

COMMENT

SOCIETY How water has shaped the history of south Asia **p.186**

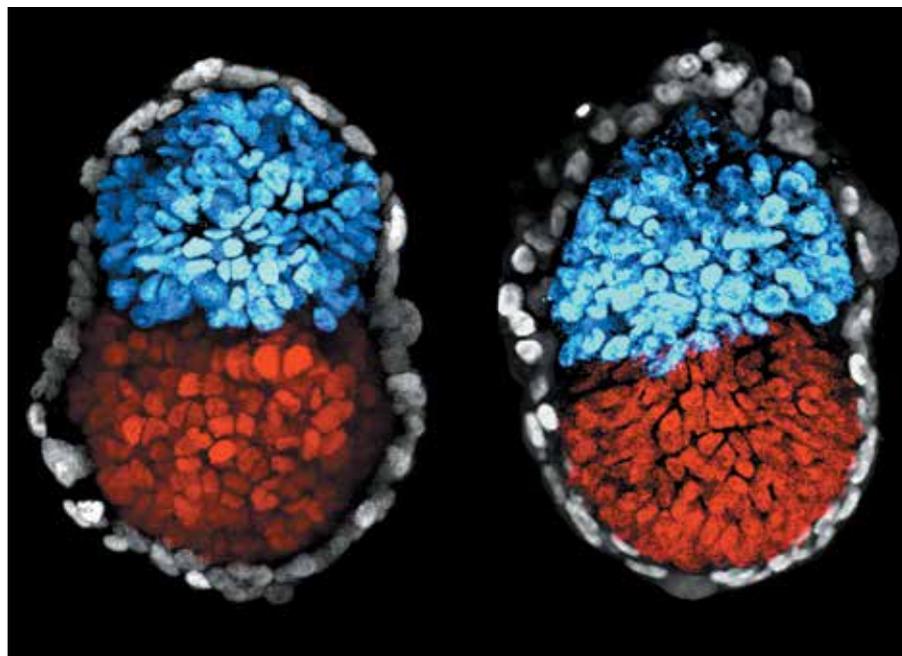


DEXTERITY A chef, a musician and a chemist walk into a conference bar... **p.188**

CRISPR TWINS Gene-therapy regulation offers good model **p.190**

DIVERSITY Redress disparities in NIH funding between institutions **p.190**

B. SOZEN ET AL./NATURE CELL BIOL.



The cells of a 4-day-old artificial embryo (left) resemble those of a 5.5-day-old mouse embryo (right).

Debate ethics of embryo models from stem cells

International discussion must guide research, urge
Nicolas Rivron, Martin Pera and colleagues.

Over the past five years, various studies have shown that mouse and human stem cells can spontaneously organize in a dish into 3D structures that are increasingly similar to mouse^{1–5} or human^{6–8} embryos. All that is needed is the right number and combination of cells, growth factors and, sometimes, a means of physically confining the cells, such as in microwells⁸.

In the past 18 months, researchers have taken a significant step forward, using mouse models. They have incorporated tissues into the models that resemble those that become

the yolk sac and placenta. In mammals, these ‘extra-embryonic organs’^{1,4,5} grow in synergy with the embryo, mediate its implantation and form the interface with the mother.

In short, it now seems feasible that stem cells can be developed into models that are almost indistinguishable from embryos in the lab. Such models can also be transferred into the womb of a mouse¹, where they begin to implant.

These models open up all sorts of possibilities in research. Studying mouse and human embryogenesis in the lab could lead to

better infertility treatments or contraceptives, more-effective and safer *in vitro* fertilization (IVF) procedures, the prevention and treatment of developmental disorders and even the creation of organs for people who need a transplant (see ‘Why model embryos?’).

These models also raise profound ethical questions. What should their legal and ethical status be now, and in the future as they are refined? Do the probable insights these embryo models provide outweigh possible ethical concerns? Because of the potential benefits, is there now a moral imperative to develop this research?

In 2015, various commentators, including four of us (M.P., M.M., G.deW. and W.D.) flagged the potential ethical implications of developing embryo models from stem cells⁹. At the time, investigators had modelled only a short span of development. No precursors of the extra-embryonic tissues had been generated.

Given the pace of progress, we now think that a major international discussion is needed to help guide this research.

NEW AVENUES

So far, biologists have produced four different types of ‘embryo model’: three in mice^{1,3,5} and one using human cells⁷ (see ‘What’s been modelled?’ and ‘Model systems’). All of the models stop developing after a few days, and the extent to which their gene-expression patterns match those of natural embryos has yet to be rigorously assessed^{1,3,5}. Even with these limitations — which are likely to be overcome in the future — stem-cell models open up new avenues for exploring human development and disease.

