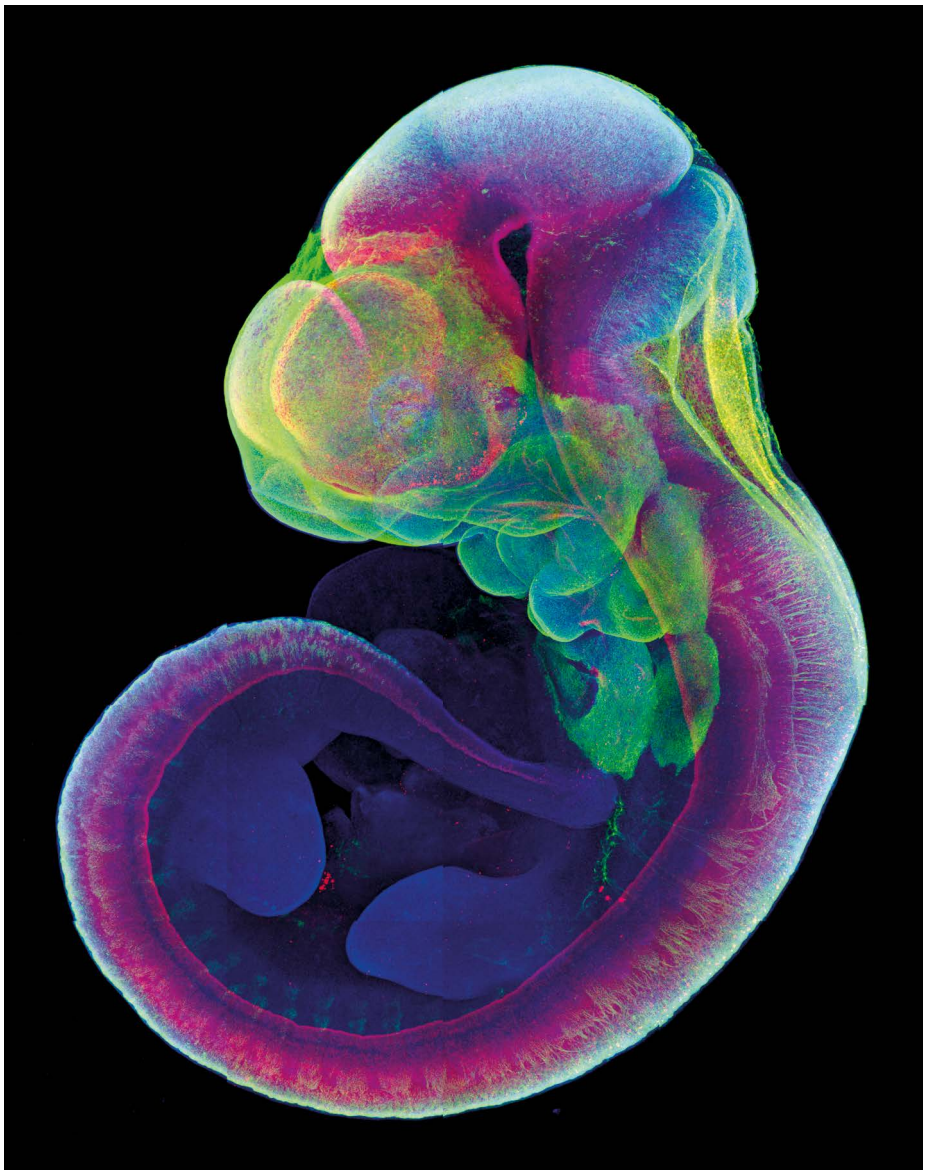


WEIZMANN INST.

A mouse embryo cultured until day 11.

Frontier of development

Keeping human embryos alive in a dish is getting easier. But as the technological and regulatory landscape shifts, fresh ethical questions are coming to the fore. **By Elizabeth Svoboda**

In a laboratory in Israel, an incubator drum spins on a bench. The two glass bottles attached to the drum contain mouse embryos, each the size of a grain of rice, with translucent, pulsing hearts.

Whole mouse embryos have typically been grown *in vitro* for only about 24 hours. But by carefully tuning the mix of chemicals that the mouse embryos are bathed in, a team at the Weizmann Institute of Science in Rehovot, Israel, managed to sustain five-day-old embryos outside the uterus for six more days[1]. This is about one-third of their normal three-week gestation and parallels some events in the first trimester of human embryonic development. Growing human embryos using similar techniques could allow scientists to study processes integral to human development that have long been hidden from view. "This may become the gold standard of looking at human embryonic biology," says Jacob Hanna, a stem-cell biologist and lead researcher on the project at the Weizmann Institute of Science.

