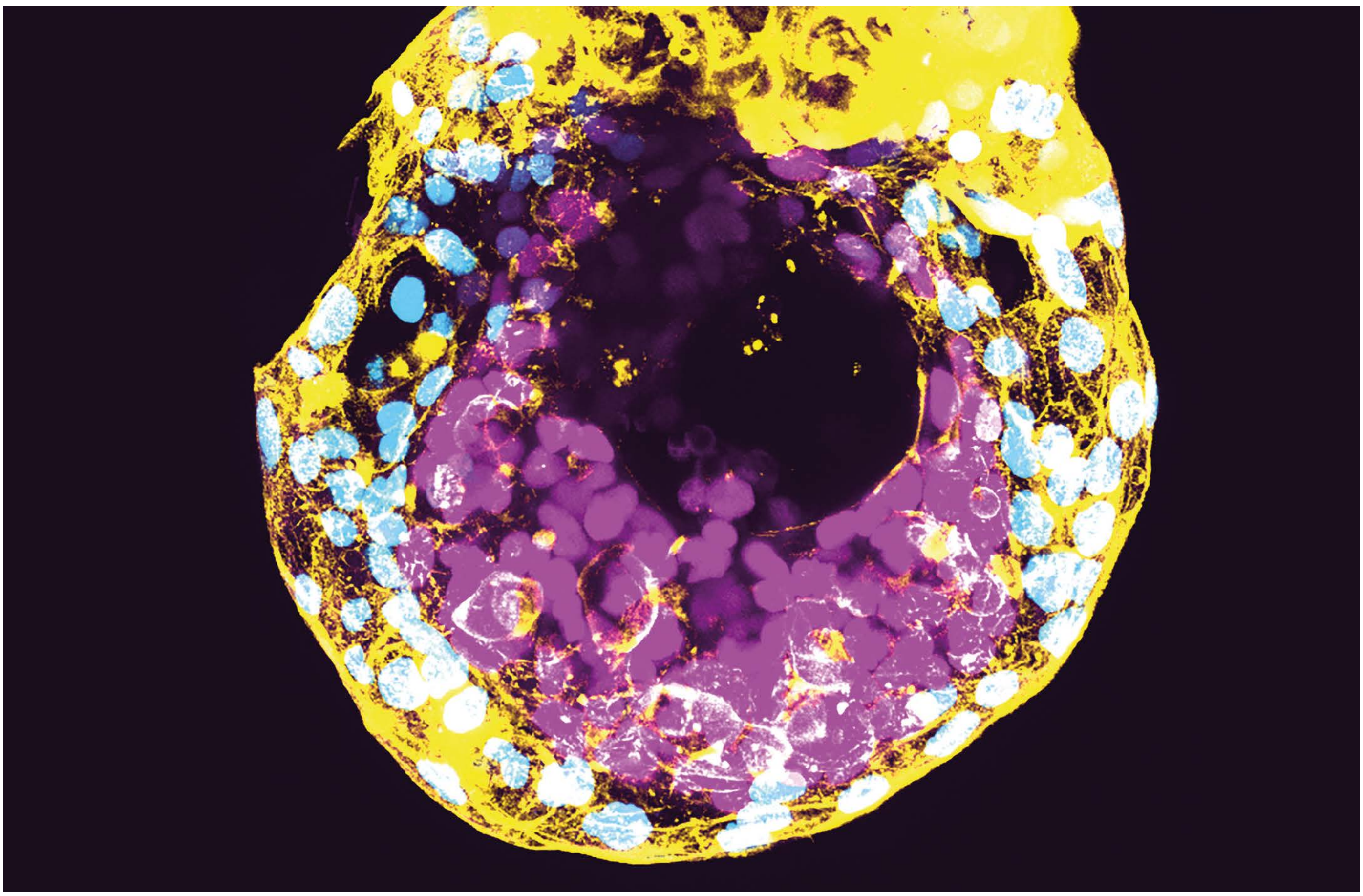
Feature

# WHAT'S NEXT FOR LAB-GROWN HUMAN EMBRYOS?

Researchers are now permitted to grow human embryos in the lab for longer than 14 days. Here's what they could learn. **By Kendall Powell**



ANTONIA WEBERLING/BAILEY WEATHERBEE/CARLOS GANTNER/MAGDALENA ZERNICKA-GOETZ

**A human embryo grown in the laboratory for 12 days, showing cells that will form the embryo itself (magenta).**

It was day 13 in a set of experiments in Ali Brivanlou's laboratory and he had an agonizing task ahead. His team of developmental biologists had thawed dozens of human embryos, placed them into individual culture dishes and watched them grow through the earliest stages of development — something that only a handful of researchers worldwide had ever seen. But he knew that it had to end.

