary tests, losing sleep. Now, on October 12, we're six hours into the experiment and all he can do is watch.

By the glow of a microscope's light, research scientist Young Chung gingerly grasps a recently harvested human egg. He does this with a micromanipulator, a microscope outfitted with several diminutive, strawlike instruments called pipettes. Using a holding pipette, he keeps the fragile egg in place while he maneuvers a second pipette into position.

Matt Gunther
Robert Lanza (left) and Young Chung

The eggs were donated by two women who were paid $4,000 each by ACT. A doctor at a clinic just outside of Boston this morning harvested the eggs - 18 in all thanks to fertility drugs - and ACT rushed them to its labs in Worcester.

Chung steps on a foot pedal to activate an ultraviolet light underneath the micromanipulator, briefly illuminating the DNA inside the egg. It's the only way he can see the genetic material without hurting the egg. If the UV is on for more than a few seconds, the egg could be damaged. Chung steps on a second pedal to control the second pipette, called a piezo, which acts like a tiny drill. The micromanipulator click-click-clicks like a minuscule jackhammer as Chung begins enucleation, the delicate task of puncturing the egg's protective membrane and sucking out its nucleus. He must be certain to retrieve all of the chromosomes inside the egg - 2 meters' worth, if they were unraveled and stretched end to end - without collapsing the membrane.

For several minutes, the only sound is the click of the machine and the tap of Chung's feet on the pedals. Finally, he looks up. "One down," he murmurs.

In recent months, Chung and Lanza have done other experiments leading up to this one, and during their last trial, an egg collapsed and died when Lanza left the room. He will not leave this time. Instead, he worries about the lab environment. There must be no unnecessary vibrations on the table where Chung works, no exposure of the eggs to fluorescent light, and no deviation from a room temperature of 85 degrees Fahrenheit. Lanza is especially concerned with CO2 levels inside the incubator; he keeps an eye on the digital display, believing that an experiment conducted in September, in this same lab, may have failed because of improper CO2 levels. Glancing at the wall-mounted thermometer, Lanza notices that the temperature has slipped to 83 degrees. He turns on a small space heater, asking Chung: "Is that OK?"

"Fine," the Korean-born scientist answers, beads of sweat forming at his brow. Chung wears black-framed glasses and blue-gray scrubs. At 42 years old, he's not as well known as Lanza, but he is highly regarded among his peers for his dexterity when manipulating cells. Nine additional freshly harvested eggs await enucleation.

Once he's emptied them, Chung performs the most critical step of the cloning procedure. In a process known as nuclear transfer, he will inject the 10 hollow eggs with donor DNA in the form of cumulus cells, which nourish the ovaries and, like other body cells, contain a full set of genetic information. These, too, were collected from the egg donors. The injected eggs will then be chemically stimulated to begin dividing as if they'd been fertilized. Taken together, it's very close to the way UK scientist Ian Wilmut created Dolly the sheep. The big difference: Wilmut implanted the sheep embryo to grow a cloned animal; Lanza will keep the resulting embryos in a petri dish to grow stem cells.

If it works, Lanza will have accomplished two amazing things: He will have cloned human embryos, and he will have harvested stem cells from them.

Embryonic stem cells are prized for their magical potential to become any type of cell in the body. Researchers see them as healthy replacements for cells damaged by diseases - including diabetes, osteoporosis, Alzheimer's, and Parkinson's - that affect more than 130 million people in the US, according to the National Academy of Sciences. Clone embryos would produce stem cells that are exact genetic matches of the donors and consequently run little chance of rejection.

But Lanza can't get stem cells until he grows an embryo that has divided into at least 16 cells, which usually takes about five days. At that stage, embryos become morulae, from which stem cells have already been obtained in animal studies. Even better would be blastocysts, embryos that have 64 to 200 cells and are distinguished by the development of an inner cell mass from which stem cells can more readily be derived. Just one problem: There have been no published reports of a human clone embryo surviving beyond a few cell divisions, let alone to 16 or 64 cells. Which raises the question of whether Lanza's project is even possible.