The first few weeks of development are crucial to the success of a pregnancy and the health of a child¹⁰. But little is known about how the human embryo forms, implants and develops in the days that follow. Embryos can be observed using ultrasound only after about five weeks. And there are strict regulatory constraints on researchers’ ability to manipulate human embryos experimentally.

What is known about this period in human development comes mainly from three lines of research. These are: studies of embryos formed through IVF, including of blastocysts cultured in the laboratory for up to 13 days^{11,12}; a small number of archival specimens of human embryos obtained decades ago through surgery and other procedures ▶

▶ that would now be considered unethical in most countries (see go.nature.com/2sufgov); and a few comparative studies on closely related primate species, such as cynomolgus monkeys (*Macaca fascicularis*)¹³.

Unlike embryos formed through the fusion of a sperm and an egg, model embryos can be generated in large numbers and tweaked, for instance by using gene editing. This means that they can be used in high-throughput genetic tests and drug screens — procedures that generally form the basis of therapeutic discoveries.

Biologists can also use model embryos to uncover basic principles. For instance, it is well known that the placenta supports and instructs the embryo's development. Yet a study this year¹ showed that, in early embryos (blastocysts), the embryo guides the formation and implantation of the future placenta. (That work was led by one of us (N.R.), building on previous observations¹⁴.)

We think that stem-cell-based models could transform medicine in at least five ways (see 'Why model embryos?'). Done properly, studies on embryo models could even obviate some of the ethical conflicts surrounding research on human development: researchers would have less need to study embryos from people or other primates.

FOUR QUESTIONS

Future progress depends on addressing now the ethical and policy issues that could arise.

Ultimately, individual jurisdictions will need to formulate their own policies and

regulations, reflecting their values and priorities. However, we urge funding bodies, along with scientific and medical societies, to start an international discussion as a first step. Bioethicists, scientists, clinicians, legal and regulatory specialists, patient advocates and other citizens could offer at least some consensus on an appropriate trajectory for the field.

Two outputs are needed. First, guidelines for researchers; second, a reliable source of information about the current state of the research, its possible trajectory, its potential medical benefits and the key ethical and policy issues it raises. Both guidelines and information should be disseminated to journalists, ethics committees, regulatory bodies and policymakers.

Four questions in particular need attention.

Should embryo models be treated legally and ethically as human embryos, now or in the future?

If the majority view is 'no', biologists could use stem-cell-based models both in basic research and in preclinical applications, unfettered by current legislation or guidelines on human-embryo research. If most stakeholders lean towards 'yes', work involving these models would be permitted in countries that allow the creation of human embryos for research, such as the United Kingdom — subject to the usual ethical and legal restrictions.

Answering this question could require testing whether these entities are capable of developing to term, but such experiments

would themselves raise ethical questions. Moreover, the worldwide ban on human reproductive cloning would prevent such a test from being conducted on models formed from induced pluripotent stem cells.

In practice, different models might need to be treated in different ways. For example, it is unlikely that current post-implantation models could ever develop fully into an organism. They mirror only some regions of the embryo, and skip over the developmental stage that normally occurs when it implants in the uterus. Complicating matters, researchers might be able to constrain or enhance the developmental capacity of a particular model using gene editing — such as by incorporating suicide genes that destroy the tissue at a certain point. In other words, what might be considered an embryo could be flipped by genetic means into a non-embryo, and vice versa.

Which research applications involving human embryo models are ethically acceptable?

Most would agree that research into the origin of infertility and genetic diseases, for example, is a worthy goal and probably achievable within current ethical boundaries. Conversely, the use of human embryo models for reproduction is much harder to justify. Such applications are a long way off, but one day it might be feasible to transfer an embryo created from (genetically edited) stem cells to a woman's uterus to treat infertility or circumvent genetic diseases. Most — including the International Society for Stem Cell Research (ISSCR) — rightly argue that it is not morally acceptable to create humans in this way, even setting aside the considerable uncertainty regarding the healthy outcome of a stem-cell-derived pregnancy.

How far should attempts to develop an intact human embryo in a dish be allowed to proceed?

The response to this will depend on the answer to our first question. If human-embryo models are deemed equivalent to human embryos, they will become part of an ongoing debate on the time limits on culturing embryos. In more than 20 countries, it is against the law for researchers to maintain intact human embryos in the laboratory past 14 days of development or beyond the initiation of gastrulation (when three different cell layers appear) — whichever comes first¹².

Does a modelled part of a human embryo have an ethical and legal status similar to that of a complete embryo?

At the moment, the following are not deemed biologically equivalent to a whole embryo: tissues sampled from embryos for diagnostic purposes; embryonic stem cells; and extra-embryonic stem cells. But it is unclear at which point a partial model contains enough

WHY MODEL EMBRYOS?

