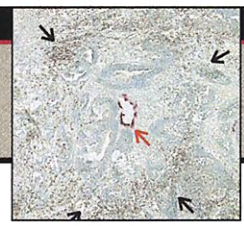

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BIOTECHNOLOGY

Perseverance Leads to Cloned Pig in Japan

Last March, PPL Therapeutics made international headlines when it announced, by press release, that it had finally succeeded in cloning pigs. At the time, the Scottish company attributed its success to a new approach—one that the scientific community is still waiting to read about in a peer-reviewed journal. But PPL wasn't the only group racing to overcome low success rates and the often-unpredictable results that have plagued other cloning researchers, particularly those trying to clone pigs (*Science*, 9 June, p. 1722). In fact, Akira Onishi, an animal breeder at Japan's National Institute of Animal Industry in Tsukuba, and his colleagues have managed to sneak under the wire ahead of PPL. On page 1188, they offer the first scientific report of a cloned pig, named Xena.

"It's a huge success," says Philip Damiani, a reproductive physiologist at Advanced Cell Technology in Worcester, Massachusetts, one that bodes well for the cloning field. "Most likely, there will be more pigs on the way," adds Damiani—although it will be some time before pig cloning becomes routine.

Cloned sheep such as Dolly, who set off the cloning frenzy in 1997 (*Science*, 7 March 1997, p. 1415), are impressive scientifically and hold the potential to become bioreactors that produce human proteins for medicine. But pigs are an even hotter commodity: They promise an unlimited supply of organs for transplantation, the result of their close physiological relationship to humans. Thanks to Xena and those piglets cloned by PPL (whose work is forthcoming in *Nature*), xenotransplantation should have moved one step closer to reality. But this week it was dealt a blow by news that pig retroviruses can infect human cells, fueling concerns about the safety of xenotransplantation.

Onishi's group produced Xena by blending the procedures used to clone mice with those used to produce clones of other livestock, such as Dolly. To make Dolly, researchers replaced the genetic material from a mature egg, or oocyte, with the nucleus from a cell of the ewe to be cloned. They activated development with electrical pulses, then implanted the embryo into a surrogate mother ewe.

A study in contrasts. Black as only a clone would be, Xena nursed from her white surrogate sow until she was ready for green pastures.

With pigs, getting a mature egg has been problematic, as has activating development. Furthermore, once implanted in a surrogate mother, a pig embryo has an even higher chance of failing than a sheep or a cow embryo, because its early fetal development requires at least three other embryos in the womb with it. "For the pig, it seems to be a real numbers game," says Damiani.

Onishi's team set out to improve the odds by doing trial runs with eggs that had not received donated genetic material. They found, for example, that eggs were more likely to become embryos if stimulated to divide by a single, strong pulse of electricity rather than by multiple, gentler shocks. They also found that mature eggs taken directly from a female pig worked better than taking the more readily available immature eggs and coaxing them to maturity in the lab.

With these conditions established, they selected fibroblast cells from 24-day-old fetuses of a black breed of pig as the source of new DNA. They deprived those cells of nutrients for 16 days to shut down cell division and most gene activity—a step some cloning experts believe increases the chances of successful nuclear transfer. Their biggest change from standard livestock cloning was in the next step, nuclear transfer. To clone Dolly, the researchers fused the donor cell with an egg whose own genetic material had been removed. "A lot of researchers tried this with pigs but without success," says Onishi. His team instead adopted the approach pioneered by a team in Hawaii to produce Cumulina, the first cloned mouse (*Science*, 24 July 1998, p. 495). Cumulina, and now Xena, were created by removing the donor nucleus in a very fine needle and injecting it into the enucleated egg. "I'm thrilled to see that they basically repeated [the Hawaii team's] stuff in another species," says Robert Wall, a geneticist at the U.S. Department of Agriculture (USDA) in Beltsville, Maryland. Adds Damiani: "It proves that microinjection can be used in large animals and livestock."

Onishi thinks that piezo-actuated microinjection, as this technique is called, worked in pigs where fusion had failed because it separates into two steps the insertion of new DNA material and the reactivation of development. In cloning Dolly, researchers used electrical shock to both fuse the nucleus to the egg and activate development. Microinjection also ensures that very little extraneous donor cell material—material that could adversely affect development—winds up in the egg.

Producing litters of pigs was yet another obstacle to overcome. An attempt to add

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cloned embryos of black pigs to pregnant white pigs resulted in only white piglets, indicating that none of the cloned embryos survived. Then they put about 110 cloned embryos into four surrogate sows that were not pregnant. One sow, which received 36 embryos, gave birth to Xena on 2 July. Not only was she black—born to a white sow—but independent DNA analyses confirmed that her DNA matched the donor fetal cell.

"It's a big achievement," says Hiroshi Nagashima, a molecular biologist at Meiji University in Tokyo, who had also been trying to clone pigs. Still, he says, getting one piglet from 110 cloned embryos could be "a matter of luck." Onishi agrees: "More work is needed to refine the techniques and secure a higher success rate." In the meantime, Onishi's successful use of an approach familiar to other scientists "will make people happy," predicts James Robl, a reproductive physiologist at the University of Massachusetts, Amherst.