If Lanza can get to morula stage, well, that's when the controversy is sure to begin. It's at this point that the embryo's genome - the donor's complete DNA - will have kicked in. No larger than the head of a pin, the morula has enormous potential. An embryo at this stage could be implanted in a uterus, a process typically done after 48 hours in the 100,000 in vitro fertilization procedures done in the US each year. Thus, a human clone - should a scientist choose that path.

But that's not what ACT wants. This experiment is central to its pursuit of therapeutic, not reproductive, cloning. Simply put, ACT wants to create stem cells, not human beings. "We have patients dying for lack of transplantable tissue on one side of the scale, and on the other someone who wants to clone a human being," explains ACT chief executive Michael West. "Do you save the lives of hundreds of thousands of people, or stop everything for fear someone would abuse this? I'd prefer to help sick people."

Back in the summer of 2001, stem cells and cloning dominated the headlines. In July, the House of Representatives passed a bill that would prohibit the cloning of human embryos for any purpose; similar legislation stalled in the Senate. The following month, President Bush announced that he would limit federal funding to the 60 or so stem cell lines already in existence. (Scientists later complained that less than 20 are viable for research purposes.) "I strongly oppose human cloning, as do most Americans," Bush said from his ranch in Crawford, Texas. "We recoil at the idea of growing human beings for spare body parts, or creating life for our convenience."

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Advanced Cell Technology's Lanza takes a break near his island home outside Boston. An avid conservationist, the 47-year-old has cloned such endangered animals as a gaur, which died two days after birth, and an Asian banteng, now at a San Diego animal park.

But there was no ban on privately funded research, so at ACT, Lanza and his colleagues pressed on. That summer, his team achieved a controversial first, the creation of several early-stage human clone embryos - one of which grew to six cells. ACT announced its breakthrough in November 2001 and immediately came under fire. "The pro-life and evangelical groups were particularly well organized with members of Congress," recalls West. "They launched an attack on these technologies as being 'brave new world.'" As criticism mounted, a crucial round of venture capital funding evaporated. Cash-starved ACT had little choice but to shut down its human research program.

Outside ACT, there was progress. The National Academy of Sciences issued a report acknowledging the medical value of therapeutic cloning. California in 2002 authorized the use of public funds for embryonic stem cell research, and several other states are considering similar bills. Last June, the American Medical Association, which sets the tone for accepted medical practices nationally, endorsed therapeutic cloning but acknowledged the right of doctors to decline any role in it.

Meanwhile, ACT centered its stem cell research on animals. The company cured spinal injuries in sheep and successfully cloned kidney cells that were a genetic match to their DNA donor, a cow. They even rejuvenated the immune system of a cow with a teaspoon of stem cells.

On the strength of these and other studies, ACT received a cash infusion and approval from its external ethics advisory board to again clone human embryos. In spring 2003, Lanza recruited two scientists: Chung, from Temple University, and Irina Klimanskaya, a Harvard researcher who played a key role in deriving 17 new stem cell lines (with private funds, of course) from frozen human embryos for the Howard Hughes Medical Institute.

By October, Lanza was ready. In an attempt to derive stem cells, he would take human cloning further than it had ever gone.

DAY ONE 5:40 pm

It takes 30 minutes for Chung to successfully enucleate six eggs, all from the first donor. He invites me to peer into the microscope. The empty eggs, gleaming in white light, resemble beautiful little sequins. Chung takes out the second batch of donor cells and turns off the lab lights. The mood is cheery. "It's not as stressful when things are going well," says Lanza. "There's no drama."

A moment later, Chung's body tenses. Lanza seems worried. Chung mutters that the eggs "look vacuolated"; their wrinkled appearance suggests an inability to withstand piercing and nucleus removal. Several long minutes pass. He clenches his jaw as he works the micromanipulator. Then, again: "One down."