The embryos would soon bump up against the 14-day rule, an international consensus that human embryos should be cultured and grown in the lab only until 14 days post-fertilization. Day 14 is roughly when the primitive streak appears, a structure that marks the point at which the embryo sets up the body axes, and begins to distinguish head from tail and left from right.

"It was one of the toughest decisions I've had to make in my life, but it was time to stop this experiment," says Brivanlou, recalling the 2015 research in his lab at the Rockefeller University in New York City. On day 13, the team peeled the embryos from the bottom of the dish and froze them — curtailing any further development. "We didn't know what was going to happen after that point," he says.

Now, Brivanlou and other developmental biologists have the chance to find out.

In May, the International Society for Stem Cell Research (ISSCR) released new guidelines[1] that relaxed the 14-day rule, taking away the hard barrier. Although only a few labs around the world have perfected the techniques needed to culture human embryos up to day 14, the science is advancing rapidly. The relaxed rule allows lab groups, in countries where it is legal, to apply to the regulators for permission to continue research past 14 days. Such studies could reveal what happens during human development after the embryo would normally have implanted in the uterus, about a week after fertilization. It then goes through a stage called gastrulation, roughly between days 14 and 22, when the body's main pattern emerges and the foundations are laid for organ generation.

Cracking open a window on these later stages would allow scientists to better understand the nearly one-third of pregnancy losses and numerous congenital birth defects thought to occur at these points in development. In addition, these stages hold clues to how cells differentiate into tissues and organs, which could boost regenerative medicine.

Most researchers expect that Brivanlou's group and a smattering of others will immediately push the technical boundary of growing human embryos in the lab for longer stretches. The few groups that can do this are in stiff competition. In other places, laws would have to be changed for such research to move forward (see 'Lifting the limit'). Some researchers in China, for example, are discussing whether to change their national policy, which currently reflects the 14-day cut-off, says Tianqing Li, an embryologist at Kunming University of Science and Technology.

So researchers do not expect a flood of new post-14-day studies just yet – nor are they all convinced such experiments are justified. "I'm cautious about using human embryos as a research system in their own right," says Naomi Moris, a developmental biologist at the Francis Crick Institute in London.

As a way of skirting the boundary, researchers have in the past five years developed an array of human embryo models, most of which are formed from mixtures of stem cells. These models mimic multiple, but brief, phases of early development and can be made without using the scarce and ethically fraught human embryos donated by people undergoing *in vitro* fertilization (IVF) treatment. So far, the 14-day rule doesn't apply to these embryo models. But, as they get more sophisticated, with the potential to form recognizable structures, or even organs, they enter their own ethical grey area.

Whether using models or the real thing, scientists say they have a lot to learn. "Embryos are the great masters," says Nicolas Rivron, a stem-cell biologist and embryologist at the Austrian Academy of Science's Institute of Molecular Biotechnology in Vienna. "They are the structures that teach us everything about how we are formed and how we fail."

## Pushing the envelope

The 14-day rule was first proposed in 1979, as IVF technology emerged and human embryos existed for the first time outside the body – although the longest they could survive at the time was a few days. By 2006, when the ISSCR issued its first set of guidelines for human embryonic stem cells (ES cells), the 14-day rule was firmly established in the research community.

The guidelines have been widely adopted around the world by researchers and funders. In several countries, including Germany and Austria, it is illegal to do any research on human embryos, and many others impose a 14-day limit by law, such as the United Kingdom, China, Japan, Australia and Canada. In a few places, including the United States and Israel, there are guidelines but no law prohibiting or limiting human embryo research, although it cannot be federally funded in the United States.

The previous update to the ISSCR guidelines came out in 2016, just before two research groups published breakthroughs.

Magdalena Zernicka-Goetz's lab at the University of Cambridge, UK, began the quest to culture human embryos beyond seven days in 2013. The group wanted to understand what happens beyond the blastocyst, or 'ball of cells', stage. Her group finessed the right recipe of hormones and growth factors, and in 2016 her team[2] and Brivanlou's[3] reported that they had grown human embryos until day 12–13.

"The discoveries we are making, such as where humans put our future head, are of major importance," says Zernicka-Goetz, who now splits her time between her lab in the United Kingdom and one at the California Institute of Technology in Pasadena. "I'm fascinated by the second, third and fourth week of development, which we cannot see with ultrasound, but starts the development of the progenitors for the main organs."