In contrast, PPL's approach is a much greater departure from the norm, because the company added a second nuclear transfer to its protocol to produce its five piglet clones. The PPL researchers fused the donor cells (in this case, adult granulosa cells) with unfertilized oocytes whose own genetic material had been removed, following in the footsteps of the Dolly team. Like Onishi, they got their mature oocytes straight from a female pig. Then, in an added step, as soon as the transferred nucleus expanded, as it typically does, the PPL team moved that nucleus into a newly fertilized egg. Although they had first removed the egg's DNA and the DNA of the sperm, the egg was still primed for cell division. In this way, "they are using the oocyte as a temporary reprogramming vehicle" in which the oocyte enables the donated DNA to direct development, Robl explains. They then circumvented the need for artificial activation by inserting the nucleus into the fertilized egg.

The jury is still out on which technique works best. What is clear, however, is that naturally matured oocytes gave both groups an edge over other would-be cloners who harvest immature oocytes from slaughterhouse pigs, says USDA's Kevin Wells. Yet neither PPL nor Onishi's team can really say they've nailed cloning for pigs. Instead, "what we're learning," says Wells, "is that anyone who suggests they know the secret to cloning is naïve."

–ELIZABETH PENNISI AND DENNIS NORMILE

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MARS EXPLORATION

Plan for Two Rovers Squeezes NASA Budget

NASA's decision last week to send two rovers to Mars in 2003 is being hailed by researchers as affirming the agency's commitment to exploring the Red Planet. But once the applause dies down, cash-strapped space science managers will be forced to make tough decisions about how to shoulder the added $200 million cost of a second mission, starting with $96 million that must come out of NASA's 2001 budget.

Two failed missions in the past year have put the agency's martian strategy under intense scrutiny. So after NASA space science chief Ed Weiler initially approved a plan to send one rover in 2003, NASA Administrator Dan Goldin pushed hard for two, to improve the chances of success, say agency sources (*Science*, 28 July, p. 521). Favorable orbital mechanics in 2003—based on a planetary alignment that won't be repeated until 2018—provided an additional incentive. "This is just an unusually good opportunity to go to the surface of Mars," says Cornell University's Steven Squyres, principal investigator for the mission's science program and chair of NASA's space science advisory panel.

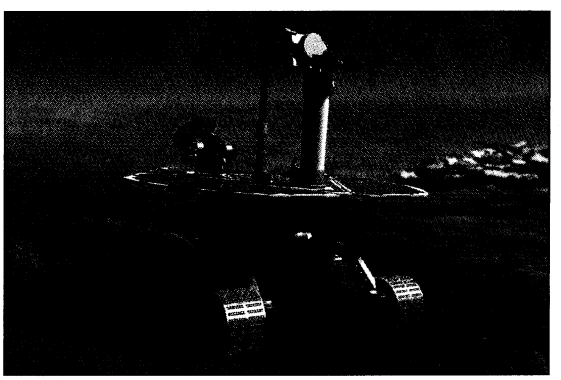
The rovers will have the same landing mechanism—a parachute and a cushion of air bags to soften the impact—as the famous Mars Pathfinder mission in 1997. But the new rovers will travel much farther from their landing sites—up to 100 meters a day, about the total distance covered by Pathfinder's rover—and carry a rack of new instruments, including a microscope and imager to analyze rocks, a device for grinding away the outer layers of rocks, and several spectrometers. Their primary job will be to understand martian geology and the possible role that water has played.

But paying for all this will entail sacrifices. The White House refused a NASA request for additional funding in 2001, and Congress seems unlikely to approve a significant boost next month in the agency's $2 billion space science budget. Weiler's office will have to cut $20 million from other programs to cover the additional costs associated with the first rover and obtain another $76 million from other parts of the agency, in a plan still being worked out. The new Mars program will cost about $600 million total through 2003—$200 million more than originally envisioned.

Weiler doesn't expect any major missions to be canceled as a result of the added Mars costs. But he acknowledged some difficult choices. "Do I try to keep all of the programs in the basket, and let launch dates slip," he wonders, "or do some of the projects have higher priority, and [do I] let one drop?"

Another agency manager says that "space science already has too much on its plate" and that big cuts are inevitable. A budget table presented by Weiler last month to NASA advisers notes "significant new content" that will grow substantially over the next 5 years. Astrobiology is slated to triple, the small Discovery missions are expected to double, and NASA's new Living With a Star program is supposed to grow from $20 million to $177 million (*Science*, 28 July, p. 528). Existing efforts, such as the Europa orbiter, face technical problems that will cost time and money to fix. And increased testing of new spacecraft will cost more money.

Politics also plays a role: A new Administration could undo all these funding decisions. In the meantime, NASA is rethinking a mission to Pluto later in the decade, and some current efforts, such as the Extreme Ultraviolet Explorer, seem vulnerable. A more expensive Mars program only makes the situation worse. "There's no question that a second rover makes a lot of sense, but we don't know what the trade-offs will be," says Bill Smith, president of the Washington-based Association of Universities for Research in Astronomy. "There could be a price to pay."



Travel costs. Two martian rovers will mean more data—but who will pay the piper?

–ANDREW LAWLER AND JOHN MACNEIL