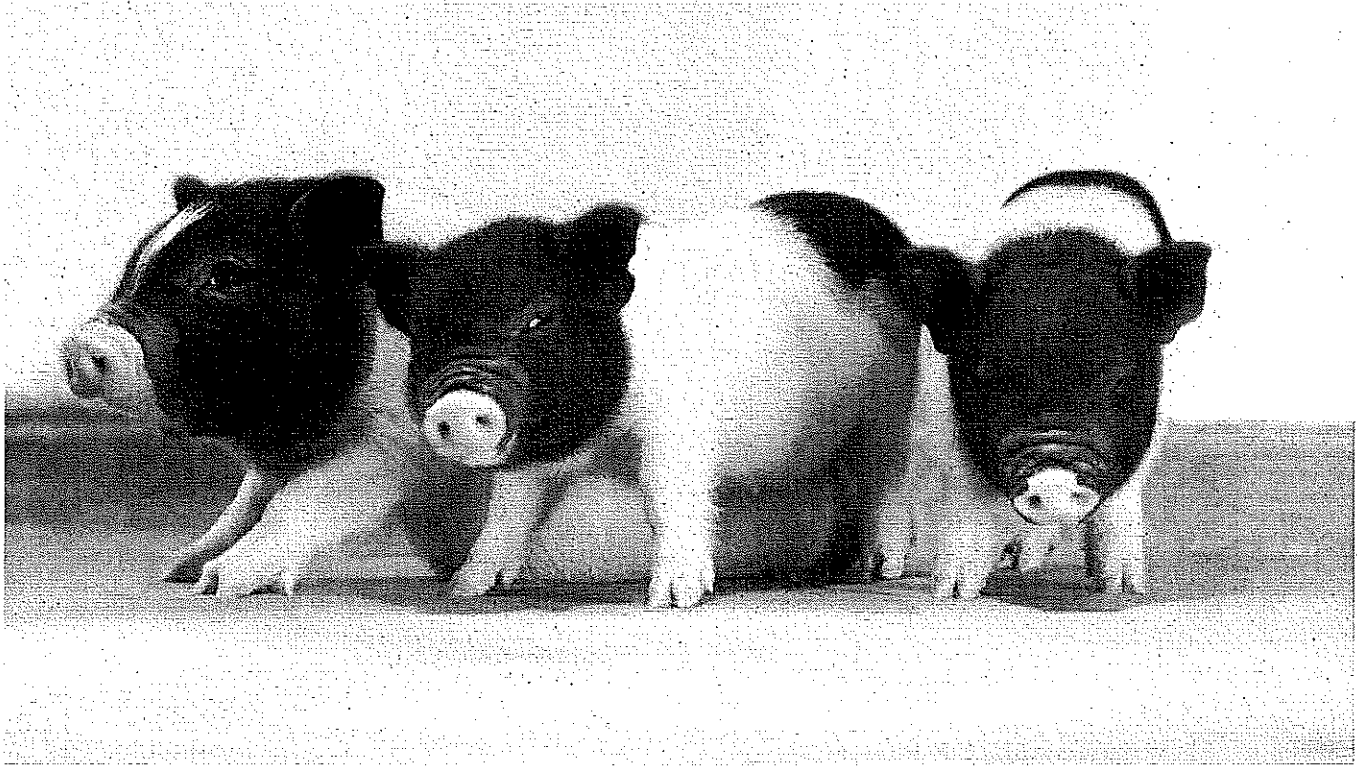


STAT

Birth of CRISPR'd pigs advances hopes for turning swine into organ donors

By Sharon Begley @sxbegle

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CRISPR'd piglets from the eGenesis study. *eGenesis*

Bioengineer Luhan Yang¹ swiped through the photos on her phone until she got to one that made her beam: It showed her crouching down by a pudgy, wide-eyed newborn she calls “my baby.”

This newborn is a pig, and it's the first to be born with dozens of genetic changes that could enable scientists to turn swine into a source of organs for human transplants, Yang and her colleagues reported² on Thursday in *Science*.

They named the piglet Laika, after the first dog to orbit Earth in 1957. The new Laika, born this year in China after numerous miscarriages and other setbacks, could be a pioneer in her own right. Using the genome-editing technology CRISPR-Cas9, Yang and her team at the biotech startup eGenesis knocked out pig DNA that has long been considered a deal-breaker for efforts to use pigs as organ donors. Laika and 36 other designer piglets are completely free of it.

There are additional Olympic-level hurdles to overcome before people facing death from organ failure get replacement kidneys, hearts, livers, or lungs from the species that provides their bacon and pork chops. Other genetic changes will be necessary. And regulators require stringent tests in lab primates before a single patient could get a CRISPR'd pig organ; that will take years.

Related Story: ¹

She's hellbent on solving the organ shortage with 'designer pigs.' Just don't keep her waiting. ¹

But after decades of dashed hopes, experts say, “xenotransplantation” might actually be in the offing.

