DEVELOPMENT

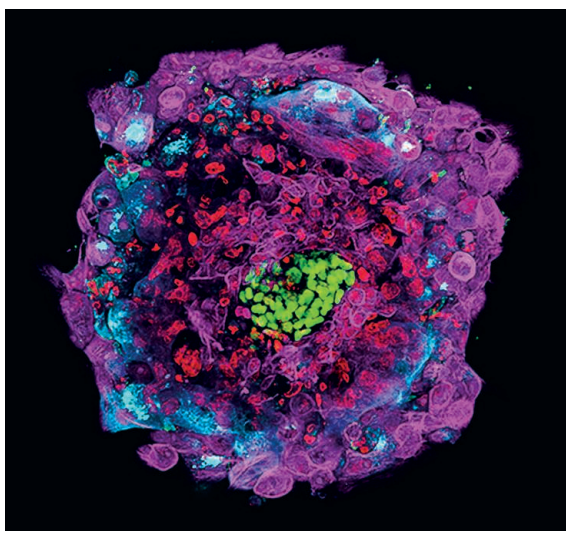
Exploring early human embryo development

Stem cell–derived models enable understanding of human embryogenesis

By **Janet Rossant**[1] *and* **Patrick P. L. Tam**[2]

Human in vitro fertilization (IVF) has been around for 40 years and yet we still do not fully understand how to ensure the generation of healthy embryos and to prevent unwanted genetic or epigenetic changes that may arise during in vitro culture. Once the embryo is transferred to the uterus to continue gestation, early implantation and the initial phases of embryogenesis and placenta formation are hidden from easy access for research. Errors in early development may lead to implantation problems, fetal defects, and placental insufficiencies, resulting in early pregnancy loss. Studying mice has provided clues about the major genetic and epigenetic events of early embryo development but, as is becoming increasingly evident, there are morphological and genetic differences between mice and humans that make cross-species comparisons problematic (*1*). Recent experimental approaches working directly with human embryos, or with embryo-derived stem cells and nonhuman primate embryos, have opened new avenues for studying the development of early human embryos.

Recent single-cell gene expression (RNA sequencing) analysis of early human embryos has provided a molecular time course of developmental progression (*2*). This has identified three distinct cell lineages in the expanded blastocyst [embryonic day 5 (E5) cavitated blastocyst]: the outer trophectoderm (TE), and the epiblast (EPI) and primitive endoderm (PrE) of the enclosed inner cell mass (ICM) (see the figure). Many key lineage specifiers, such as CDX2 (caudal-type homeobox protein 2), POU5F1 (POU domain, class 5, transcription factor 1), and SOX17 (SRY-box 17), show conserved expression with mouse embryo development, albeit with differences in timing of expression. In humans, these three cell lineages cannot be easily separated by transcriptional profiling prior to the expanded blastocyst stage, whereas in the mouse, the ICM and TE transcriptional profiles are distinct before the morphological events of cavitation of the blastocyst. The outer cells of the blastocyst are committed to a TE fate in the 32-cell-stage mouse embryo (*3*). By contrast, TE cells of the fully expanded human blastocyst are still capable of regenerating an entire blastocyst (*4*). These observations suggest that lineage segregation takes place after blastocyst formation in humans instead of progressively as in mice.



A human blastocyst cultured in vitro to 12 days postfertilization shows cell layers reminiscent of postimplantation development.

If true, the difference in lineage segregation between mice and humans has implications for the cross-species extrapolation of the upstream signaling events that drive lineage specification. For example, differences in Hippo signaling between the outer and inner cells of mouse embryos prior to blastocyst formation are important in specifying ICM versus TE fate. However, in humans, it is not clear whether the same pathway is involved. Additionally, fibroblast growth factor (FGF) signaling differences are key to the specification of EPI and PrE in the mouse ICM, but blocking FGF signaling in human embryos does not affect PrE formation (*1*), suggesting that other signaling pathways may be involved in this lineage decision, with implications for providing the critical signaling conditions in vitro to ensure correct development of IVF embryos.

Direct assessment of gene function in early embryos could provide insights into the importance of differences between mice and humans, but this was not considered feasible until the advent of CRISPR-Cas gene editing. Several jurisdictions have allowed experimental gene editing in early human embryos to explore gene function and the potential utility of germline correction of genetic diseases. Gene editing of *POU5F1* in human embryos has shown unexpectedly that mutant embryos in which this gene was ablated failed to reach the blastocyst stage, suggesting a role for *POU5F1* earlier in human than in mouse development (*5*).

Although it is feasible to study early development up to the blastocyst stage directly in human embryos cultured under IVF conditions, extension of such studies to implantation and the early postimplantation period requires new technical capabilities to maintain the embryo beyond the blastocyst stage. Recently, human blastocysts have been grown over the implantation period in a two-dimensional (2D) culture system and achieved the initiation of EPI, amnion, and yolk sac formation (*6, 7*). Although these cultured embryos are not properly organized and fail to progress beyond 10 to 12 days, advances in the fabrication of extracellular matrices and improved culture conditions could support extended human embryo development toward the next key milestone of development that occurs at around E14, gastrulation. Such experiments have not taken place, because there is an almost universal prohibition on growing intact human embryos beyond 14 days of development in culture. This time point marks the onset of nervous system development. Given the potential for such cultures to provide new insights into the key events of early development, there are emerging discussions about whether the 14-day rule should be revisited.

