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Self-organization of stem cells into embryos: A window on early mammalian development

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Abstract

Embryonic development is orchestrated by robust and complex regulatory mechanisms acting at different scales of organization. In vivo studies are particularly challenging for mammals after implantation, owing to the small size and inaccessibility of the embryo. The generation of stem cell models of the embryo represents a powerful system with which to dissect this complexity. Control of geometry, modulation of the physical environment, and priming with chemical signals reveal the intrinsic capacity of embryonic stem cells to make patterns. Adding the stem cells for the extraembryonic lineages generates three-dimensional models that are more autonomous from the environment and recapitulate many features of the pre- and postimplantation mouse embryo, including gastrulation. Here, we review the principles of self-organization and how they set cells in motion to create an embryo.

Vertebrate development deploys orthologous sets of genes that first create the body axes—anterior-posterior (AP), dorsal-ventral (DV), and left-right (LR)—as well as the germ layers—endoderm, mesoderm, and ectoderm—and ultimately refines these patterns to the diverse adult forms we know. How are these spectacular feats of self-organization possible?

Although we have an inventory of genes that confer cell identity, we are far from understanding how their products communicate to generate embryonic patterns.

Multiple levels of regulation add robustness to embryonic development, but this redundancy makes the regulatory network difficult to decipher. Using stem cells as a model system to study embryology, we are now able to start peeling back these layers of regulation to reveal the dynamic organization of the embryo. Technical advances that created stem cell embryology were reviewed in (1). Here we focus on the principles of how cell communication at the molecular level enables embryonic self-organization in mouse and human embryos.

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Mammalian embryo development

Preimplantation development is fairly conserved among mammalian species (2). Fertilization leads to a stepwise process of cell fate specification that culminates with the blastocyst comprising three cell types: the embryonic epiblast and the extraembryonic primitive endoderm and trophectoderm (3–6). Blastocyst implantation initiates a dialogue between the uterus and the embryo, which leads to the reorganization of both the embryo and the maternal tissues. Across diverse mammalian species, the basic relation between tissues is conserved, but postimplantation conceptuses present distinct embryonic architectures, from the cylinder-like shape of mouse embryos to the bilaminar disc of human embryos (2) (Fig. 1). How these different shapes evolved remains unknown.

Interactions between embryonic and extraembryonic tissues are critical to reshaping the developing embryo. In the mouse, the polar trophectoderm proliferates in response to fibroblast growth factor 4 (FGF4) secreted by the epiblast to form the extraembryonic ectoderm (7), which will form the placenta. Concomitantly, the epiblast and extraembryonic ectoderm undergo a process of lumenogenesis in response to extracellular matrix (ECM) secreted by the primitive endoderm–derived visceral endoderm (8, 9). The fusion of the extraembryonic ectoderm and epiblast cavities leads to the formation of the proamniotic cavity (10), fundamental for the establishment of the body plan. This coincides with a symmetry-breaking event to form the anterior signaling center in the visceral endoderm (AVE) that defines the AP axis and the site of gastrulation (11–13).

In human embryos, the epiblast undergoes lumenogenesis in a similar way to that of the mouse, with one important difference: Epiblast in contact with the trophoblast forms the amniotic epithelium, whereas epiblast in contact with the hypoblast (visceral endoderm-equivalent) forms the epiblast disc (1, 2, 14). The mechanisms of symmetry breaking leading to AP axis formation in human embryos remain unknown, but mechanical and chemical cues are clearly involved. In cynomolgus monkey embryos, a population of hypoblast cells that expresses Wnt and Nodal inhibitors (DKK1 and CER1), characteristic of the mouse AVE, has been identified (15).

Is a dialogue between mother and embryo required for this morphogenesis? Comparative embryology provides a preliminary answer. In mammalian embryos such as pig, rabbit, and cow, embryonic morphogenesis and gastrulation take place before implantation (16, 17). Mouse and human embryos can undergo early postimplantation morphogenesis without maternal input (8, 18–21). Even if the uterine environment could help to modulate these events (22, 23), the self-organizing capabilities of mammalian embryos (and stem cells) are becoming increasingly apparent.

