Perspective

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Stem cell-derived embryo models: a frontier of human embryology

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Abstract: Studying human development remains difficult due to limited accessibility to human embryonic tissues. Prompted by the availability of human stem cells that share molecular and cellular similarities with embryonic and extraembryonic cells in peri-implantation human embryos, researchers have now successfully developed stem cell-based human embryo models that are promising as experimental tools for studying early human development. In this Perspective, we discuss the current progress in mouse and human stem cell-derived embryo models and highlight their promising applications in advancing the fundamental understanding of mammalian development.

Keywords: embryo models; embryonic development; pluripotent stem cells

Overview of mouse and human blastocyst formation

After fertilization, the zygote undergoes multiple rounds of cell division, giving rise to the morula that contains identical blastomeres. The inner and outer blastomeres of the morula gradually loose totipotency and are specified into the inner cell mass (ICM) and trophectoderm (TE), respectively (Figure 1). During this lineage bifurcation process, developmental signaling pathways regulate activities of key transcription factors (TFs) to specify ICM vs. TE cell fates. Specifically, ICM vs. TE lineage bifurcation occurs with position-dependent expression of lineage-specifying TFs such as *Sox2* and *Cdx2* in prospective ICM and TE cells, respectively, resulting in fully committed ICM and TE [1]. Continuous development of the morula leads to the formation of a structure known as the blastocyst. In the blastocyst, individual ICM cells show upregulated *Nanog* or *Gata6* expression, respectively, in a salt-and-pepper pattern, preceding epiblast (EPI) and primitive endoderm (PE) lineage specification and segregation [2]. After implantation, the EPI gives rise to the embryo proper, whereas the PE contributes to development of the yolk sac. The TE also contributes to the embryonic compartment of the placenta. In humans, however, cell lineage-specific TFs *CDX2*, *OCT4*, *NANOG*, and *GATA6* are not expressed until the late blastocyst stages (embryonic day 6/7) [3]. Furthermore, studies have shown that isolated human ICM cells can reconstitute blastocysts [4], suggesting their unrestricted lineage potential and developmental plasticity. Hence, further studies are warranted to determine whether and how these TFs function in pre-implantation human development.

Morphogenetic differences in post-implantation mouse and human embryos

Morphogenesis in post-implantation human embryos differs greatly from that in mice (Figure 1). When a mouse blastocyst implants in the uterus via mural TE, the EPI and polar TE (TE cells at the embryonic pole) generate an egg cylinder structure, a characteristic of rodent embryos. Implantation of the human blastocyst is initiated by the cross-talk between the polar TE and uterus tissue. Subsequently, the EPI undergoes lumenogenesis, forming a nascent cavity (the prospective amniotic cavity) surrounded by a single layer of EPI cells. Thereafter, EPI cells next to the polar TE spontaneously differentiate into the amniotic epithelium (or amnion). The amnion does not exist in pre-gastrulation mouse embryos. Development of the amniotic cavity leads to the formation of a two-layered embryonic structure containing the EPI and hypoblast, collectively known as the bilaminar disc. An additional role of the amnion has been suggested in recent studies, showing that primordial germ cells

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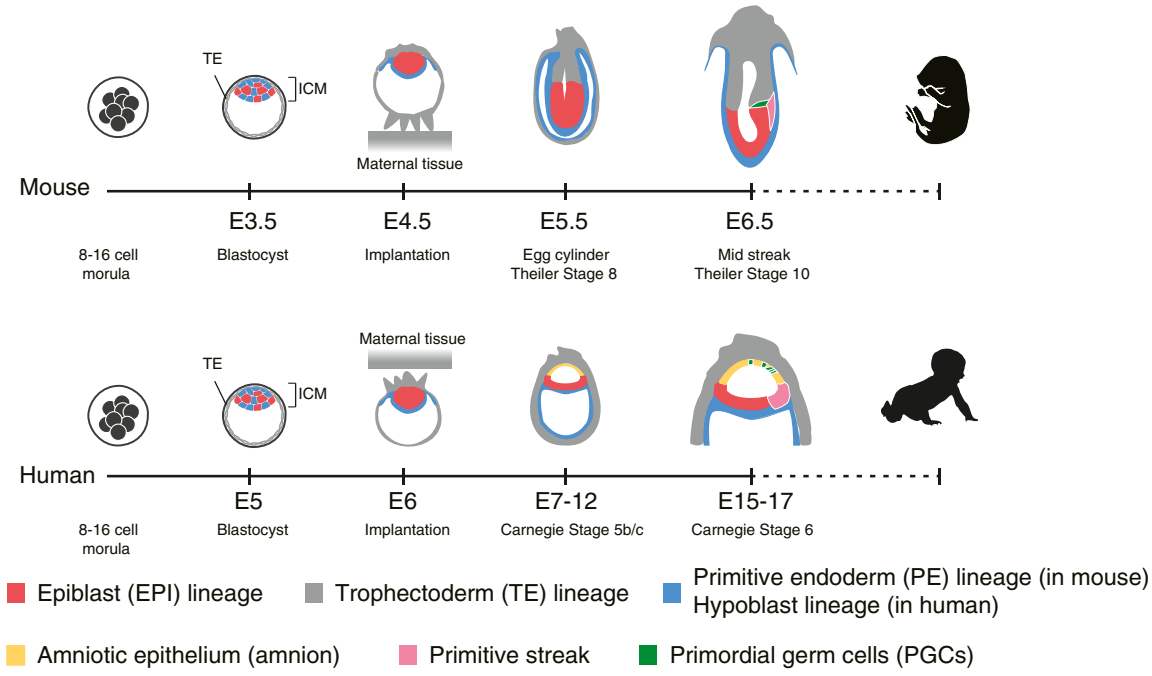