In view of the ethical concerns around the extended culture of human embryos and the inaccessibility of the early implantation stages in vivo, alternate models need

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to be considered for research on early human development. The similarities between the morphological features of human and nonhuman primate embryos have long been apparent (see the figure). A study on cynomolgus monkey postimplantation embryos has revealed details of the process of amnion formation, trophoblast development (which forms the placenta), germ cell formation and gastrulation, and the timing and spatial location of expression of some of the key genes underpinning these events (*8*). Although nonhuman primate embryos may be amenable for direct experimentation and, potentially, for genetic modification, such studies are technically challenging and come with concerns of animal ethics and care. There are thus still limitations on the extent to which nonhuman primate embryos can be considered a surrogate for human embryo experimentation.

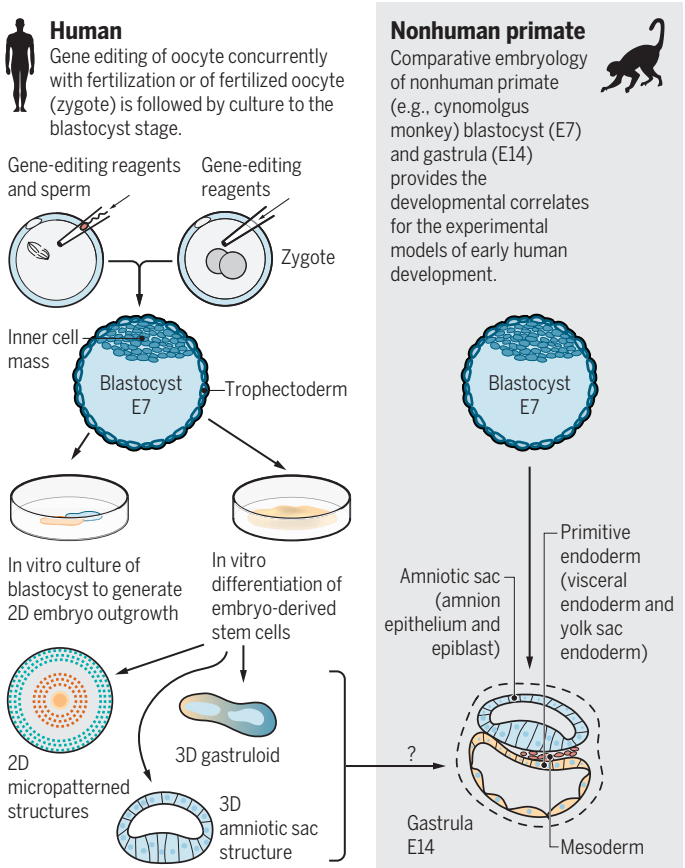
Recently, it has been shown that embryo-like structures (embryoids) can be generated in culture from human pluripotent embryonic stem (ES) cells. In micropatterned cultures of human pluripotent embryonic stem cells, organized 2D patterns of cell types and associated gene expression profiles, reminiscent of the ordered pattern in the germ layers of mouse embryos, can be discerned (*9*). These patterned constructs do not fully recapitulate the 3D organization that is the hallmark of the embryo at gastrulation. By contrast, cultures of pluripotent stem cells in 3D matrices can generate embryoid structures that appear to recapitulate development of the EPI and amnion formation (*10*).

In mice, ES cells grown as aggregates in 3D supporting matrices generate gastruloids (*11*). These entities not only mimic EPI organization but also display a localized primitive streak-like structure and anterior-posterior tissue patterning, which are the distinctive features of a gastrulation-stage embryo. This degree of organization in the absence of the extraembryonic tissues (such as the trophoblast and PrE), which normally provide localized signals to establish the body plan at gastrulation, is remarkable. Furthermore, combining ES cells and trophoblast stem (TS) cells can mimic some of these embryonic-extraembryonic interactions and enables the generation of blastoids and embryoids with architecture that resembles that of blastocysts and pregastrulation embryos, respectively (*12*, *13*). These findings raise the possibility that the combination of human ES cells and human TS cells (*14*) may provide similar experimental models for human development. At this juncture, the pressing question is whether the development of these embryo-like structures mirrors that of the human embryo in vivo closely enough that the outcomes are scientifically relevant to early human development. Further study directly on human embryos is needed to establish a paradigm of developmental correlates to guide the evaluation of the findings from these embryo-like entities.

The goal of research on human embryology is to gain scientific knowledge on the fundamentals of the cellular and molecular control of early human development, and its application to assisted reproductive technologies, gene editing, stem cell research, and the prevention of genetic birth defects. Although such knowledge would ideally be gleaned from studying the human embryo per se, the ethical, legal, and practical issues concerning the provenance of "excess" and "consented" human IVF embryos for research must be observed. The prohibition in many jurisdictions on creating embryos for research also precludes the feasibility of certain experimentation, such as germline gene editing prior to fertilization and zygote formation. In the future, stem cell–derived embryo-like structures could be the model systems, in place of human embryos, that offer the most useful insights into early human development.

As the technology for the generation of stem cell–derived embryo-like structures improves, these biological constructs will more closely mimic human embryos. This raises the question of whether the emergent properties of these entities should bring them under the same ethical limitations as human embryos. Should the 14-day limit of in vitro development be applied to these embryo-like cultures (*15*)? The embryological knowledge that informed the 14-day rule is gleaned from studies of mouse development and morphological studies of a rare archive of human embryonic materials, but not on experimental findings of the defining attributes of human embryos. The 14-day rule might lose its relevance as a meaningful and practical defining limit of the extent of development for the study of embryoids and gastruloids if they can advance beyond the nominal signposts of a 14-day human embryo. There is, therefore, a strong imperative to perform robustly controlled studies of human postblastocyst embryo development to test the scientific merit, and the ethics and legal practicality, of the 14-day rule before the consideration of whether to uphold or modify this rule for research on early human embryos and alternative embryological models. ■

Experimental studies of embryo development



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