Chimeric organ transplants: Science and ethics of growing human organs in pigs

Each day, 22 people die waiting for an organ transplant in the United States, leading to 8,000 deaths per year. The numbers are similar in Europe and other countries, but scientists may soon be in a position to offer a possible solution to the shortage: growing human organs in pigs.

The animals would be sacrificed when the organs are needed. That concerns some people, but this is a society that kills 117 million pigs each year for food, so an additional 8,000 or so for direct salvation of human lives might not pose an ethical dilemma for most of us. But people are worried about the ethics of creating a human-porcine chimera. Society is understandably resistant to the idea of pigs with human physical characteristics, such as human-like brains–but researchers are ahead of society on this, and to a large extent so are government regulators.

Chimeric organs: Where does the science stand?

Image not found or type unknown Chimera_Apulia_Louvre_K362 The Chimera of ancient Greek literature exhibited recognizable anatomic parts of a lion, goat and snake, but in modern biology the meaning of the word chimera varies, depending on the particular biological field. It can apply to transgenic organisms, to the RNA used in CRISPR genome editing (called chimeric RNA, because it's a fusion different RNAs that exist in a bacterial CRISPR system that researchers simplified), or to a hybrid animal, such as a mule (hybrid between a horse and donkey). In embryology, a chimera is an organism made from a combination of cells from different individuals, but cells that mixed together in the embryo.

When we talk about a chimera in connection with growing organs of one species within another, we're talking about a chimera in the embryonic sense. Thus far, no chimera has even been produced harboring the cells of a human and a pig, but—perhaps due to the meaning of the word 'Chimera' as people know it from literature—the internet is awash with alarmist stories of secret porcine-human experimentation, complete with pictures of hybrid animals that rival the imagination of the ancient Greeks.

The reality is that scientists have created chimeric rodents. Six years ago, scientists at the University of Tokyo's Center for Stem Cell Biology and Regenerative Medicine demonstrated that mice could be created that carried a rat pancreas. In 2010, they did it by first generating mice embryos that lacked a gene called *Pdx1*, which is needed for the embryo to generate its own pancreas. Perviously this was quite difficult, but the advent of CRISPR-Cas9 has made it easy today. One needs only a CRISPR kit, plus a strand of RNA that is complimentary to the *PDX1* gene. You can buy that RNA strand too; in fact, you can order it today and have it delivered to your lab tomorrow.

Now, if a mouse embryo that is *Pdx1* negative is allowed to mature, the baby mouse will develop diabetes and die. But if you inject what are called induced pluripotent stem cells (iPSC) into the developing embryo, it grows a pancreas and lives, and that happens whether the iPSCs are from a mouse, or a different animal. Using rat iPSCs results in a mouse that has a working pancreas consisting of rat cells. That pancreas can be removed form the mouse where it formed, then transplanted into a rat and it won't be rejected by the rat's immune system, because the pancreas cells don't have mouse marker molecules on their surface that would stimulate the rate immune system.

Why pigs?

When it comes to farming human organs, it may be obvious why mice can be nothing more than laboratory models for proof of concept, but why pigs? Great apes are closer to us phylogenetically, and they have been considered as potential blood donors. But apes not on the short-list for organ-growing They're endangered and so human-like that few people in our society would accept the idea of breeding them for eventual sacrifice. Also, ironically, because they're similar to humans, apes are potential sources of new zoonotic infections; in fact, the leading theory on the origin of HIV is that it was introduced into humans from chimpanzees.

We could consider lions and tigers and bears, but when going through the Animal Kingdom, medical researchers have been relying on pigs for decades, both for clinical experimentation and as sources of tissue. Internal organs of pigs are roughly the same size as their human counterparts. For this reason, many people are walking around with porcine valves that were transplanted into their hearts to replace failing human valves. Thus, if a mouse can accommodate a rat organ, it makes even more sense that a pig could accommodate a human organ. Take this together with the enormous number of pigs that are maintained anyway, mostly for food, and our porcine relatives do stand out.