One question concerns how genes are expressed in the embryo's cells as it grows. In one of the largest studies of human embryos so far, Zernicka-Goetz's group analysed 4,820 single cells from 16 embryos developing in the lab from the stage when an embryo would normally implant in the womb (day 5) to the preparations for gastrulation at day 11. Single-cell RNA sequencing revealed which genes switch off and which switch on as embryonic cells transition from totipotency, when they can still become any cell in the body, to pluripotency – a more differentiated state[4].

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Although these developmental signals are known in mice, this study is one of the first to reveal the molecules that underwrite human development.

In other experiments that can be done only with human embryos in culture, both Brivanlou and Zernicka-Goetz and their teams followed the fate of human embryos with aneuploid cells. These are cells that have an abnormal number of chromosomes, a condition thought to cause up to half of early pregnancy losses.

IVF clinics typically test just a few of an embryo's cells to analyse its genetic health. But experiments suggest that this could be misleading. Zernicka-Goetz's group found that embryos diagnosed with some types of aneuploidy go on to develop normally in the lab[5]. Brivanlou's group have analysed gene expression in human embryos from days 3 to 14, and found that cells with abnormal numbers of chromosomes were eliminated – perhaps by developing into supporting tissues or being culled through cell death[6]. Both studies suggest that the testing for aneuploidy commonly done on IVF embryos probably results in many embryos being wrongly deemed 'unhealthy'.

Working with human embryos is essential for these insights, says Brivanlou. "We cannot learn this other than from watching it play out," he says. He is planning experiments – some of which might stretch past day 14 – to try to find out exactly how an embryo containing some aneuploid cells adjusts.

## Beyond two weeks

Researchers working with animal embryos have already cultured them beyond the developmental equivalent of 14 days, which could pave the way for similar advances in human embryo culture. In March, Jacob Hanna's group at the Weizmann Institute of Science in Rehovot, Israel, doubled the time that mouse embryos can be cultured in the laboratory[7], from day 5.5 to day 11 (roughly equivalent to human days 13 to 30). Although other researchers describe the process as finicky, the embryos go well into the process of developing organs.

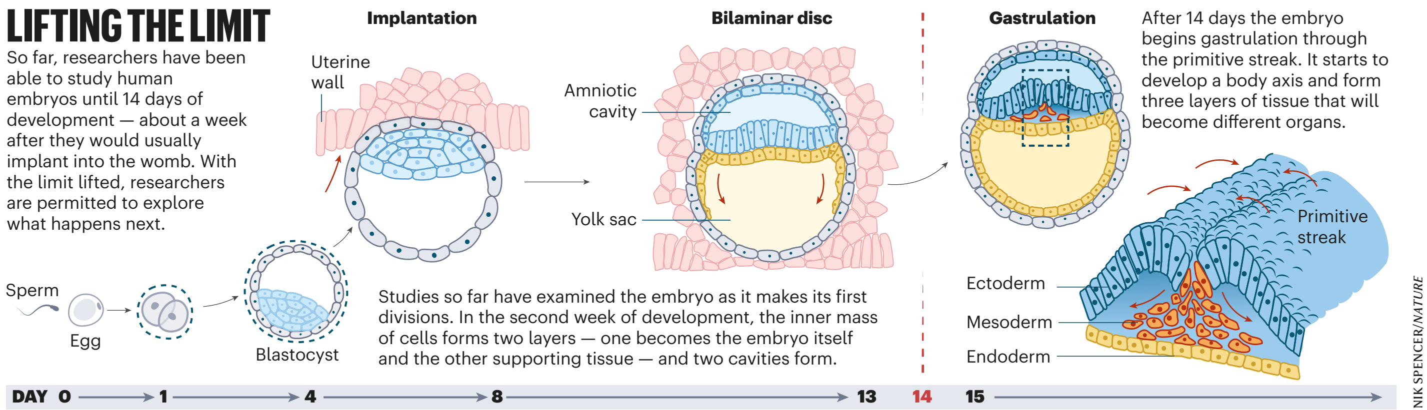
Both Brivanlou and Zernicka-Goetz plan to push the envelope for culturing human embryos further, doing for humans what Hanna's team has done for mice. Brivanlou wants to unlock the genetic program that turns stem cells into the first brain cells, and also wants to reveal the molecular instructions for a four-chambered beating heart. Both events arise after day 14 and unravelling them could prove key to understanding neurodevelopmental disorders and common congenital heart defects.

Brivanlou and others say that, in the future, researchers will be technically capable of watching a human embryo implant into uterine tissue in the lab. His group is applying to his university's regulatory committee to extend their research on human embryos to day 21. In that extra week, the entire body plan is set, including the structures that will become spinal cord, brain, bone, heart, blood, muscles and face. "I don't know if we can push a human embryo past day 14," he says. "But once we get there, we should move very gently."