"Wow," Lanza whispers with relief.

Chung removes the nuclei from the three remaining eggs without incident and by 6:10 they have 10 enucleated eggs. The scientists are jubilant. A 100 percent success rate is practically unheard of. "I'm pretty sure they'll all survive," Chung says.

"I just did some arithmetic," Lanza says, trying to nail down the best time to perform the injection of cumulus cells into the enucleated eggs. He knows the optimal window for nuclear transfer hinges on exactly when the donors received the medicine that induced ovulation. The scientists quickly agree on a cutoff of 8 pm.

Chung removes vials of cultured cumulus cells from the locked incubator. They've been sitting for several weeks in a nutrient-rich solution. It's a special sauce, one of many variables critical to this experiment.

"Cooking with Young," Lanza laughs.

"It's a cable show, How to Make Your Own Stem Cells," Chung jokes back. He places the cultured cells next to a red ice bucket, which holds another batch of vials, freshly harvested cumulus cells from the egg donors. Both groups of cells will become the genetic payload for the enucleated eggs. Neither Chung nor Lanza is sure whether cultured or fresh cells work best, so they will try some of each.

At 6:40, Chung starts on the empty eggs from the first donor. This time he uses an even smaller piezo to drill into the egg. The micromanipulator produces a clicking that's more insistent, and seems to last longer than during the enucleation process.

"OK, great," Chung finally says. He has penetrated the membrane of the first egg, and seconds later he injects the cumulus cells through the piezo, moving a copy of donor DNA into the enucleated egg. It's the essence of cloning.

Chung wipes his palms on his pants. The clicking resumes, then suddenly he eases off the pedals. Lanza grimaces. Chung swears under his breath. The second egg's protective membrane is surprisingly tough; he cannot pierce it with the piezo. He quickly switches pipettes. Every second counts; it's 7:10 and the 8 pm deadline looms. It's taken nearly 30 minutes to inject the first two enucleated eggs. The next two go more smoothly. By 7:15, four of ten eggs are done. Chung smiles at me, relieved.

Thirty minutes later, the nuclear transfer is finished. Ten eggs - six from one donor and four from the other - have been injected with DNA and now have the potential to become human clone embryos. But first the eggs need to be activated, which is basically a jump-start to prod them to begin dividing. In the early days of cloning, researchers did this with electric shocks. Chung's approach: dribbling a prepared chemical reagent into their petri dishes. It's the easiest part of the process. So far, not a single egg has been lost, and he has beaten the deadline.

Chung directs his attention to the eight remaining eggs. Instead of cloning, now he's going to try a different process to get at the all-important stem cells. It's called parthenogenesis, a form of reproduction that occurs naturally among aphids, snakes, even turkeys - but not mammals. Chung's goal is to create parthenotes, embryo-like balls of cells that, like clone embryos, can lead to stem cells. But we're not cloning here; Chung uses a different reagent to fool the embryos into dividing as if they were fertilized.

Parthenotes lack the necessary male chromosomes to form a placenta, making it unlikely they could ever become a human. Because of this, some scientists see

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parthenotes as a way to avoid the ethical issues surrounding therapeutic cloning. ACT hopes to use parthenogenesis to derive hundreds of broadly transplantable stem cell lines, enough to match the tissue types of nearly everyone in, say, the US.

On the Front Lines

At 8:30, the waiting game begins. If all goes according to plan, the eggs will grow into early-stage clone embryos and parthenotes over the next 72 hours. It is possible, however, that nothing will happen and they all will die.

Lanza announces that he's taking the incubator key to guarantee peace, quiet, and darkness for the nascent cells. The incubator is not to be opened for three days. He is convinced that prematurely opening it can kill embryos. It has happened to his experiments before.

