HUMAN ORGANS FROM ANIMAL BODIES

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BIOLOGY

Scientists are taking the first steps toward growing replacement parts for people inside pigs, cows and other animals

By Juan Carlos Izpisúa Belmonte

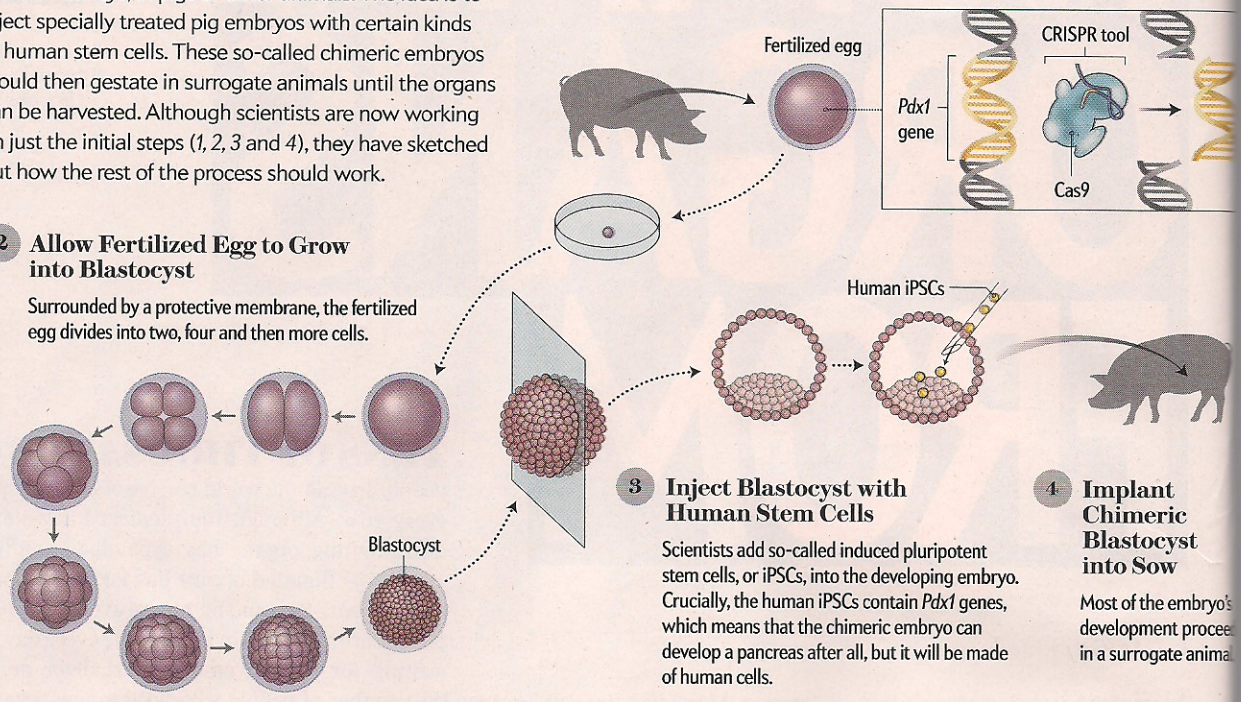
TENS OF THOUSANDS OF people around the world receive organ transplants every year. Although the medical know-how for transplanting organs has expanded rapidly, the number of donated organs has lagged. Global figures are hard to come by, but an average of 16 people in Europe and 22 in the U.S. die every day while waiting for a replacement heart, liver or other organ. Moreover, the gap between the number of people who need a new organ and the number of organs available for donation keeps widening.

One way to alleviate the shortage would be to grow replacement organs in the laboratory. A few years ago scientists thought that they could do that by using stem cells, which are progenitor cells that can give rise to different kinds of tissues, and an artificial scaffold to create a new organ. Investigators have struggled, however, to orchestrate the development of stems cells to produce a fully functioning human organ. Research continues on this approach, but progress has been slow.