Modes of self-organization

Although a system composed of invariant parts might be induced to self-assemble, here we focus mainly on self-organization that encompasses both patterning (fate change) by exchange of signals, as well as cell rearrangements. To further refine terminology, consider a supersaturated vapor that is spatially homogeneous until droplets nucleate and grow. The

immediate trigger for a drop may be a speck of dust, but its subsequent expansion is reproducible. This is an example of spontaneous symmetry breaking, because the initially homogeneous vapor (the symmetric state) becomes an inhomogeneous mist of droplets. Analogous, self-organization occurs in systems of chemical reactions with diffusion where Turing showed that inhomogeneities with a characteristic spatial scale result from a random trigger to a uniform but unstable system (24). Embryology generally avoids spontaneous symmetry breaking because the outcome is too fragile; rather, it proceeds by progressive refinement of prior asymmetries, still suggestive of Turing's ideas.

The requirements for Turing instability are intuitively transparent: An activator induces the production of its own inhibitor, but the inhibitor diffuses more rapidly than the activator and confines the activator in space. This has the seemingly paradoxical consequence that the peak expression of both the activator and inhibitor are in the same place, rather than being opposed. Both modes of regulation are seen in the mouse embryo (25).

Because signaling pathways often involve secreted inhibitors, Turing phenomena are frequently posited. However, there are many confounding influences as exemplified by studies of digits and feather follicles in the skin (26–28). Reaction-diffusion systems can also account for the "community effect," articulated by John Gurdon, whereby a tissue forces the majority fate on cells within it (29–31).

In quantitative analogy to the surface tension–driven separation of oil and water, cells of different types can sort by differential adhesion (32). Chemotaxis can also contribute to pattern formation as in sporulation in *Dictyostelium*, and signaling pathways themselves can provide chemotactic signals (33).

Self-organization in embryonic stem cells

The disc shape of the human epiblast suggests the possibility of a two-dimensional (2D) model. Embryonic stem cells (ESCs) naturally supply the epiblast. The extraembryonic hypoblast and spatial confinement are modeled by micropatterns: Slides with arrays of disks where ECM proteins bind and control where cells adhere. The extraembryonic trophoblast is modeled by addition of BMP4 to the media to provide the morphogen trigger (34). As envisioned by Tam (35), the cells pattern with concentric rings of endoderm and mesoderm and a central disk of anterior epiblast (Figs. 2 and 3). The mesendoderm cells express the same markers and require the same signals (Wnt and Activin/Nodal induced downstream of BMP4) as does the mouse primitive streak, and the same secreted inhibitors are required to spatially confine the streak and shield the central epiblast from morphogens. Thus, a homogeneous layer of human ESCs (hESCs) can self-pattern on a scale of ~2000 cells, without contribution from extraembryonic lineages. Similarly, micropattern culture guides self-organization of ectodermal derivatives (36).

The micropattern system facilitates deciphering how cell fates are defined by distance from the colony boundary (37–40). hESCs are apicobasally polarized, and the BMP, Activin, and Nodal receptors are basolateral and not accessible to apical ligands except at the colony boundary. The secreted BMP inhibitor NOGGIN also restricts signaling to the colony

boundary and is active from the apical side, suggesting complex signal transmission in polarized epithelia (41).

Receptor localization in the micropattern system, when folded into a cup-shape as in the mouse, helps explain why the proamniotic cavity (facing the apical side of the epiblast) does not short-circuit the proximal-distal patterning and why the initial BMP response is proximal only (41). This conjecture was confirmed by mistargeting the BMP receptors in the embryo (42).

Micropattern culture has been extended to the mouse (43). When mouse ESCs (mESCs) are differentiated to a postimplantation-like state (44) and transferred to micropatterns, they display properties similar to those of the pregastrulation epiblast. Differentiation with Wnt, Activin and BMP gives fates indicative of distal versus proximal streak derivatives.

Embryonic and extraembryonic stem cells have been shown to self-organize in 3D culture (45–49). When ESCs are cultured in a 3D gel supplemented with ECM, they form an apicalbasal polarized shell that eliminates the boundaries of micropattern culture, allowing control of substrate mechanics and chemistry. Human pluripotent cells cultured in such a system respond to BMP by polarizing and breaking symmetry into anterior epiblast and posterior primitive streak in a Wnt-dependent manner (50).

