RESEARCH HIGHLIGHT

Studying human embryo development with E-assembloids

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Stem cell-based models of early human embryo development can provide important insights into key stages of human pregnancy. In a recent *Cell Research* study, based on data obtained from their detailed analysis of gene expression in intact human embryo cultures, Ai and colleagues document the formation of stem cell 'E-assembloids' that mimic morphogenetic events of early human post-implantation development in vitro.

In vitro fertilization (IVF) has become a standard form of reproductive technology worldwide: it is estimated that 3% of babies born in China each year are produced by IVF. Yet we still have very little understanding of why IVF only succeeds in 40% of cases and why early pregnancy loss remains a persistent problem worldwide. IVF allows access to early human embryos in the first days of their development up to the blastocyst stage. The blastocyst marks the stage when the progenitors of the fetus itself become separate from the outer trophoblast and hypoblast cells that will go on to make the placenta and yolk sac membranes. After this stage, the embryo implants into the mother's uterus. The next key stages of development, including amnion and yolk sac formation, gastrulation, and placental initiation, all take place out of reach of experimental observation or intervention. There is an urgent need to understand these key stages in terms of both fundamental understanding of human biology and insights into improving IVF success, preventing early pregnancy loss, and exploring the onset of congenital defects and developmental origins of adult disease.

Many groups worldwide are tackling this problem in different ways, based on direct study of human embryos, comparative study of non-human primate embryos and generation of stem cell-based models of human development. In a paper recently published in *Cell Research*,[1] Ai et al. make use of their previously published 3D culture system for human blastocysts[2] to develop a detailed single-cell transcriptomic profile of the human embryo in culture over the early implantation period. By probing this dataset they were able to identify signaling pathways that drive extraembryonic lineage formation from human pluripotent stem cells. They then aggregated naïve hESCs with cells treated with BMP that provide a 'signaling nest' for the pluripotent cells. Interactions between the two cell types and a careful time course of application of Wnt, BMP, and Nodal agonists and antagonists resulted in the formation of E-assembloids that could grow and develop in culture for up to 9 days. The E-assembloids contained an outer layer of hypoblast-like cells enclosing the epiblast structures derived from the ES cells. Importantly, morphological events typical of the early post-implantation stages of development were observed, albeit not always as coherently as in the embryo itself. The bilaminar disc structure of the epiblast, and the formation of the amniotic and yolk sac cavities were all observed to occur, along with extraembryonic mesenchyme. Initiation of primitive streak formation and germ cell development were not so easily recognizable, although single-cell RNA sequencing analysis of the E-assembloids did indicate some possible candidate cells. This study provides strong pilot data supporting the relevance of these structures to normal development, based on the authors' own careful comparative analysis of cell profiles from human embryos themselves. Further improvements in the culture system and in defining the different starting cell components should enhance the efficiency and reproducibility of the E-assembloid approach over time.

These E-assembloids join a now-growing list of stem cell-derived models aimed at modeling these early human post-implantation stages in vitro. Some of these, like the E-assembloids, combine epiblast progenitors with extraembryonic endoderm/hypoblast-like cells,[3, 4] which seem to be the key components for initiating cavitation and morphogenesis within the epiblast. Surprisingly, perhaps, the inclusion of trophoblast-like cells does not seem to be necessary to initiate differentiation of the post-implantation epiblast towards gastrulation. Two papers did include both trophoblast and hypoblast-like cells in the mix,[5, 6] but in one case the putative trophoblast cells failed to maintain trophoblast gene expression profiles,[5] making the role of trophoblast uncertain. The second study did propose a key role for trophoblast derivatives for full embryonic development.[6] However, at this early stage in the research, we still lack clarity on all the key components needed for full organization of these embryo-like structures into true embryo models. Every protocol is different from the next: the starting cell state often differs, and the culture conditions are far from fully defined.

While there has been media attention on some of these models proclaiming them as 'synthetic embryos', it is important to point out that none of the stem cell embryo models produced to date are in any way complete replicas of a functioning embryo. And, indeed, that is not and should not be the goal of the experiments. The goal is to provide tractable model systems to explore specific aspects of early human development and translate that knowledge into improved IVF and better pregnancy outcomes. There are many exciting challenges ahead in this field.

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ADDITIONAL INFORMATION

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