While Lanza and Chung work in the lab, CEO West goes looking for money. The controversy surrounding cloning - and the simple fact that there's little revenue in it - has made it difficult to keep Advanced Cell Technology afloat. West attributes the financial woes to two years of plummeting biotech stocks and private investor disdain for the sector. "Little biotech companies can't do it on their own," he laments. Several times during recent months, the 16-person ACT barely made payroll.

For months, West has been speaking with potential investors, and today he's talking to someone about taking a large stake in ACT, perhaps even buying the company outright. A source close to the possible deal says West's target is Exeter Life Sciences, a biotech holding company owned by American billionaire John Sperling. Sperling also owns pet cloning firm Genetic Savings & Clone, which pays royalties to ACT for use of its cloning technology.

Money problems have plagued ACT for years. Founded in 1994 as Avian Farms, an agbio concern engaged in poultry genetics and animal cloning, the company appealed to West, who had a growing interest in stem cell therapies. In 1998, West left Geron, a biotech firm he founded, and joined ACT as CEO; two years later, he led a group of investors to buy the company. Under West, ACT expanded into human research and hired several top biomedical scientists, including Lanza. By 2001, the company was preparing for what would be its breakthrough experiment: the successful cloning of several human embryos.

That summer, West and Lanza traveled to Washington to meet with regulators at the Food and Drug Administration. Their point: that ACT's research did not violate a 1998 agency directive prohibiting cloning experiments where "human subjects are or would be exposed to unreasonable and significant risk of illness or injury." The FDA, recalls Lanza, said "this would be easier for us if you put it in writing." So he and West composed a letter stating that ACT was interested only in stem cells and had no intention of engaging in the risky business of cloning a human.

Since that time, the FDA has left ACT alone, allowing its therapeutic research to continue provided it draws the line at stem cell treatments. "If we actually wanted to do a human clinical trial where we made cells and put them into a patient who was sick, we'd go back to the FDA for approval," West says.

In fact, ACT's future depends on developing additional technologies - for creating embryos and parthenotes and for deriving stem cells - that it can patent and license. But first the medical value of stem cells must be proven and therapeutic cloning embraced by the marketplace. "We'll need tens of millions to carry the research through to clinical trials and to the successful launch of the product," admits West.

DAY FOUR 3 pm

"It would be foolish for us to expect a home run," Lanza cautions. After three days of suspense, he and Chung are about to unlock the incubator.

Of the 10 clone embryos, five have reached the four-to-eight cell stage - quite an accomplishment. (In natural reproduction, the embryo would be at 8 to 16 cells after 72 hours, but clone embryos often develop at a slower pace.) Two have divided past ACT's previous record of six cells. "That's not trivial," Lanza remarks, with classic scientific understatement.

The parthenotes look equally promising. All eight have divided, with several of them cleaving into early-stage embryos. Lanza expects that within two days he'll have two or three blastocysts. But he's wary: "With parthenotes or clones, the embryos can look really good at one point and then nose-dive." Chung shuts the incubator, and the wait continues.

DAY FIVE 10 am
I wait in a hotel next door to ACT's offices. Though today is the big day, Lanza is calm as we sit in the lobby, talking about his personal biocentric theory of the universe. We joke about his wild ideas and don't mention a word of what is growing back in the lab or his plan to open the incubator at 2 pm. He bids good-bye simply by saying, "I'll let you know what happens."

I have witnessed nearly every minute of the experiment Lanza and Chung set in motion. Only today, because of Lanza's increasing obsession with not disturbing the embryos, I have been banished, sentenced to sit in my room and stare at my cell phone. At 2:14 it rings.

"Are you sitting down?" Lanza asks.

My voice rises, almost to a shout. "What have you done?"

"We're so surprised. We did it!" Lanza laughs. "We have one clone at 16 cells, it's a beautiful compacting morula! We could get stem cells now if we want!" Though it's not yet a blastocyst, which is the ideal stage for harvesting stem cells, the clone embryo is at the critical point of development where the injected genes become functional. It's evidence that both the donor DNA and the embryo are thriving, that normal cell division is happening. "Only when you get to a morula are you sure it's occurring," says Lanza.