A synthesis of signals and mechanics can be achieved by placing hESCs on a 3D soft gel and doping the media above with a low concentration of ECM components (51, 52). This treatment induces the patches to fold into closed polarized shells, which, depending on the initial cell density, can generate squamous, asymmetric, or columnar cysts. Squamous cysts represent an amnion-like tissue based on gene expression, cell shape, and BMP signaling activity (51), also active in the amnion of cynomolgous monkeys (15). Columnar cysts represent the epiblast, and asymmetric cysts undergo a symmetry-breaking event to form an amnion-like hemisphere and an epithelia-like disc (Figs. 2 and 3). The morphogenesis of the resulting amniotic sac plausibly requires BMP that induces Brachyury expression and EMT in the putative epiblast, but the mechanisms of symmetry breaking remain unknown. With the ability to define a gel surface in 3D, future studies will shed light on how morphology influences cell-cell signaling.

Embryoid bodies offer an alternative approach for eliciting the self-organizing potential of stem cells (53, 54). When a clump of mESCs is given a pulse of Wnt agonist, it elongates into a tube showing markers for AP, DV, and LR axes (55, 56) (Fig. 2). These so-called gastruloids recapitulate the spatiotemporal patterns of gene expression of embryos after gastrulation, such as homeobox (*HOX*) genes, in the correct temporal order and in nested telescoping domains (57).

Self-organization of embryonic and extraembryonic stem cells

These ESC-only based models are informative in revealing how homogeneous populations of cells can give rise to different cellular fates through the process of self-organization. However, these models differ from those of natural embryos in their lack of extraembryonic tissues, which are critical for development and provide spatial context for signaling

interactions. For this reason, new stem cell embryo models have been developed that incorporate interactions of ESCs with extraembryonic cells (58–61) (Figs. 2 and 3).

Combining mESCs and trophoblast stem cells (TSCs) (Fig. 1) in ECM (58) to substitute for the basal membrane produced by the primitive endoderm leads to the generation of postimplantation embryo-like structures. In this model, cells polarize and form lumens in the ESC-derived embryonic and TSC-derived extraembryonic compartments that then join, in response to Nodal signaling (58, 60). A domain of asymmetric Brachyury expression develops at the boundary between the ESC and TSC compartments. These polarized embryo-like structures induce mesoderm formation but do not proceed through gastrulation (Figs. 2 and 3). This event has been observed after substituting the ECM with the third stem cell type, extraembryonic endoderm (XEN) stem cells (Fig. 1), which provide the natural basement membrane (59, 60). As a result, the formed structures look markedly similar to early postimplantation embryos in morphology, gene expression, and signaling communication. They break symmetry at the embryonic and extraembryonic boundary with the induction of AP patterning and EMT, leading to mesoderm and definitive endoderm formation (59). This self-organization occurs in response to BMP and Wnt signaling active during the cavity fusion process (58, 59). Finally, the markers of primordial germ cells become expressed in a spatial-temporal manner characteristic of development. These structures induce decidualization upon their transfer to mouse foster mothers but do not develop further.

The self-assembly and subsequent self-organization into so-called gastrulating embryo-like structures are possible because the different stem cell types not only establish signaling among themselves but also provide the building blocks for spatial morphogenesis. The migration of ESCs to form the mesoderm layer, sandwiched between ESC-derived epiblast and XEN-derived visceral endoderm, and replacement of the XEN-layer with definitive endoderm are the hallmarks of early-to-mid gastrulation (59). This points to the essential requirement for the correct choreography of cells from embryonic and the two extraembryonic tissues to achieve correct form.