Illustration by Peter Horvath

The Research Plan

Recent advances in stem cell technology may one day allow researchers to grow human organs, such as a pancreas or kidneys, in pigs or other animals. The idea is to inject specially treated pig embryos with certain kinds of human stem cells. These so-called chimeric embryos would then gestate in surrogate animals until the organs can be harvested. Although scientists are now working on just the initial steps (*1*, *2*, *3* and *4*), they have sketched out how the rest of the process should work.



1 Change Fertilized Egg's Genetic Makeup

Researchers interfere with a pig embryo's ability to grow a pancreas by delet the *Pdx1* gene using the CRISPR/Cas9 enzyme as a pair of genetic scissors.

2 Allow Fertilized Egg to Grow into Blastocyst

Surrounded by a protective membrane, the fertilized egg divides into two, four and then more cells.

3 Inject Blastocyst with Human Stem Cells

Scientists add so-called induced pluripotent stem cells, or iPSCs, into the developing embryo. Crucially, the human iPSCs contain *Pdx1* genes, which means that the chimeric embryo can develop a pancreas after all, but it will be made of human cells.

4 Implant Chimeric Blastocyst into Sow

Most of the embryo's development proce in a surrogate anima

A small but increasing number of investigators, myself included, think that there may be another way: let nature do the heavy lifting. Evolution has already created an exquisite process for turning a handful of identical cells into all the specialized organs and tissues needed to build an entire complex organism—whether it is a mouse or a person. That virtuoso performance occurs in the weeks and months after a fertilized egg gives rise to an embryo that grows and—without having to rely on an artificial scaffold—develops into a full-grown animal with a well-formed heart and lungs, kidneys and other tissues. We believe we can figure out a way to harvest organs from animals, such as pigs, for use in people.

A normal pig heart would, of course, be of little use to a human in need of a transplant. For starters, our immune systems would overwhelmingly reject a direct cross-species implant. (Pig heart valves are suitable substitutes for human tissue only after they have been chemically treated to prevent this immune reaction—a process that would destroy a complex organ's ability to function.) My colleagues and I believe that it may be possible t grow human organs—made entirely, or almost entirely, of human cells—in an animal such as a pig or cow. The resulting animal would be a chimera—a creature that combines the parts o two different species, much like the mythical griffin, which sports the head and wings of an eagle and the body of a lion. Our dream is to create a chimera by injecting human stem cells int carefully prepared animal embryos so that when they become fully grown, they contain some organs made up of human cells After sacrificing the animal, we would then harvest the single heart, liver or kidney made up of human cells and give it to a person in need of a transplant.

The idea might sound far-fetched, but researchers in the U.S and Japan have already shown that it is possible in principle. Several different teams injected custom-designed mouse embryos with rat stem cells and then allowed the resulting chimeras to develop in surrogate mouse mothers. After a few weeks of gestation the surrogates gave birth to animals that looked and acted like