Taking things to the next step

Researchers at several institutions are now working on scaling up the mouse-rat technique to create a human pancreas inside a pig. But there are potential problems. The biggest concern—though it may not generate headlines and artwork as interesting as those predicting a human-looking pig—has to do with diseases. The risk is lower compared with chimpanzees, but still significant with pigs that retroviruses might be introduced into the human population through transplants, leading to a new infectious disease. For this reason, one area of study is the use of CRISPR editing to remove retroviruses during production of chimeric organs. But another issue is that a significant fraction of the cells in the modified mice—almost 20 percent—are rat cells. Most such rat cells are in the pancreas, where they're supposed to be, but other tissues in the mouse can show a certain degree of chimerism.

Based on the rat chimerism in the mouse model, there's a concern about what would happen in a pig embryo treated using CRISPR to prevent a pancreas from forming were it given human iPSCs. The details of the mouse experiment are known well enough to say that such an altered pig would grow a pancreas fit for a human and consisting of human cells. Extension of the technique, through CRISPR knockout of other genes, also should work to create human kidneys, livers or even a heart. But what would happen in the rest of the pig's body? If a few human iPSCs were to get into the developing porcine brain, could the brain develop such that it is more conscious than a normal pig brain? Furthermore, could the iPSCs produce human gametes in pig reproductive organs that could then produce a pig-human hybrid in the next generation?

In June, researchers at the Université de Montpellier in France published a comprehensive assessment of such exotic, but scary-sounding, possibilities in the journal *Stem Cell Research Therapy*. The authors pointed out that hybrids produced from human gametes would be close to impossible given the obstacles that nature itself has evolved to prevent embryogenesis from species as different as humans and pigs. It's important to keep in mind, for instance, that humans have 46 chromosomes, while pigs have only 38. That's in contrast to horses and donkeys, which differ by only one pair (64 for horses and 62 for donkeys). As for the humanized brain concern, the authors point out–and cite other publications backing the idea—that, even if contaminated by human stem cells, a pig brain wouldn't have time to develop additional sophistication, since the gestation period is only three months—roughly one third that of human gestation.

Second, they cite growing evidence that pig cells would dominate the development process in all organs, except for organs that were edited out purposefully in order to be replaced with the human-counterparts using the iPSC technique. This means that a pig designed to grow a human internal organ such as a pancreas or kidney might have some neurons whose nuclei harbor a human genome, but the gross and molecular architecture of the resulting brain would still be 100 percent pig.

Third, in order to achieve the proof of concept, experiments with mice purposefully used very high numbers of rat IPSCs, possibly much higher than actually needed to produce the needed organ. Research is now trying to quantify the needed amounts and this will be a major focus as the technology scales up to creating a human pancreas in a pig. Researchers could eventually find that there are thresholds, below which the amount of chimerism in the pig central nervous system is minimal. This type of knowledge can form the basis for legal regulations and policies become more sophisticated in parallel with the research. Currently, laws in countries where this organ research is flourishing–the USA, UK, France, Germany and Japan—vary, but they have certain things in common in that they seek to prevent the creation of human/non-human hybrid. Thus, the US prohibits the use of human cells in non-human primates, while Germany allows human cells in non-human embryos during research, but prohibits use of non-human cells in human embryos.

Fourth, it is possible to engineer suicide genes into human cells, so they'll be programmed to die if they find themselves in certain environments, such as in the developing pig brain. Taking this together with the fact the environment would be ripe for human iPSCs to form an organ only where there is an anatomic vacancy, due to the knockout of genes for that organ in the host embryo, a scenario in which this research leads to a pig with human-like traits becomes extremely far-fetched.

The research has a way to go until such pigs are generated, but when they finally come to life, these pigs will be pigs with just one different from their parents. Each will carry an organ with human cells and human DNA that will act the same in the pig as would the pig's own organ have acted. If the organ is a pancreas, it means producing insulin, other hormones, and digestive enzymes. But, importantly, a pig can carry such an organ without having the ability to contemplate its place in the Cosmos.

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