Will stem cell models ever pass the ultimate test of function, which is development following implantation? Combining mESCs and TSCs in a nonadherent platform leads to the generation of preimplantation embryo-like structures markedly similar to blastocysts, both in terms of shape, gene expression, and intercellular communication (61) (Fig. 2). These so-called blastoids can also induce decidualization, but then their development stops (Fig. 3). The derivation of extraembryonic stem cells that better match the expression signatures of real embryos should improve the morphology of these embryo-like structures (62, 63). Similarly, the recent generation of expanded potential stem cells, which have the ability to form both embryonic and extraembryonic tissues, represents a promising tool for future research (64, 65)

Conclusions and perspectives

Stem cell models of embryogenesis allow independent control of shape, mechanics, and means to juxtapose embryonic and extraembryonic tissues. Therefore, they represent

powerful systems with which to address classic questions of embryology. For example, does gastrulation proceed in embryo-like structures of anomalous size, or does size have to be regulated first? Which combination of chemical and mechanical signals suffices to trigger primitive streak formation? To what extent can we induce gastrulation without AVE? Do the genetic barriers to chimerism operate through the same pathways as intraspecies cell competition? Stem cell systems will thus illuminate the genetic determinates of size and timing control.

Stem cell–derived embryos are models of development, and therefore they cannot fully recreate all the complexity of developing organisms. The field of stem cell embryology is in its infancy and will expand by tuning chemical and physical parameters, and using stem cell lines with broader developmental potential (64, 65). Particularly interesting would be the combination of hESCs with human TSCs (66), and potentially human hypoblast stem cells, to recapitulate the human conceptus.

However, in devising these studies, it is important to consider when stem cell models of embryos acquire the protections attached to human embryos. Is a collection of cells that mimics gastrulation any more human than a brain organoid that might one day be endowed with sensory primordia (67)? It is clearly unethical to implant a stem cell–derived embryo into a human, yet many pregnancies fail or are impaired by placentation. Can stem cell models help to address this problem?

The promise for basic science is clear. Building embryos from stem cells, like the in vitro reconstitution of biochemical systems from purified components, is the test of whether we can understand the whole from the parts.

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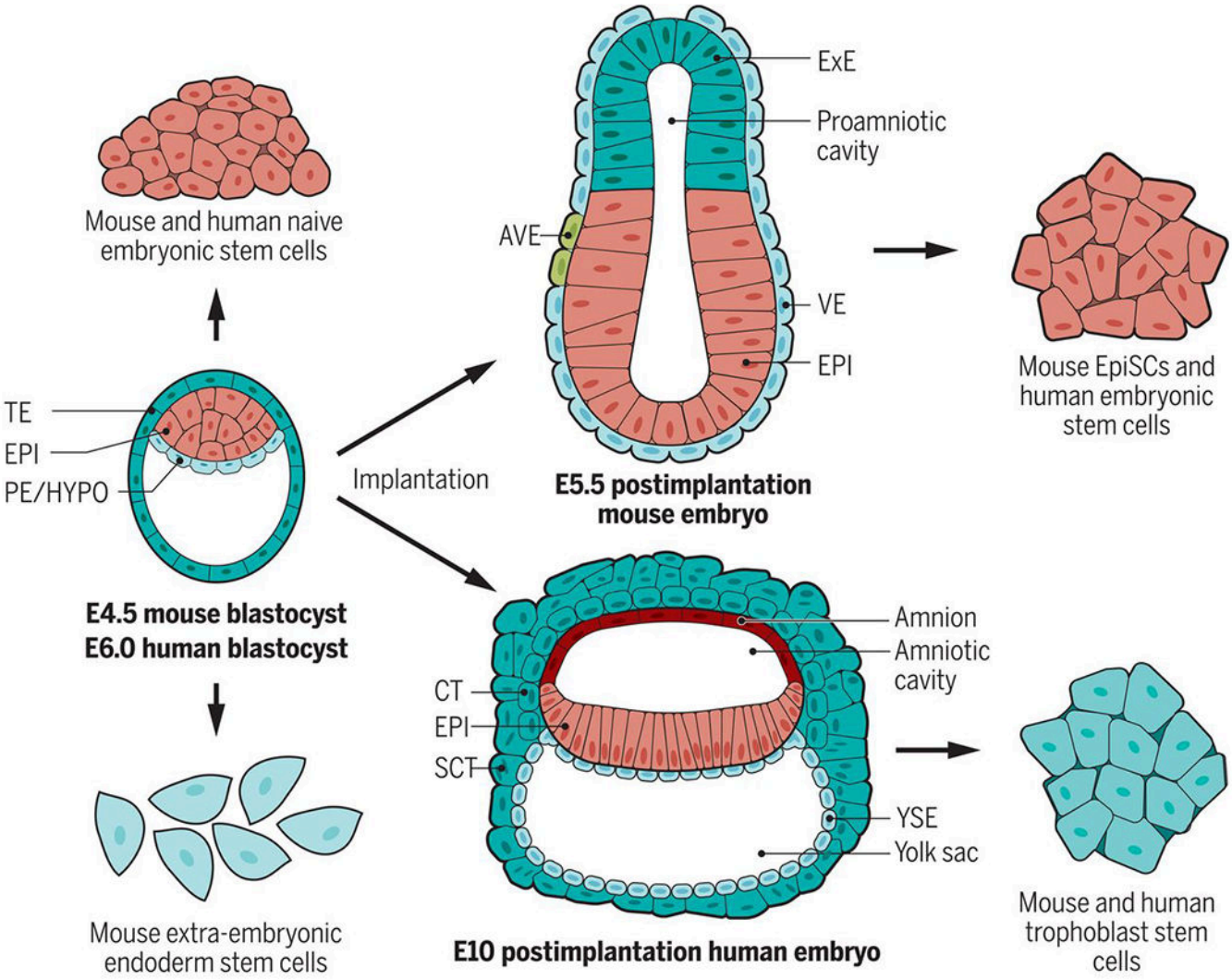


