Human development

Stem cells model a two-week-old human embryo

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Researchers have used stem cells to create models that resemble human embryos at two weeks old, but bypass the earliest developmental stages — paving the way for studies that are not possible in human embryos. See p.562, 574 & 584

Ask a person on the street what a 13-day-old human embryo looks like, and you might hear a broad range of answers. Some might imagine a formless clump of cells, others a fetus-in-miniature with recognizable features such as limbs, eyes or a heart. In truth, the architecture of the two-week-old embryo is more like a series of nested cellular bubbles, or fluid-filled cavities, that support the development of a relatively simple cell layer that will eventually give rise to a fetus. Similar structures have been made entirely from stem cells, as reported in *Nature* by Oldak *et al.*[1] (page 562), by Pedroza *et al.*[2] (page 574) and Weatherbee *et al.*[3] (page 584).

The textbook anatomy of the human embryo has been painstakingly sketched out by researchers hoping to unlock the secrets of how humans are formed, using donated material that is both rare and valuable. But this work is challenging, not least because of the ethical limitations and technical obstacles. Currently, scientists lack dynamic information about how cells communicate and how gene expression changes during the stages of development. This is mainly because human embryos — donated from *in vitro* fertilization (IVF) or formed from donated sperm and eggs — can be grown in the laboratory only for 14 days[4]. Reaching this cut-off is itself challenging because the human embryo nestles into the uterus between days 7 and 10, and it is difficult to mimic this implantation in the lab. For stages beyond 14 days, scientists must rely on donated material from pregnancies terminated in the earliest stages, which is extremely difficult to access.

This is where the burgeoning field of stem-cell-based embryo models comes in. The models are sometimes incorrectly given the moniker 'synthetic embryos' — there is nothing synthetic about them, and nor are they embryos. Instead, they rely on the remarkable, but natural, potential of stem cells to give rise to any cell type in the body — a feature known as pluripotency. Pluripotent stem cells can form an entire embryo, as long as they are placed in the correct environment. And that's the sticking point. Although evidence has shown conclusively that mouse pluripotent stem cells injected into an early embryo and put back into a mouse can make a mouse that survives after birth[5,6], researchers are only beginning to scratch the surface of how to get stem cells to undergo the same process in a dish.

The latest work builds on several years of research across multiple labs that are all actively seeking the perfect conditions to support stem cells in their natural goal of generating an embryo. For this to happen, the stem cells need to exist at the right starting point along the developmental path so that they can differentiate into specific cell types, including supporting cells that are essential for the formation of tissues such as the placenta.

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Researchers also need to concoct the correct conditions, using the appropriate medium and methods, to grow the structures. But once those two requirements are met, the hard work is done by the cells themselves.

The three teams that created the latest human-embryo models used 'naive' human stem cells, which are reset to resemble the earliest stages of pluripotency[7]. Pedroza *et al.* aggregated these cells under specific chemical conditions to allow them to spontaneously become other cell types. Weatherbee *et al.* forced higher than normal expression of a particular set of genes so that cells were biased towards becoming the different cell types, before aggregating the cells to allow an embryo-like structure to form. Oldak *et al.* identified chemical cocktails that allowed the naive cells to assume the identity of two supporting cell types, before combining these with naive cells in defined ratios.

In the model by Weatherbee *et al.* (Fig. 1a), the cells at the centre of the structure formed a small ring of epithelial cells (which sit tightly side by side) that resemble the layer of cells that form the embryo proper (which goes on to form the fetus), surrounded by supporting cells. The authors noticed that the outermost supporting cell layer in their model was not equivalent to that of an actual embryo, but it still had some of the same functions.

Likewise, Pedroza and colleagues' model (Fig. 1b) had a ring of cells made up of cell types that resemble the embryo proper, and frequently formed an organized neighbouring population called the amnion that would, in an embryo, later form the amniotic sac. Their model also had other types of supporting cell, but no trophoblast — a cell layer found on the outside of actual embryos that would normally give rise to the embryonic portion of the placenta. By contrast, in the model by Oldak *et al.* (Fig. 1c), the cells rearranged so that the populations on the outer surface resemble the trophoblast, and cells on the inside even formed organized cavities that look similar to those of an actual human embryo.

The authors of all three papers then characterized the gene and protein expressions of the resulting structures. All three groups saw evidence of key populations of cells, including mesoderm (the precursor to muscle and bone tissues). Weatherbee *et al.* and Oldak *et al.* observed the precursors of germ cells (cells that give rise to sperm or eggs). Oldak and colleagues' model also contained cells resembling early blood progenitor cells. The work described in all three papers is undoubtedly a remarkable technical achievement, and the stem-cell model from Oldak *et al.* arguably leads the field as the most embryo-like in terms of its structure, specifically at post-implantation stages.

But there are several difficulties to overcome in future work. First, the rate of successful generation of these models is vanishingly low, at just 1–2% in the case of Oldak and colleagues' system. This must improve before the models can be used to explore mechanisms of human development, let alone for efficient screening for chemicals or genetic perturbations.