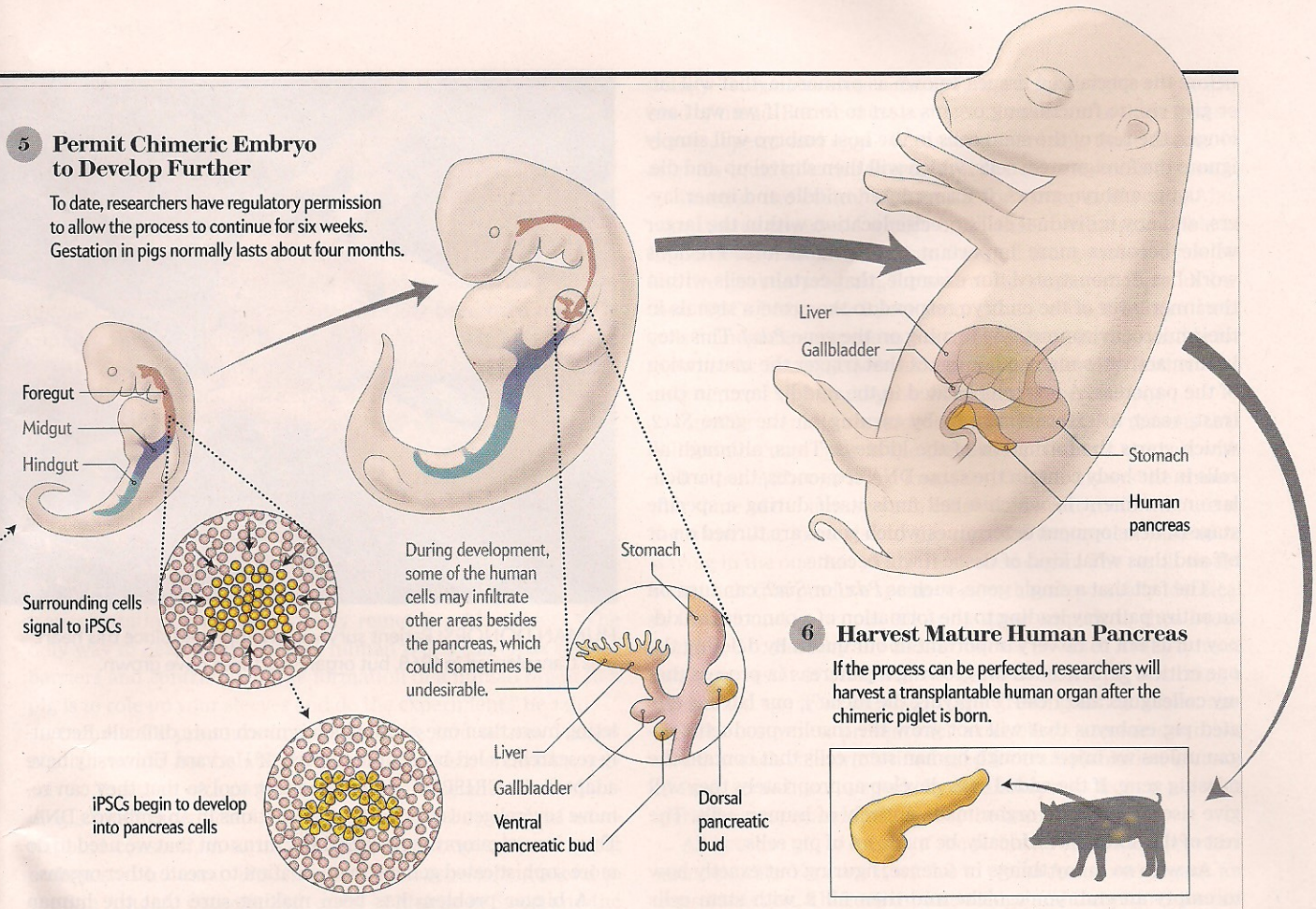
IN BRIEF

Biologists are trying to figure out how to grow human organs inside of animals, such as pigs, using the latest advances in stem cell technology. Such an achievement could dramatically decrease current organ-transplant shortages.

The idea is to take human stem cells and implant them, under the right conditions, into specially prepared pig embryos so that the resulting organism, known as a chimera, develops into an animal with a human pancreas, kidney or other organs.

If early experiments are successful and investigators obtain the necessary regulatory permissions from local and national authorities, the goal is to allow the chimeras to develop full-term (about four months for pigs) to see if they produce usable human organs.

Graphic by Amanda Montañez



mice—except that they had the pancreas of a rat. Researchers in my lab and in other groups have taken the next step and injected human stem cells into porcine embryos. A few of these injections "took," and we confirmed that the human tissue had started to mature normally. Then we transferred the chimeric embryos into surrogate sows, where we have allowed them to develop for three to four weeks. After completing several more intermediate experiments, we will permit the embryos to grow a couple of months, at which point we will determine how many of their cells are of human origin. Provided these experiments are successful—and we receive permission from state and local authorities to continue—we expect to enable the embryos to grow full-term (which for pigs is about four months).

We are nowhere near ready to take that final step of producing chimeric piglets. We still have much to learn about how best to prepare human stem cells and animal embryos so that the chimeras will remain viable throughout pregnancy. A lot could go wrong. But even if we are unable to create fully formed organs, the techniques we discover should help us better understand the onset, progression and clinical outcome of many complex and devastating illnesses, including cancer. If successful, this approach could have enormous implications for organ-transplant therapies. Waiting lists could become a thing of the past as we develop a bountiful supply of replacement parts from farm animals for tens of thousands of suffering people around the world.