Fig. 1. Schematic representation of mouse and human pre- and postimplantation embryos and the stem cell lines that can be derived from them.

Extraembryonic tissues are shown in different shades of teal, and epiblast derivatives in different shades of red. EPI, epiblast; TE, trophectoderm; PE, primitive endoderm (mouse), HYPO, hypoblast (human); ExE, extraembryonic ectoderm; VE, visceral endoderm; AVE, anterior visceral endoderm; CT, cytotrophoblast; SCT, syncytiotrophoblast; YSE, yolk sac endoderm.

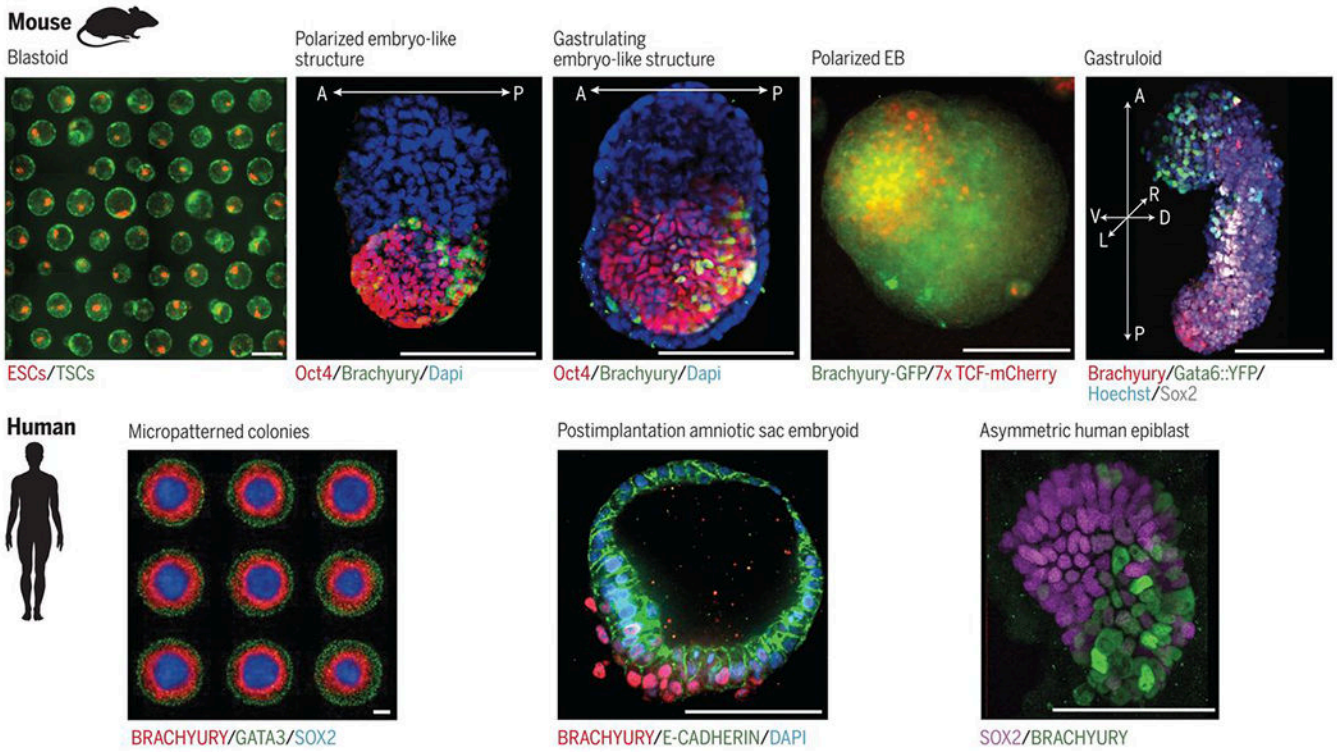


Fig. 2. Images of stem cell embryo models.

Oct4 labels pluripotent epiblast cells; Brachyury marks mesoderm; Gata6 marks endoderm; Gata3 marks extraembryonic cells; Sox2 labels both ectoderm and pluripotent cells; 7xTCF-mCherry is a reporter of Wnt signaling activity; E-Cadherin labels cell-cell adhesion sites; and Dapi and Hoechst label nuclei. ESCs, embryonic stem cells; TSCs, trophoblast stem cells. Scale bars, 100 μm. These models are described in (34, 50, 52, 53, 56–59, 61).

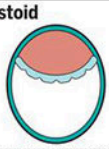
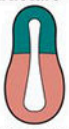
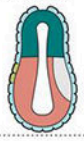
EMBRYO-LIKE STRUCTURE	STARTING COMPONENTS	SPECIES	EMBRYONIC STAGE	ADVANTAGES	DISADVANTAGES
Blastoid 	ESCs + TSCs	Mouse	Blastocyst	Self-assembly and self-organization between 2 cell types Morphogenesis + cell fate	Cellular interactions by chance (decreased efficiency) Limited developmental potential
Polarized embryo-like structure 	ESCs + TSCs	Mouse	Early post-implantation	Self-assembly and self-organization between 2 cell types Symmetry breaking in the absence of AVE	Cellular interactions by chance (decreased efficiency) Lack of EMT and gastrulation