## Model embryos

Researchers already have some ways of looking at the process of gastrulation in mammals, not by using real embryos, but by constructing

## LIFTING THE LIMIT




models of them from 3D mixtures of stem cells.

In the past five years, researchers have crafted a variety of embryo models in the lab that can be used to glimpse stages beyond day 14. In most cases, these embryo models are not subject to the 14-day rule or any special review.

In 2017, Jianping Fu, a biomedical engineer at University of Michigan in Ann Arbor, and his team made the first human embryo model simply by putting ES cells into 3D culture, where they self-organized to form the amniotic sac and the first signs of a primitive streak[8]. The finding generated a great deal of excitement in the field, Fu says. "That such possibilities exist within human ES cells – they're amazing."

Models like Fu's proliferated, and can now mimic portions of the earliest embryonic stages in mouse and human – implantation[8], gastrulation[9,10], and the rudimentary beginnings of brain[11], spinal cord[12] and heart development. The mixtures of cells often look and behave like embryos at the same stage, but whether they recapitulate the molecular and cellular events of normal development remains a yawning gap in knowledge.

Fu and others acknowledge that, as human embryo models form more complex structures and push further along the developmental timeline, they raise new ethical questions. For instance, their neurons could begin to fire or their heart cells to beat. Or they could acquire the potential to develop beyond the limited stages they mimic so far. Most models lack the full complement of life-supporting tissues that they would need to form a whole embryo, but in the past few years a handful of labs have constructed mouse and human models of the blastocyst stage, called 'blastoids'. These contain the precursors to these support tissues and could theoretically form the entire organism[13–15].

The ISSCR has its eye on the area, and its guidelines state that models that contain these supporting tissues must be subject to special oversight and grown for the minimum time necessary to meet the scientific aim. As they evolve, these models will need ethical reconsideration, says Robin Lovell-Badge, a stem-cell biologist at the Francis Crick Institute who chaired the ISSCR steering committee. "Obviously, this space has to be watched."

As work in real and model embryos moves forward, scientists are keen to know how similar the two really are. Finding out how models differ in their molecular details, and how their cells behave, is the main reason researchers wish to push beyond 14 days in real embryos. "We can learn a lot from a model," says Jesse Veenvliet, a developmental biologist at the Max Planck Institute of Molecular Cell Biology and Genetics in Dresden, Germany. "But it's important to know where it goes wrong."

In 2020, Moris and colleagues ran a side-by-side gene expression comparison of mouse embryos and mouse gastruloids and found striking similarities in the timed waves of signalling that set up the body plan[16]. She says that this exact kind of benchmarking should be done for human gastruloids, too, which would require culturing human embryos up to around day 21. Rivron envisages molecular maps for each stage of human development.

Once enough benchmarking is done, researchers could reduce the number of human embryos used, only turning to them when there is a strong justification.

### Stop the clock

But for just how long should researchers watch human development play out in a dish? Critics have said it was irresponsible for the ISSCR to relax the 14-day rule without giving researchers a new stopping point – that it gives the appearance of a green light for embryo research.

Fu was on the ISSCR committee that developed the new guidelines over 18 months and in more than 100 Zoom meetings. "The science is progressing so fast, it was hard to draw another stop sign as a scientific community," he says. Instead, the guidelines left the door open to do research as long as the appropriate review processes occur.

Brivanlou concurs with the decision. "In my conscience, I know there are great benefits in pushing past 14 days," he says, pointing to the work on abnormal chromosomes. "It may literally save lives in the next generation." He and Lovell-Badge argue that it would be unethical not to allow some post-14-day research because it could unlock how organ cell types arise and how miscarriages and birth defects occur.

It's also becoming fuzzier as to which experiments and models should be accorded the same status as post-14-day embryo research. The ISSCR guidelines draw a clear ethical division, placing only models that include supporting tissues – and that theoretically have the potential to develop fully – in the same category as post-14-day embryos. (The guidelines also ban the transfer of human research embryos, human–animal chimaeric embryos or human embryo models into an animal or human uterus.)

Some researchers are conservative about the lifting of the 14-day line. Moris, for instance, doesn't think that the public has had a real chance to weigh the consequences of post-14-day experimentation.

Bioethicist Josephine Johnston goes one step further: "I think it's a mistake to drop the 14-day rule and not propose another rule." A limit signals that the scientific community understands that society values human embryos and respects that, says Johnston, a bioethicist at the Hastings Center in Garrison, New York. They also need to be upfront about the details of research on embryos, Johnston says. "A lot of this research feels very distant, but it's careless not to have some limits."

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