LEARNING FROM NATURE

IN RECENT YEARS biologists have learned so much about how embryos grow that we have tentatively begun to tailor the process to our bidding. We also recognize how much this growth is guided by the precise location of different cells at various times within the developing organism. The cells make and release specialized proteins called growth factors that, depending on their concentration within distinct regions of the embryo, in turn activate and silence a raft of internal genetic programs. Relying on this still incomplete understanding and a lot of trial and error, researchers in our lab and elsewhere are manipulating pig embryos so that they produce tissues that would eventually give rise to a human kidney, pancreas or other organ.

The raw materials we use include porcine eggs and sperm (taken from animals) and human stem cells (grown in cell cultures). We fertilize a pig egg with pig sperm, and a few hours later the combined cell, now known as a zygote, divides into two and then four seemingly identical cells. Each of these cells activates the same groups of genes in its DNA, which leads to the production of various proteins that coax the cells, among other things, to divide even further.

Thanks to the complex interplay of genes and proteins, these once identical cells soon start to move and behave differently as they divide. Within a few days several hundred cells have formed a kind of ball within a ball, known as the blastocyst. This is the latest point at which we can inject the human stem cells—

before the specialized tissues, known as primordia, that will later give rise to functioning organs start to form. If we wait any longer, the rest of the stem cells in the host embryo will simply ignore the foreign stem cells, which will then shrivel up and die.

As the embryo grows, it forms outer, middle and inner layers, and any individual cell's precise location within the larger whole becomes more important than ever before. Previous work has demonstrated, for example, that certain cells within the inner layer of the embryo respond to the protein signals in their microenvironment by turning on the gene *Pdx1*. This step in turn activates many other genes that trigger the maturation of the pancreas. A few cells located in the middle layer, in contrast, react to external signals by turning on the gene *Six2*, which starts the formation of the kidneys. Thus, although all cells in the body contain the same DNA sequences, the particular environment in which a cell finds itself during a specific stage of development determines which genes are turned on or off and thus what kind of tissue it will become.

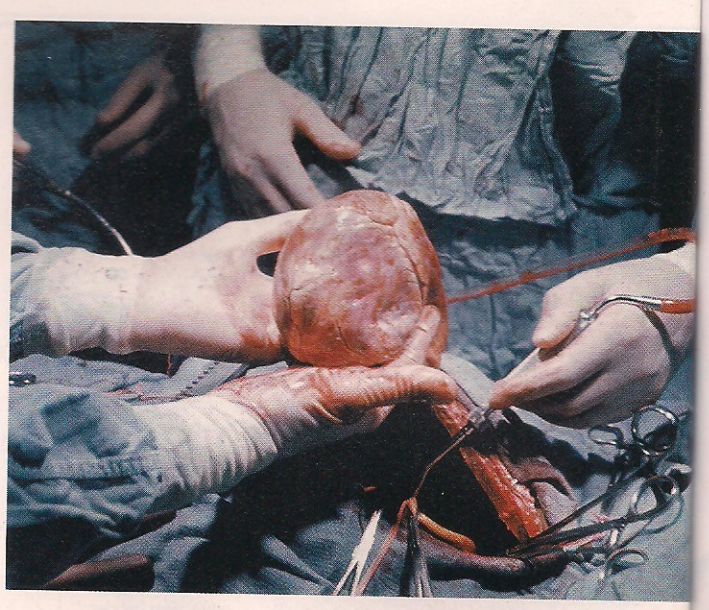
The fact that a single gene, such as *Pdx1* or *Six2*, can turn on an entire pathway leading to the formation of a pancreas or kidney turns out to be very important in our quest. By deleting the one critical gene needed for growing a pancreas (a process that my colleagues and I call "emptying the niche"), our lab has created pig embryos that will not grow the insulin-producing organ unless we inject enough human stem cells that contain the missing gene. If the added cells develop appropriately, they will give rise to a mature organ made entirely of human cells. The rest of the animal will, ideally, be made up of pig cells.