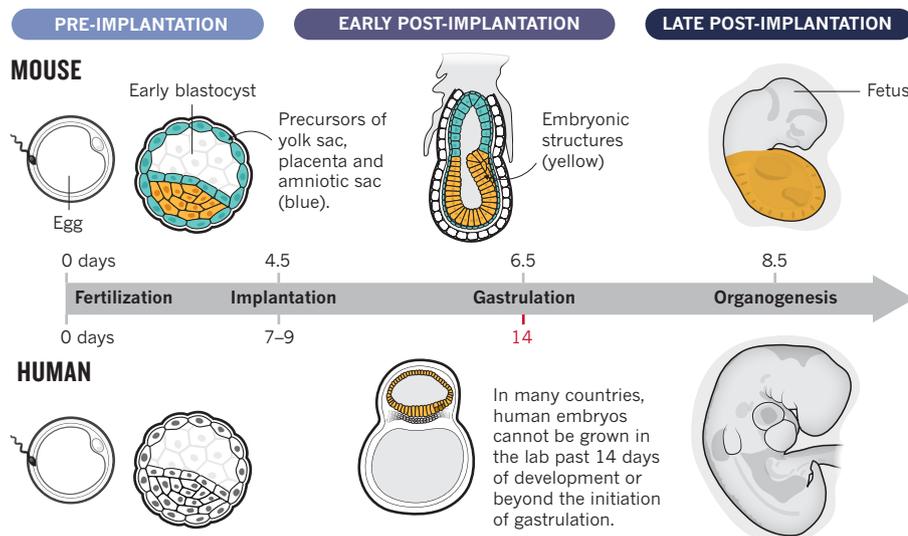
Five ways in which embryo models could improve health

- **Treating infertility.** Embryo models could give researchers a better understanding of implantation and gastrulation, and lead to better infertility treatments. (It is thought that at least 40% of pregnancies fail by 20 weeks, and that 70% of those that fail do so at implantation¹⁵.)
- **Improving IVF.** Only around 20% of IVF procedures result in a birth¹⁶. Using stem-cell models, researchers could optimize implantation and minimize cellular abnormalities, such as an aberrant number of chromosomes. As well as safeguarding the health of children conceived *in vitro*, this could reduce the number of procedures.
- **Designing new contraceptives.** Embryo-model work could improve drugs that prevent implantation (as the oral contraceptive pill or intrauterine devices do, in part). Women and health professionals need drugs and devices that are easier to use and that have fewer side effects. Family planning is central to sustainable, global development (see go.nature.com/2rdqpwv).

- **Preventing disease.** Subtle cell abnormalities during the first weeks of pregnancy, such as those caused by the use of alcohol or medications, can do damage throughout pregnancy and beyond¹⁷. They can alter development of the placenta and restrict embryo growth, affecting the baby's birth weight and propensity for chronic diseases (such as those of the heart) decades later¹⁸. Entities based on stem cells could help researchers to pinpoint the genetic and epigenetic changes involved¹⁸, and assess the effects of diets or drugs^{10,16}.
- **Creating organs.** Mini brains, livers, kidneys and other organoids made from stem cells are highly simplified. Initiating organ development in an environment as similar as possible to the developing embryo might enable researchers to reliably generate structures that more closely resemble mature, functional organs, for drug screens or even for transplantation. *N.R., M.P. et al.*

WHAT'S BEEN MODELLED?

Using stem cells, researchers have produced entities in the lab that mimic certain structures found in the mouse embryo (coloured parts). Work with human stem cells is less advanced.



MODEL SYSTEMS

How stem cells are used to study embryo development

Mouse stem cells can form 3D structures that resemble the 3.5-day-old mouse embryo (the blastocyst) before it implants in the uterus. These ‘blastoids’ contain analogues of the three cell lineages thought to form the embryo, placenta and yolk sac. Blastoids implanted into female mice trigger a uterine response. Currently, development stops shortly after implantation¹.

Mouse stem cells can also form entities that are similar to specific regions of the 6.5–8-day-old mouse embryo after it has implanted in the uterus^{2–5}. A process called gastrulation, during which the body plan is established, occurs in these regions.

These models are termed ETS/X embryo-like structures^{4,5} and gastruloids^{2,3}. In the

first type, some interactions between the embryonic and extra-embryonic tissues are repeated^{4,5}, the anterior–posterior body axis is laid down and analogues of gastrulating cells are generated⁵. In gastruloids, the three basic germ layers are laid down^{2,3} and the precursors of organs develop³.

Work with human stem cells is less advanced, but is on a similar trajectory. Currently, human stem cells can model aspects of gastrulation¹⁹ and the formation of the beginnings of the amniotic cavity²⁰. They can also form 3D asymmetric cysts that model the development of the epiblast–amniotic ectoderm axis^{6,7}. As far as we know, this structure arises during the second week, soon after implantation. *N.R., M.P. et al.*

material to ethically represent the whole, so this must also be discussed by regulators.