The results of the experiment pose a challenge to a widely embraced report published by Gerald Schatten, reproductive science professor at the University of Pittsburgh. Last April, he wrote that using current techniques, human cloning would be almost impossible due to errors in early cell division, which are caused by removing the nucleus of the egg.

Lanza's experiments seem to show otherwise. One of his embryos divided successfully to at least 16 cells. That means he has found not simply a path to stem cells, but made a significant if unintended step toward human cloning. After all, if thriving clone embryos can grow to 16 cells and beyond in a lab, those cells could theoretically be tested for genetic abnormalities (as they routinely are in IVF procedures) and then be implanted in a uterus - reproductive cloning. Lanza insists he's not going there, but others surely will.

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Meanwhile, Lanza is also excited about his parthenotes. That part of the experiment has led to two blastocysts with inner cell masses, which means they contain stem cells. Lanza is positively ecstatic. "The parthenotes are unbelievable."

DAY SIX 10:15 am
Lanza and Chung reopen the incubator and move the parthenotes to the microscope for observation. Another morula has become a blastocyst overnight; now there are three. Inside their circular membranes is what looks to be a crescent moon with a clump of cells facing inward from its middle. It is the inner cell mass.

Chung retrieves the dish cradling the most advanced clone embryo and places it under the microscope. He and Lanza observe at least 16 cells inside a circular outer membrane. The embryo is at rest in the morula stage, just as it was the day before. Lanza refuses to tell me more. Stunned by the progress of his experiment, he insists that he would be condemned by his peers if he allowed Wired to report the fate of the clone embryo. Lanza says he'll publish the outcome in a scientific journal. When I protest, he allows me to follow the less-controversial parthenotes a bit further.

The stem cell specialist Irina Klimanskaya arrives at noon to harvest the inner cell masses of the parthenote-derived blastocysts. She'll "plate out" the ICMs into petri dishes, a process that involves encouraging stem cells to grow on a layer of feeder cells.

Klimanskaya plates out the three blastocysts. She notes that two other parthenotes continue to thrive. By the time she leaves, it's nearly midnight.

DAY SEVEN 11 am
Back at the lab, Klimanskaya opens the incubator to check on the petri dishes. Lanza is forced to watch and wait as his experiment unfolds. Two more parthenotes have grown into blastocysts. The ACT team has obtained five blastocysts from eight eggs. Better still, the ICMs Klimanskaya plated out yesterday have already attached to the feeder cells. Lanza and company cross their fingers that they'll get stem cells.

Meanwhile, I've learned nothing more about the human clone embryo and, as I return to my hotel, I am left pondering a host of questions. Did the embryo progress beyond 16 cells to a blastocyst? Will it yield stem cells? Or has it already died?

For answers, I'll have to wait, like everyone else, until Lanza publishes his results.

But this much is clear: ACT is pioneering new methods to grow stem cells - and along the way, bringing us closer to a fascinating, if ethically complex, future.

Countdown to a Human Clone

The 1978 birth of Louise Brown, the world's first test-tube baby, spurred a series of medical advances - and government restrictions. The past decade's milestones:

May 1990: The Human Genome Project, an international effort led by the US, is launched.

September 1990: USC medical school professor W. French Anderson conducts the first somatic gene transfer experiments.

January 1992: First baby conceived through intracytoplasmic sperm injection - a fertility treatment in which a single sperm is injected into a single egg - is born in Belgium.

July 1996: Dolly, the cloned sheep, is born in Scotland.

March 1997: President Clinton issues an executive order banning federal funds for cloning experiments.

October 1998: The FDA asserts jurisdiction over human cloning.

January 2001: ACT announces birth of a cloned gaur, an endangered relative of the cow.

August 2001: President Bush restricts federal research funds to existing stem cell lines.