As with so many things in science, figuring out exactly how to empty an embryonic niche and then fill it with stem cells from a different species first took a lot of experiments on rodents. Finally, in 2010, Hiromitsu Nakauchi, then at the University of Tokyo, and his colleagues reported that they had successfully grown a mouse with a rat pancreas. More recently, my lab has been able to genetically reprogram mouse embryos so that they will use stem cells from rats to grow cells in their eyes. After three weeks of gestation in surrogate mouse mothers, these embryos become fetal mice with rat cells in their eyes.

CHALLENGES

EACH STEP ON OUR JOURNEY requires careful consideration of different potential problems. Because mice are too small to generate organs that would be a useful size for human patients, we have now concentrated our efforts on creating pig embryos. Pigs, and their organs, can grow to almost any proportion that transplant surgeons might need to help people of varying builds. Pigs also have a longer gestation period than mice do (about 20 days for the latter). Because normal human embryos require nine months to develop fully, researchers are inventing certain biochemical tricks to help human stem cells speed up their internal clocks so that they mature, or differentiate, on the host embryo's schedule. Adapting human cells to the somewhat longer pig timeline should require less effort than aligning with the much shorter mouse timeline.

Currently my colleagues and I have focused on growing a pancreas or kidney made of human cells because we know that a single gene kicks off its development in the embryo—a fairly straightforward process. Other organs, such as the heart, however, may depend on several genes to initiate the process, which means that emptying the niche for these organs will require deleting more than one gene, which is much more difficult. Recently researchers led by George Church of Harvard University have adapted the CRISPR/Cas9 gene-editing tool so that they can remove several genes from different locations in an embryo's DNA. Thus, investigators are prepared if it turns out that we need to do more sophisticated genetic manipulation to create other organs.



HUMAN DONORS: Patient survival has improved since this heart was transplanted in 1968, but organ shortages have grown.

A bigger problem has been making sure that the human stem cells that are used are pristine enough to give rise to any kind of tissue. Biologists refer to this physiological state as being "developmentally naive." Human embryonic stem cells, which could be harvested from the leftover zygotes generated by in vitro fertilization clinics, would fit the bill, but their use would prove highly controversial.

Over the past decade researchers made a number of technical advances that looked, at first glance, as though they might solve the dilemma. They figured out how to coax mature cells taken from the skin or gut of an adult into becoming a kind of stem cell called an induced pluripotent stem cell, or iPSC. Experimenting on human iPSCs instead of human embryonic stem cells would certainly be more ethically acceptable. Using iPSCs would offer the added advantage of one day allowing scientists to create organs that are a genetic and immunological match for individual patients.

Closer study of the human iPSCs created to date, however, shows that they are not as naive as they need to be to survive inside a chimeric embryo. They are already so far along to becoming one of several specific cell types that they can no longer react to any of the biochemical signals coming from the embryo that tell them to grow into something else. Because these iPSCs do not respond correctly, the developing embryo ejects them as foreign.

Recently Jun Wu in my lab has begun treating human iPSCs with a unique combination of growth factors that allow a few of them, at least, to react appropriately to a wider range of embryonic signals. To date, our group has obtained preliminary results showing that our treated human iPSCs can, in fact, integrate into blastocysts. My colleagues and I stopped the experimental embryos from growing at different times after fertilization and ana-

GETTY IMAGES

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lyzed them under the microscope to check how well the host and donor cells had mixed. Next, we plan to allow the embryos to develop a little longer—until they are six weeks old, and the primordia can be seen. At that point, the embryos will begin generating the precursors of the body's various tissues and organs.

Even if we are able to produce human iPSCs that can fully integrate into pig embryos, however, we are not home free. Humans and pigs are not as closely related, evolutionarily speaking, as the mice and rats that have already been used to create chimeric animals. Thus, human iPSCs simply may have lost the ability to perceive all the biochemical signals from a more distantly related species such as pigs. If we cannot figure out a biochemical work-around to this problem, we may need to start testing our ideas in other species, such as cows.