This and other recent breakthroughs, such as the creation of human-embryo-like structures from pluripotent stem cells, give scientists an arsenal of tools with which to probe further into early human development. Hanna's drum incubator and these human-embryo models promise to allow more detailed study of processes such as gastrulation – in which three germ-cell layers develop into an array of tissues – and organ formation. Hanna and others say that understanding these crucial embryonic phases is essential to devising therapies that correct developmental errors, as well as to creating transplantable human organs.

But ethical guidelines on human embryo use have halted most research into these phases of development – until now. This May, the International Society for Stem Cell Research (ISSCR) lifted its long-standing rule stating that human embryos should not be cultured past the 14th day post-fertilization. That change, in concert with the flurry of advances, is making scientists confront questions about how far is too far when it comes to growing human embryos in the name of science. The rapid pace of discovery has led both biologists and ethicists to call for a broader public conversation about which techniques are justified given the medical benefits they promise – and which ones threaten long-standing ideas about the moral status of human life.

"This is the beginning, not the end, of controversies," says William Hurlbut, a bioethicist at Stanford University in California and a member of the President's Council on Bioethics during George W. Bush's presidency of the United States. "Unless we get hold of these questions, unless we ponder them, we're not going to be able to contend with the future as it unfolds."

A spate of breakthroughs

Researchers have long referred to the first several weeks of human development as a black box, because they could not typically study what was taking place inside the uterus without disrupting the embryo's growth. "After implantation, you really have a hard time seeing what is happening," says Matthias Lütolf,

a biomedical engineer at the Swiss Federal Institute of Technology in Lausanne. Without a way to grow embryos for extended periods outside the uterus, the details of this stage of development would remain a mystery.

Hanna's incubator drum represents an important step towards resolving the problem. His innovation was the product of several years of trial and error. "There was no eureka moment," Hanna says. "It was just painful optimizations." To keep the mouse embryos alive *in vitro*, Hanna's team tweaked inputs to the surrounding environment – including human umbilical cord blood serum, glucose and a flow of oxygen bubbles – until the embryos were able to survive for one day, then two days, then six, up to the point of organ formation.

Around the time Hanna's incubator research went public, scientists at two other institutions – Monash University in Melbourne, Australia, and the University of Texas Southwestern Medical Center in Dallas – reported successfully generating human-embryo models, known as blastoids, from lines of stem cells[2,3].

Although these blastoids, made of about 100–400 cells, are incapable of developing into fully fledged humans, their growth is strikingly similar to that of natural embryos. Some blastoids even adhered to the glass dishes they were growing in, as if burrowing into the lining of the uterus. So far, the blastoids have been kept viable in the lab for only a few days, although that interval could lengthen in continuing trials.

The blastoid experiments show that human stem cells can generate multicellular structures that have many of the same features as natural embryonic tissue. Because these cells self-organize and differentiate much as embryonic cells do, blastoids could provide a window into processes such as gastrulation that are normally hard to access, Lütolf says. Unlike real human embryos, which are donated by people undergoing fertility treatment and are therefore in short supply, these artificial embryo models can be grown in large numbers, allowing many more experiments to take place.

Other ways of growing near-human embryonic structures are also gaining traction. In April, an international team of researchers reported injecting human pluripotent stem cells into six-day-old monkey embryos, creating hybrid embryos known as chimaeras[4]. Although none of the chimaeras survived for more than 20 days, the experiment supplied proof of the concept that large numbers of human cells can be kept alive for more than two weeks in a primate embryo. (In older studies of chimaeras made with pig or sheep embryos, a vanishingly small percentage of human cells survived.) If the human–monkey chimaeras could be kept alive for a longer time, they could be used to grow transplantable human organs in a non-human substrate – although the use of other host species, such as pigs, is also being explored (see page S12).

As embryonic models and cultivation methods become more refined, scientists are using genetic analysis to zero in on early developmental events with greater precision. In June, a team of researchers at the University of Cambridge, UK, reported sequencing the RNA of embryos left over from *in vitro* fertilization (IVF) at 9 and 11 days post-fertilization[5]. The researchers also identified key signalling processes between a layer of cells bordering the embryo, known as the hypoblast, and the developing embryo itself.

"This may become the gold standard of looking at human embryonic biology."

These signals, the team reported, determine which cells will become the embryo's head and tail – a differentiation event that lays the groundwork for intricate developmental sequences that follow. Molecular biologist Marta Shahbazi, part of the Cambridge team that worked on the study, now wants to investigate related processes that occur further along in embryonic development. "At gastrulation, so many important things are going on," she says. Observing these transitions could provide clues about why some early human embryos stop growing.