Second, the three models — like others described this year[8–10] — have different compositions of supporting cells, ranging from just one or two cell types to several well-organized structures that closely resemble those seen in human embryos. In 2021, the International Society for Stem Cell Research (ISSCR) proposed guidelines for research that distinguish between integrated models (those that include supporting cells) and non-integrated models (those that don't include supporting cells)[11]. However, this distinction becomes problematic because the latest models lie somewhere on a spectrum, each having varying

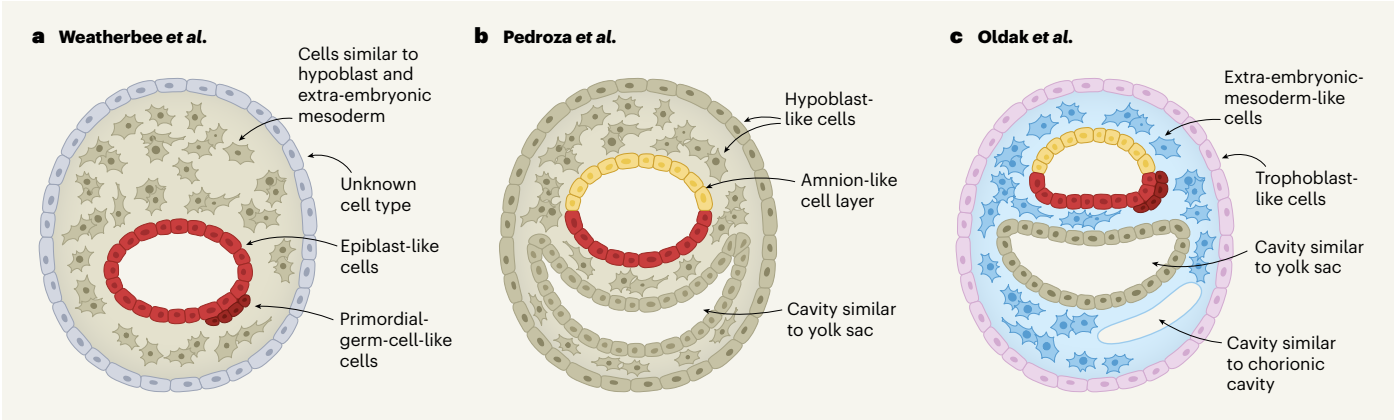

Figure 1 | Stem-cell-based models of a post-implantation human embryo. Human-embryo models were created by Weatherbee *et al.*[3] (**a**), Pedroza *et al.*[2] (**b**) and Oldak *et al.*[1] (**c**) from naive human stem cells, which, under the correct conditions, self-organize to form structures with features that resemble those of a real embryo at days 13 to 14 (not shown). Each model contains a layer of cells that is similar to the epiblast (which will form the embryo proper and, later, the fetus), and two of the models (**a**, **c**) contain cells that resemble the primordial germ cells (which will form sperm or eggs). The three models have various representations of supporting cell layers, such as the amnion (which will form the amniotic sac), hypoblast (which surrounds the yolk sac), extra-embryonic mesoderm (which forms the chorionic cavity that later surrounds the growing embryo) and trophoblast (which will form part of the placenta). Not all features of the models are shown, and some features vary within each model. Illustrations were adapted from individual micrographs from Fig. 3h of ref. 3, Fig. 3e of ref. 2 and Fig. 4g of ref. 1.

representations of the different supporting cell populations. In the human embryo, these cell types exist in the correct proportions, have the correct gene signature, show structural characteristics and perform specific functions. This is not always the case in embryo models, even when such cell types are deliberately added.

Third, it is important to remember that the models completely circumnavigate the way in which the human embryo is established. No sperm or egg cells are used, no fertilization takes place, there is no formation of a blastocyst (the hollow ball of cells that forms 5–6 days post-fertilization) and there is no early implantation. Instead, the stem cells skip straight to post-implantation stages of development, completely bypassing the earliest stages. This means that the embryo models are unlikely to shed any light on events occurring at the earliest stages of development, including the first interactions between the embryo and the uterine tissue.

The big question for many people will be, 'What next?'. Can these models be cajoled to develop to the next stages of development, in which the sculpting of the body plan begins? Could they include a beating heart, which forms on day 21–23 of human embryo development, or a spinal cord, which is finalized around day 30? These stages have already been achieved with mouse stem cells in extraordinary work[12,13] by researchers in the same groups as Weatherbee *et al.* and Oldak *et al.* For now, the three human embryo models stop short of such landmarks, but it is probably only a matter of time before they are reached.

All signs suggest that such advanced structures are scientifically possible, but just because we can do something doesn't necessarily mean that we should. Because stem- cell models seem to fall outside the current legal definitions of a human embryo, they are not subject to the same regulation — yet the distinction between the two is blurring, and the astonishingly rapid advancements in the field of embryo models have left little time for regulatory and legal frameworks to catch up. Scientists are resolutely calling for up-to-date governance that will allow them to work confidently within publicly acceptable limits[14–18]. Efforts by scientific communities, including the ISSCR and the UK-based project the Governance of Stem Cell-Based Embryo Models will probably fill some of these gaps. But with renewed public interest, discussions will need to include the voices of the general public as well as scientific, legal and ethical perspectives.

Although we celebrate the technical achievements showcased by the work of the three teams, such advances must also give us pause for thought. Pushing embryo models to be ever more embryo-like and towards more developmentally advanced stages might help scientists to understand how cells build embryos, but pushing the field too far risks jeopardizing public support for such research. As in any field of biology, researchers should be mindful to use models that best fit the research question. Is a complete embryo model at late stages of development always necessary, or would a less-advanced model suffice? Insights from simpler models could justify to the public the power of — and the need for — embryo models, without instigating widespread concern.

Perhaps the most useful embryo model, then, is one that is complex enough to aid understanding, but not so similar to an embryo that ethical boundaries are crossed. Exactly where this line falls will inevitably depend on the questions being asked and the regulatory frameworks under which research is conducted. But it behoves us to decide, in advance, how far we should go.

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