NEXT STEPS

IN 2012 I DISCUSSED THESE and other concerns with my collaborator Josep Maria Campistol, general director of the Hospital Clinic of Barcelona, which is internationally known for its organ-transplantation services. I vividly remember his advice: "The only way to determine whether human iPSCs can cross species barriers and contribute to the formation of a human organ in a pig is to role up your sleeves and do the experiment," he said.

Campistol's pronouncement jolted me into action. I knew our lab would not be able to accomplish such a task by ourselves. Together with embryologists, veterinarians, stem cell biologists and bioethicists, my colleagues and I created an international consortium to test our ideas. We began injecting pig embryos with human iPSCs in 2015. I am especially grateful to the San Antonio Catholic University of Murcia in Spain and the Moxie Foundation for supporting this early work when no one thought our approach was even feasible.

To date, most of our experiments have been conducted in California and Spain—under the supervision of local and national regulatory agencies. So far we have allowed the chimeric pig-human embryos to gestate in a sow for about four weeks—at which point we sacrifice the animals. (The guidelines that we have worked out with regulatory authorities require us to sacrifice both surrogates and embryos.)

Overall, the results obtained from these and other experiments have helped us to gain some basic knowledge about the development of chimeric embryos. We are starting to learn the best number of human iPSCs that need to be implanted for the embryo to develop successfully and the time at which we need to implant them. We have also begun tracing the way the human cells start to migrate to different parts of the embryo.

AN ETHICAL BALANCE

EVEN AS WE SCIENTISTS perfect our procedures, however, we must work with the larger public to address the new ethical, social and regulatory challenges created by this emerging field. Our consortium worked closely with ethicists and regulators in California and Spain for a year and a half to develop the guidelines that govern our research.

It goes without saying that we abide by the standard rules regarding animal welfare that should apply to all research with sentient creatures—to avoid unnecessary pain and to provide adequate living space and exercise, among other things. There are additional concerns, however, that are specific to this technology. Truly naive stem cells, as I have said, can give rise to any kind of tissue. But we must pay special attention to three types—nerves, sperm and eggs—because humanizing these tissues in animals could give rise to creatures that no one wants to create.

Imagine the ethical nightmare, for example, if enough human nerves populated a pig's brain that it became capable of higher-level reasoning. We can forestall that problem by deleting the genetic program for neural development from all human iPSCs before we inject them. Then, even if human stem cells managed to migrate to the embryonic niche responsible for growing the brain, they would be unable to develop further. The only neurons that could grow would be 100 percent pig.

Another scenario researchers want to avoid, for reasons that will soon become clear, is the breeding of chimeric animals with each other. Although it is a long shot, there is always the chance that some of the human stem cells we implant could migrate to the niche that gives rise to the reproductive system instead of staying in the one that yields the desired organ. The result would be animals that produce sperm or eggs that are virtually identical to those found in people. Allowing these animals to then breed could lead to the ethically disastrous case in which a fully human fetus (the result of a humanized sperm from one pig fertilizing a humanized egg from another) starts growing inside a farm animal. The best way to prevent such a troubling outcome is to make sure that each chimeric animal used for transplantation is created from scratch, so to speak, by fertilizing eggs from a pig with sperm from a pig and then adding the human stem cells.

All bets are off, of course, if the technical challenges prove insurmountable. Yet even if we fail to create functional organs for transplantation, I believe the knowledge and techniques we discover along the way will prove enormously valuable. One of the first fields to benefit will most likely be cancer research. Studies show that many tumors grow uncontrollably in a child or adult by reactivating some (but not all) of the genes that once allowed the embryo to grow into a fetus. Thus, the better investigators understand the normal cellular signals that allow embryos to grow—and tell them when to stop growing—the better they may be able to coax cancer cells into abandoning their treacherous path.

Scientists are people, too, of course. We get excited about new ideas and novel ways of doing things. And we can be overly optimistic about what our discoveries may imply—not just for our own fields but also for humankind. But the preliminary results I have described in this article make me cautiously optimistic that we may generate human organs from chimeric animal embryos in the next couple of decades. SA

MORE TO EXPLORE

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