Treatment implications

Much of the excitement surrounding such discoveries stems from their potential to upend the medical landscape. One goal now within reach, says Jianping Fu, a biomedical engineer at the University of Michigan in Ann Arbor, is to create human-embryo models that represent moments of transition through a key stretch of human development – from pre-implantation, implantation and gastrulation, all the way to early organogenesis. Developing accurate embryonic models of gastrulation and organogenesis, however, will probably require researchers to grow natural embryos beyond 14 days – one factor that motivated the ISSCR to revise the limit (see *Nature* **594**, 18–19; 2021).

Studying embryos or embryonic models past the 14-day mark, perhaps inside an incubator system similar to Hanna's, would enable researchers to improve their understanding of the origins and progression of conditions that arise from genetic or developmental mutations. These might include β-thalassaemia, which reduces the blood's ability to carry oxygen, or spina bifida, in which the developing spinal bones do not fuse together in the typical way during neurulation – the developmental process that follows gastrulation.

That knowledge of gastrulation and later processes, in turn, could allow scientists to develop and test targeted therapies that reverse these defects. Early-stage embryos or embryonic models with β-thalassaemia, for instance, could be modified with the CRISPR–Cas9 gene-editing technique, then placed into an incubator system where researchers could monitor the impact of their intervention for weeks at a time. In a future treatment setting, the edited embryos could be returned to the uterus to continue developing. The US National Academy of Sciences has expressed cautious support for clinical trials that use CRISPR to edit embryos when the purpose is to treat serious disease, provided that trials are done under strict oversight.

Researchers say that studying post-fertilization development will also help them to pin down causes of early pregnancy loss, a phenomenon currently shrouded in mystery. During pregnancy, the developing embryo initiates a cascade of signalling processes, and Shahbazi's embryo experiments show that disruptions in this molecular crosstalk underlie at least some early miscarriages[6]. Her team studied embryos that had three copies of chromosome 16, a common cause of miscarriage. "We found a defect that was specific to the tissue that will make the placenta," Shahbazi says, which allowed them to identify molecular processes that could be responsible. By studying signalling processes beyond the 14-day limit, Shahbazi and her colleagues hope to find more mechanisms driving pregnancy loss, and ultimately correct some of them.

In the pharmaceutical realm, human-embryo models might be suitable for drug screening trials, in which researchers could identify medications' toxic effects on development without having to test the drugs on natural embryos. Even further down the line, non-viable human-embryo models – such as blastoids or animal–human chimaeras – could be used to grow entire human organs for transplant, which would require months of development. Reaching these ambitious goals will involve further study of both real human embryos and embryonic models long after the original 14-day limit, Hanna says. "There's a big gap in knowledge. Filling that gap is critical to advance stem-cell research and organ regeneration."

Ethical wrestling

The medical revolution seemingly at hand was an important factor motivating the ISSCR's decision to lift its 14-day limit on growing

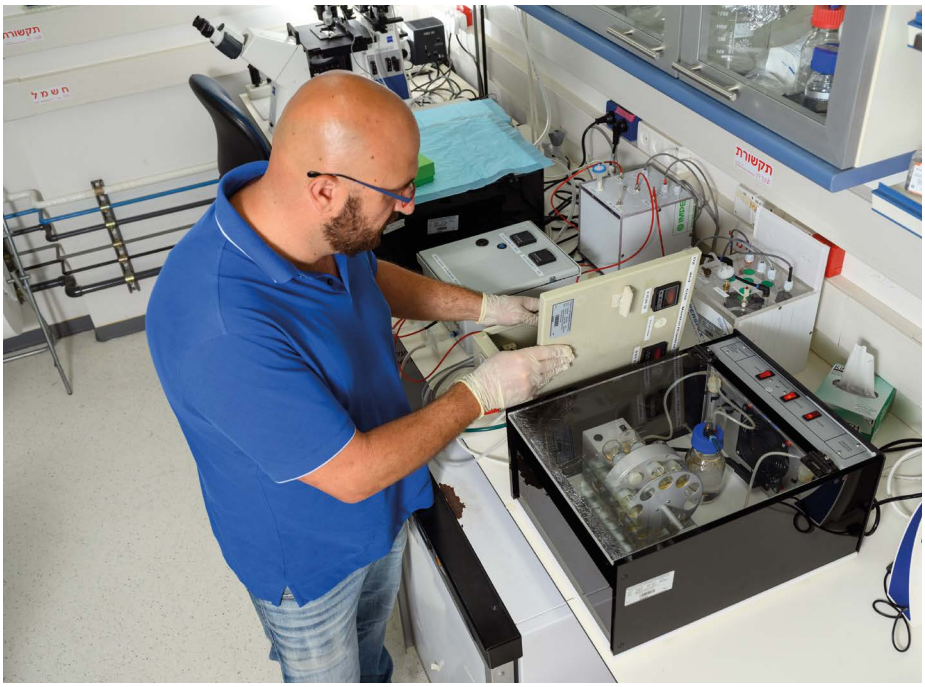
human embryos. In part because the science is so fluid and ever-changing, ISSCR officials opted not to replace the 14-day guideline with another specific limit. Instead, the society proposes an ethical review in which each research proposal "should be judged individually, on whether the research is justifiable in terms of the value of the information obtained", wrote stem-cell biologist Robin Lovell-Badge, who chaired the recent ISSCR task force, in *Nature* (see *Nature* **593**, 479; 2021). The reasoning is that the longer embryos are grown for, the stronger the justification will have to be for growing them.