Figure 1: Schematic of mouse and human embryo peri-implantation development.

(PGCs) might be first specified in the nascent amnion in the cynomolgus monkeys [5]. Formations of the amniotic cavity and amnion are soon followed by the gastrulation process, with the appearance of a primitive streak in the EPI. Through gastrulation, the EPI generates the three germ layers: endoderm, mesoderm, and ectoderm. Thus, through gastrulation, the EPI transforms from a one-dimensional layer into a trilaminar disc structure, setting the stage for subsequent organogenesis events.

To advance the existing knowledge of organogenesis as well as of congenital abnormalities, studying human gastrulation and early organogenesis is important. However, natural specimens of peri- and post-gastrulation human embryos are very scarce [6]. Additionally, existing *in vitro* culture protocols remain suboptimal for prolonged cultures of human blastocysts to allow progression to the gastrulation stage [7]. It is foreseeable that a significant future emphasis will be placed on studying the peri- and post-gastrulation human development, using either *in vitro* cultured human embryos or *in vivo* primate monkey embryonic specimens [6–8].

Challenges and opportunities for stem cell-derived embryo models

There are mouse and human pluripotent stem cells (PSCs) that exist in different pluripotent states, including naïve and primed pluripotency. Naïve PSCs correspond to the pre-implantation EPI, whereas primed PSCs are considered equivalent to peri-gastrulation EPI [8]. As our understanding of the self-organizing properties of PSCs advances, Three-dimensional (3D) culture protocols for PSCs to generate multicellular structures mimicking embryo and organ developments are also improved. In parallel, advanced bioengineering tools are developed to allow dynamic controls of proper physical and chemical environments of 3D cultures. Based on improved knowledge of early mammalian development, PSC properties and 3D cultures, researchers have successfully used PSCs with naïve and primed pluripotency to develop mammalian embryo models to recapitulate pre-and post-implantation stages of mammalian development, respectively.

Mouse blastocyst models (or blastoids) were successfully developed by sequential aggregation of naïve mouse PSCs (mPSCs) and mouse trophoblast stem cells (mTSCs) in microwells [9]. mTSCs enveloped mPSCs, resulting in the formation of blastoids that are comparable to mouse blastocysts in terms of morphology, size, and cell lineage composition. Although attempts have been made by researchers to transfer mouse blastoids into the uteri of pseudo-pregnant mice, they have not yet succeeded in mimicking the *bona fide* post-implantation embryonic development *in vivo* [9]. Recent studies by Hanna and Zernicka-Goetz groups, using a combination of an “assembloid-like” approach and *ex utero* embryo culture systems,