“It’s an elegant tour de force of genetic engineering, so my hat is off to them,” said Dr. A. Joseph Tector, of the University of Alabama, Birmingham, who has also made genetically modified pigs aimed at producing transplantable organs. “But if you want to move xenotransplantation to the hospital, there are many more things you’ll have to do.”

Doctors won’t have to do much persuasion, however, to get patients to accept organs from another species. “There is so much desperation among people on transplant lists, and 20 a day are dying as they wait,” said Dr. Adam Griesemer, a xenotransplantation researcher and transplant surgeon at Columbia University Medical Center. “This could be a path to a transplant for them. Colleagues keep asking me when we’re going to do it.”

Pigs are scientists’ first choice because their organs and physiology are pretty close matches to humans’, and they come with less ethical baggage than, say, chimps or baboons. But for years, the path to xenotransplantation has been paved with disappointment. Pig organs with genetic changes, transplanted into baboons and other lab animals, kept failing within weeks, even though the recipients received immune-suppressing drugs to prevent organ rejection.

Yang believes that CRISPR can accomplish what previous approaches have not: make multiple, simultaneous changes in pig DNA so that the animals’ organs work, and work safely, in people.

The team at Massachusetts-based eGenesis, working with scientists in China, used the Dolly recipe⁴ to clone pigs. They started with cells from adult pigs, and used an electrical jolt to fuse them with pig ova whose DNA had been removed. They grew the resulting embryos in lab dishes and then transferred healthy ones to sows, hoping for pregnancies.

The adult cells were not as nature made them, however. In a key step, the scientists used the genome-editor CRISPR to cripple all 25 copies of “PERV” genes — DNA in the pig genome that makes potentially dangerous viruses that could infect anyone who receives a pig organ. (PERV stands for porcine endogenous retroviruses.) Initially, in about one-third of the CRISPR’d pig cells, the PERV genes were almost all gone. In most of the rest, CRISPR missed its mark. That wasn’t unexpected; for all the hype around CRISPR, it isn’t perfect.

The unwelcome surprise was that cells that were effectively CRISPR’d — the ones the scientists needed to clone designer pigs — were dying like orchids in the tundra. Apparently, in its zeal to attack so many PERV genes, CRISPR had shredded the cells’ genomes — fatally.

“It’s quite a problem, when you move to so many targets,” said Yang, the chief scientific officer at eGenesis. “If there are multiple cuts in the genome at the same time, chromosomes rearrange themselves. That can happen when you make two or three [CRISPR edits], and we’re dealing with 25.”

The eGenesis scientists, many of them alums of George Church’s lab at Harvard Medical School, scrambled for a solution. They eventually stumbled on a cocktail of molecules that both increased the

number of PERV targets that CRISPR hit and, even better, kept the well-CRISPR'd cells alive. "We were able to get cells to grow even with very aggressive gene editing," Yang said: 100 percent of the cells doused with the chemical cocktail were 100 percent PERV-free.

Related Story: ⁵

U.S. scientists edit genome of human embryo, but cast doubt on possibility of 'designer babies' ⁵

As is typical with cloning, very few of the cloned embryos were healthy enough to implant into sows, and few implanted embryos resulted in births. Crucially, however, of the 37 piglets born from 17 sows, all were PERV-free. And CRISPR did not change any DNA it wasn't supposed to; there were no "off-target" effects ⁶.

The oldest pigs are nearly 5 months old, or adolescents; 15 remain alive. The rest were killed so the scientists could see whether their organs were developing normally.

So far, so good, Yang said, showing that pigs don't need PERVs to live: "We've shown you can produce PERV-free pigs which could serve as a source for future xenotransplants."

Among eGenesis's next experiments: see if the pigs are fertile and, if so, whether their CRISPR'd genetic changes, including inactivating PERVs, are inherited. That could provide an easier source of transplantable organs than cloning.

Other scientists have also used CRISPR to produce pigs with altered genomes, including pigs in which a gene ⁷ that triggers organ rejection was eliminated. Last year, scientists announced ⁸ that hearts from genetically-modified pigs survived in baboons for up to 945 days, a record.

UAB's Tector and his colleagues, with financial backing from United Therapeutics Corp., are using CRISPR not on PERVs but on other pig genes. Knocking out three ⁹ in particular could protect pig organs from being attacked by the human immune system, he said; lab macaques that received kidneys from the pigs have survived as long as 499 days. "We have a pig we are very confident we can make work for kidney transplants," Tector said.

There is disagreement about whether pig organs would have to be PERV-free to be successfully transplanted into people. Tector said transplant patients could take anti-retroviral drugs, just as they take immune-suppressing drugs, to kill the viruses.

Nevertheless, eGenesis scientists' achievement — with their 25 DNA edits, the eGenesis pigs set the record for genome modifications — suggests that however many edits are needed to make pigs into organ donors might be feasible. The challenge is to identify which pig genes are necessary and sufficient to change so that the animals' organs have a shot at working in people.

About the Author



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