Gastrulating embryo-like structure 	ESCs + TSCs + XEN cells	Mouse	Gastrulation	Self-assembly and self-organization between 3 cell types Morphogenesis + cell fate	Cellular interactions by chance (decreased efficiency) Limited epiblast patterning
Embryoid body 	ESCs	Mouse	Post-gastrulation	Self-organization of ESCs Symmetry breaking in the absence of exogenous cues	Limited patterning Lack of proper tissue organization
Gastruloid 	ESCs	Mouse	Post-gastrulation	Self-organization of ESCs Cell fate specification and tissue patterning	Limited morphogenesis Lack of proper tissue organization
Micropatterned colonies 	ESCs	Human	Post-gastrulation	Self-organization of ESCs Quantitative cell fate specification and tissue patterning	Limited morphogenesis 2D platform
PASE 	ESCs	Human	Gastrulation	Self-organization of ESCs Spontaneous amnion-epiblast fate split	Lack of precise control (decreased efficiency) Limited epiblast patterning
Asymmetric human epiblast 	ESCs	Human	Early post-implantation	Self-organization of ESCs Spontaneous symmetry breaking	Limited morphogenesis Lack of morphological in vivo equivalent

Fig. 3. Summary of stem cell models of the mouse and human embryo.

For each model, the starting cell types, the corresponding embryonic stage, and the main advantages and disadvantages are shown. AVE, anterior visceral endoderm; EMT, epithelial-to-mesenchymal transition; PASE, postimplantation amniotic sac embryoid; ESCs, embryonic stem cells; TSCs, trophoblast stem cells; XEN cells, extraembryonic endoderm stem cells.