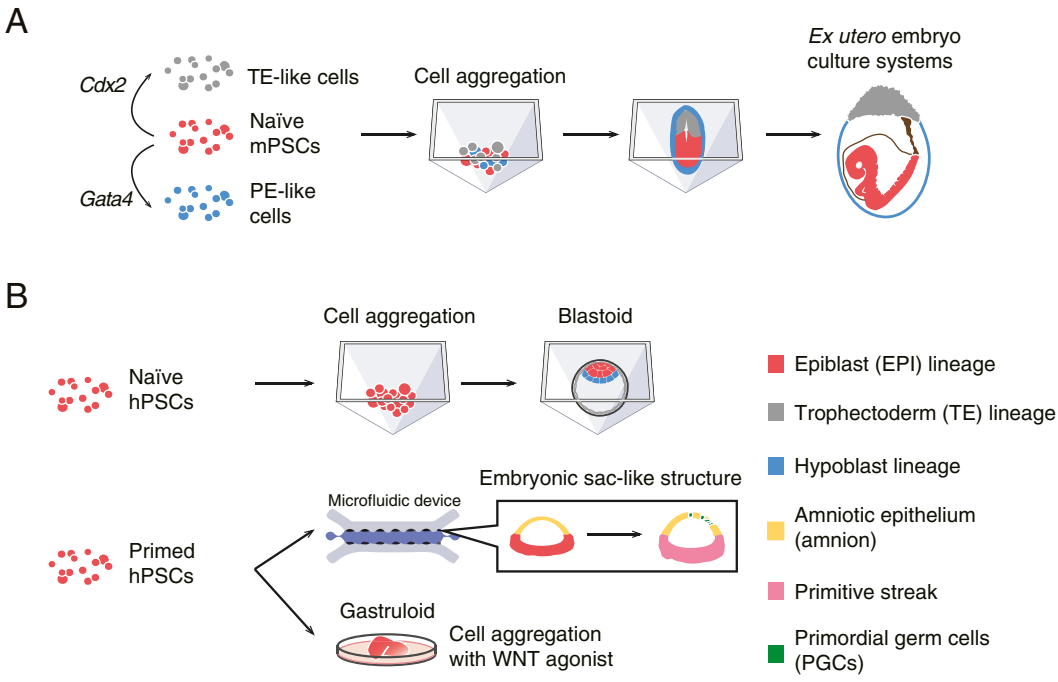

Figure 2: Graphical abstract showing synthetic mouse and human embryos. (A) Recapitulating mouse embryogenesis from mPSCs *in vitro*. Using a combination of an "assembloid-like" approach and an ex utero embryo culture systems, mouse stem cell-derived embryos showed the ability to grow and resemble mid-gestation mouse embryos [10, 11]. (B) Modeling of post-implantation human embryos *in vitro*. Reconstructed stem cell-derived embryo models can serve as reliable models for elucidating human embryo development *in vitro* [12–15].

reported the formation of peri-implantation mouse embryo-like structures and their continuous development into early organogenesis stages [10, 11] (Figure 2A). Both groups derived PE-like cells by transient overexpression of *Gata4* and TE-like cells via transient overexpression of *Cdx2*, respectively, from mPSCs, and subsequently aggregated them with mPSCs. Remarkably, after mixing, the cells self-organized and formed egg cylinder-like structures. These assembloids were allowed to develop for eight days in *ex utero* embryo culture systems, also known as rotator-type bottle culture systems, leading to the development of primitive organ rudiments, including the neural tube, primitive gut, beating heart, and bilateral and paired somites. Histological and transcriptome analyses suggested that these post-gastrulation mouse embryo-like structures resembled 8.5-day *in vivo* mouse embryos. Thus, post-gastrulation mouse embryo-like structures, despite their low efficiency, appear to have substantial, if not complete, potency for *bona fide* development.

As with mice, induction of self-organization of naïve human PSCs (hPSCs) could lead to the formation of human blastoids [12, 13]. Several groups have attempted to develop implantation models with endometrial cell cultures using human blastoids, in hopes of such implantation models allowing progressive development of human blastoids to recapitulate post-implantation human development [13] (Figure 2B). Primed hPSCs have been used for modeling post-implantation developmental events associated with the EPI lineage. Using a bioengineered microfluidic device and hPSCs with primed pluripotency, Zheng et al. devised an embryonic-like structure resembling the bipolar patterned amnion-EPI sac before gastrulation [14]. Importantly, the onset of gastrulation and lineage specification of PGCs are also evident when the patterned amnion-EPI-like sac continuously develops. Another human embryo model, termed "gastruloid," has been developed by exposing primed hPSCs with exogeneous Wnt signals. Human gastruloids have been shown to undergo symmetry breaking and morphogenetic events that mimic certain developmental features during post-gastrulation development and even early organogenesis [15].

The studies by Hanna and Zernicka-Goetz groups suggest that stem cell-derived mouse embryo models have substantial developmental potency [10, 11]. Following their work on mouse embryo models, it is expected that stem cell-derived human embryo models will soon follow suit. Culturing tools for natural primate embryos are continuously improved, with a clear goal of prolonging the culture and development period of cultured natural primate embryos or embryo models beyond the gastrulation stage. Important efforts also

include studies on human and non-human primate (NHP) embryos in post-implantation stages *in vivo* [16–18] and the development of monkey (NHP) blastoids that can be utilized for implantation studies with a surrogate uterus to promote their continuous development [19].

Human blastoids remain imperfect, and there are still important technical and ethical limitations making human blastoid research very challenging. Current human blastoids show very limited peri-implantation development potential [13]. It has also been reported that the starting cells and methods used to generate human blastoids strongly impacts the faithfulness of the model [13, 20, 21]. A suboptimal medium or defects during human blastoid formation could lead to the precocious differentiating into non-blastocyst-stage cells [20]. *Bona fide* human trophectoderm and hypoblast stem cells remain to be developed to be incorporated into human embryo models. It is also not acceptable to perform implantation studies of human embryo models using surrogate uterus. To address these challenges regarding human blastoids, monkey blastoids may be an ethically more acceptable alternative to model post-implantation primate development while providing useful insights to improve human blastoid techniques.

Together, mammalian embryo models will provide essential experimental tools to dissect the molecular and cellular events driving mammalian development. This emerging research field will also be promising in improving assisted human reproduction and prevention of pregnancy loss, birth defects, and teratogenesis.

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