November 2001: ACT announces that it grew a six-cell human clone embryo.

December 2002: Clonaid, a company formed by the Raelian sect, claims to have created "Eve," the first human clone.

February 2003: For the second time in two years, the US House passes a bill to outlaw human cloning; no action from the Senate.

February 2003: Dolly dies from a lung infection.

April 2003: The mapping of the human genome is completed.

October 2003: ACT grows a 16-cell human clone embryo in order to derive stem cells.

November 2003: The United Nations defers voting on two proposed bans on human cloning.

6 Steps to a New You

The science isn't a mystery. The question: Are you cloning to get stem cells, or to give birth?

1. Harvest Collect eggs and cumulus cells from female donors. The normal function of cumulus cells is to nourish eggs in the ovaries, but, like other body cells, they also contain a person's complete genetic information.

2. Enucleate Puncture the egg's outer membrane with a pipette and remove the nucleus and, thereby, its DNA.

3. Transfer Inject a cumulus cell - which contains a full copy of the donor's DNA - into the enucleated egg. The result: a renucleated egg.

4. Activate Place the renucleated egg in a chemical solution. This tricks the egg into dividing as if it had been fertilized normally.

5. Incubate The clone embryo begins mitosis. After three days, the embryo typically has four to eight cells, and after five, 16 cells. At this point, the embryo is called a morula, and stem cells can be derived, according to animal studies. If the embryo progresses to a blastocyst, stem cells can be more readily obtained. **Now you've done it: therapeutic cloning.**

6. Implant The early-stage embryo is inserted in the uterus, where it attaches to the lining. It's a long shot, but if all goes well, the embryo will develop a placenta and eventually become a viable fetus. **Now you've done it: reproductive cloning.**

On the Front Lines

By Wendy Goldman Rohm

Matt Gunther

Robert Lanza spent 20 years researching heart transplantation and diabetes treatments before joining ACT in 1998. As medical director, Lanza is on a mission to clone human embryos and perfect other techniques in pursuit of stem cells. ACT's ultimate goal: hundreds of stem cell lines to treat everything from Alzheimer's to diabetes to Parkinson's.

WIRED: What have you accomplished here?
LANZA: There was a growing consensus in the scientific community that human cloning both for reproductive and medical purposes was impossible. These studies clearly show that it is indeed possible to generate early embryos via cloning.

What's your position on reproductive cloning?
Aside from the moral and ethical issues, it would be dangerous and scientifically irresponsible. I don't know of a reputable scientist who'd consider using this technology to clone for reproductive purposes.

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What's the benefit of your experiment?
This is extremely important if we are to harness genetically matched cells and tissues for human transplantation. For example, if someone has a heart attack, that person would need a ready supply of stem cells and you wouldn't have time to derive those from scratch.

How did you succeed?
This has been a process, a continuation of our ongoing efforts. We've had extensive experience cloning other species and have been troubleshooting problems for years. Also, we have an incredible scientific team. Young Chung is extremely skilled at cloning techniques.

Do you worry about the ethical implications of your research?
Cloning is currently regulated by the FDA. Our intent is to use this technology to generate stem cells to treat serious and life-threatening diseases, not to create a child. The American Medical Association agrees that this research is consistent with the ethical goals of medicine, namely, healing, prevention of disease, and helping to alleviate pain and suffering.

What's next for ACT?
The goal of this research is to generate embryonic stem cells. This is an ongoing research project and there are many steps ahead, including developing the cells into viable therapies. It will require many years of research.

Are you concerned that others might use your results for reproductive cloning?
We can't stop this valuable research from going forward for fear of the few bad apples out there. That's why there are laws.

Wendy Goldman Rohm (wwendyrohm@cs.com) wrote about the 25th anniversary of in vitro fertilization in Wired 11.10. She is the author of the soon-to-be-published Miracle Cells: Adventures on the Front Lines of a New Science.