FOUR RECOMMENDATIONS

These are complex questions, and discussions about all these issues and others will need to be regularly revisited as the field evolves. The pace of progress, however, prompts us to recommend the following.

First, we think that the intention of the research should be considered the key ethical criterion by regulators, rather than surrogate measures of the equivalence between the human embryo and a model. This was the approach taken with cloning. In the late 1990s and early 2000s, many nations prohibited human reproductive cloning, but did not ban the transfer of nuclear material

from a somatic cell to an egg to produce a blastocyst and generate lines of stem cells. Here, the key consideration was the intention of the study rather than whether the clone was equivalent to a natural embryo.

Second, we urge regulators to ban the use of stem-cell-based entities for reproductive purposes.

Third, in our view, current stem-cell models that are designed to replicate only a restricted part of development, or that form just a few anatomical structures, should not have the ethical status of embryos.

Finally, we urge any scientist using human stem cells for research to abide by existing guidelines, such as those of the ISSCR. They should send their research proposals to a stem-cell oversight committee or a local

independent ethical review board before undertaking any studies, submit their results to peer review and publicize their findings.

As part of ensuring good practice, stem-cell researchers, developmental biologists, human embryologists and others need to reach consensus on what terminology accurately captures the properties of the different models. (Currently, several terms are used interchangeably to describe the various types.) Ideally, terms should reflect the cellular composition and tissue organization of each, and indicate their developmental stage and potential.

Such provisions will help to ensure that this research is conducted ethically. Crucially, the recommendations will also help citizens to understand what researchers are doing, and why. Transparency and effective engagement with the public is essential to ensure that promising avenues for research proceed with due caution, especially given the complexity of the science. ■

Nicolas Rivron leads the laboratory for synthetic embryology both at the MERLN Institute, Maastricht University, Maastricht; and at the Hubrecht Institute (KNAW), Utrecht, the Netherlands. **Martin Pera** is a professor at the Jackson Laboratory, Bar Harbor, Maine, USA. **Janet Rossant**, **Alfonso Martinez Arias**, **Magdalena Zernicka-Goetz**, **Jianping Fu**, **Susanne van den Brink**, **Annelien Bredenoord**, **Wybo Dondorp**, **Guido de Wert**, **Insoo Hyun**, **Megan Munsie**, **Rosario Isasi**.
e-mails: n.rivron@maastrichtuniversity.nl; martin.pera@jax.org

- Rivron, N. C. *et al. Nature* **557**, 106–111 (2018).
- van den Brink, S. C. *et al. Development* **141**, 4231–4242 (2014).
- Beccari, L. *et al. Nature* **562**, 272–276 (2018).
- Harrison, S. E., Sozen, B., Christodoulou, N., Kyprianou, C. & Zernicka-Goetz, M. *Science* **356**, eaal1810 (2017).
- Sozen, B. *et al. Nature Cell Biol.* **20**, 979–989 (2018).
- Shao, Y. *et al. Nature Mater.* **16**, 419–425 (2016).
- Shao, Y. *et al. Nature Commun.* **8**, 208 (2017).
- Shahbazi, M. N. & Zernicka-Goetz, M. *Nature Cell Biol.* **20**, 878–887 (2018).
- Pera, M. F. *et al. Nature Meth.* **12**, 917–919 (2015).
- Bianco-Miotto, T., Craig, J. M., Gasser, Y. P., Van Dijk, S. J. & Ozanne, S. E. *J. Dev. Orig. Health Dis.* **8**, 513–519 (2017).
- Deglicert, A. *et al. Nature* **533**, 251–254 (2016).
- Hyun, I., Wilkerson, A. & Johnston, J. *Nature* **533**, 169–171 (2016).
- Nakamura, T. *et al. Nature* **537**, 57–62 (2016).
- Gardner, R. L. & Johnson, M. H. *J. Embryol. Exp. Morphol.* **28**, 279–312 (1972).
- Norwitz, E. R., Schust, D. J. & Fisher, S. J. *N. Engl. J. Med.* **345**, 1400–1408 (2001).
- Chen, M. & Heilbronn, L. K. *J. Dev. Orig. Health Dis.* **8**, 388–402 (2017).
- Cha, J., Sun, X. & Dey, S. K. *Nature Med.* **18**, 1754–1767 (2012).
- Burton, G. J., Fowden, A. L. & Thornburg, K. L. *Physiol. Rev.* **96**, 1509–1565 (2016).
- Warmflash, A., Sorre, B., Etoc, F., Siggia, E. D. & Brivanlou, A. H. *Nature Meth.* **11**, 847–854 (2014).
- Taniguchi, K. *et al. Stem Cell Rep.* **5**, 954–962 (2015).

Full affiliations for all authors are listed online (see go.nature.com/2sxyqdt).