Hurlbut, however, thinks the ISSCR made a mistake in scrapping its original rules without creating clear replacement guidelines. He sees human-embryo studies past the 14-day limit as a political powder keg, and urges scientists to seek less fraught ways to explore early development. He and others pushed for the creation of pluripotent stem cell lines not derived from embryos. "We should be able to do these studies, at least at a very good approximation, without creating embryos," Hurlbut says. "We treat living human beings after they're born as having a distinct inviolability. Now we're talking about a few months before that — we can't just say it's nothing."

Some biologists, however, argue that scientists must first directly study human embryos to be able to create models that accurately depict early developmental stages. "We want to build alternative systems, to not have to use real embryos," Lütolf says, "but at the same time, we still need to know how the real thing behaves." Hanna agrees, adding that mouse embryos cannot take the place of human embryos in most cases because they develop differently after gastrulation.

Complicating the issue further, experiments on non-embryos — such as blastoids or animal–human chimaeras — come with their own ethical baggage. Although these structures might not have the potential to develop into humans, their likeness to human embryos has prompted Fu and others to call for careful oversight of how they are used in the lab. Questions have also been raised about how closely embryo-like structures must resemble natural embryos before these structures, too, are considered human. For now, the ISSCR states that if a human-embryo model represents "the integrated development of the entire embryo", rather than only a part of the embryo, it should be subject to the same detailed review process as natural embryos before it can be studied past the 14-day stage.

Fu, Hurlbut and others say that future discussions about the moral precepts guiding research on early development should involve not just the international scientific community, but society at large. "We need to make sure that we remain transparent," Fu says. "We should always have public conversations on the scientific significance of such research, as well as on the societal and ethical issues."



WEIZMANN INST.

Stem-cell biologist Jacob Hanna adjusts a custom-made system for culturing embryos.

An unsettled future

On top of the ethical hurdles, there are practical and financial obstacles to overcome. Right now, Fu says, scientists in the United States are unsure whether the National Institutes of Health will maintain its current reluctance to fund most embryo experiments, given this year's update to ISSCR rules. The situation is similar in Europe, where prohibitions on funding embryo research past 14 days still stand in many countries, despite the ISSCR's guideline changes. Some countries, such as Germany, Austria and Russia, continue to forbid any experimentation on human embryos at all.

This uncertainty has prompted researchers around the world to call for more clarity from funding agencies. "Science is moving so fast in this particular area that regulations are always lagging behind," Lütolf says. Yet the regulations that will govern funding decisions going forwards are shaped in a political and legislative context. That makes it all the more crucial, Hurlbut says, to educate the public as much as possible about the basic biology behind embryo cultivation techniques, as well as about the medical innovations these techniques could make possible.

In the current unsettled climate, most researchers are focusing on immediate next steps out of necessity. Hanna is seeking approval from Israeli oversight boards to grow unused IVF embryos in his incubator drum for up to 40 days past fertilization. "These are embryos that are going to be disposed [of] anyway, because they've been frozen for a very long time," he says.

Even if oversight authorities grant Hanna's request, he and other researchers will face continued backlash from critics who cite the ethical repercussions of growing human embryos past the 14-day mark. "I'm concerned about where it's going," Hurlbut says. "I think this is going to affect the character of our culture." But in Hanna's view, the potential rewards, such as being able to fix embryonic mutations so that newborns can live long and healthy lives, provide ample justification for orchestrating human development on his lab bench. "We're not dismissing the ethical issues," he says. "Is there sufficient benefit to take a look? I think, very much."

Elizabeth Svoboda is a science writer in San Jose, California.

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