

Reconstructing Mammalian Embryogenesis In-Vitro Through Lineage Specification, Morphogenetic Self-Organization and Programmable Stem Cell-Based Embryo Models

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Abstract: Stem-cell-based embryo models (SCBEMs) have emerged as powerful experimental systems for investigating how mammalian embryos acquire cellular identity, establish spatial organization and undergo coordinated morphogenesis. This review traces the development of SCBEMs from embryoid bodies and gastruloids to ETS, ETX and ETiX/EiTiX models, highlighting how the progressive incorporation and engineering of embryonic and extraembryonic lineages has improved the reconstruction of early developmental events. Attention is given to the molecular and cellular mechanisms that regulate lineage specification and developmental competence, including OCT4, SOX2, NANOG and CDX2 regulatory networks, together with Hippo-YAP-TEAD4, WNT- β -catenin, FGF-ERK and BMP-NODAL signalling. Recent advances in CRISPR-based transcriptional activation, transgene-free lineage induction and chemically induced pluripotent or totipotent-like states are further expanding the capacity to manipulate developmental trajectories. Single-cell transcriptomics, spatial multi-omics and quantitative morphological profiling now provide increasingly detailed approaches for evaluating cellular heterogeneity and developmental fidelity. Developmental competence is increasingly recognized as a system-level property emerging from the coordinated regulation of cellular metabolism, genome stability and epigenetic state. Nevertheless, incomplete trophoblast maturation, limited extraembryonic tissue development and the absence of authentic embryo-maternal interactions remain major challenges. Collectively, these advances are reshaping SCBEMs from phenotypic mimics of embryogenesis into experimentally tractable and increasingly programmable systems for dissecting the causal principles of mammalian development.

Keywords: Stem Cell-Based Embryo Models; Mammalian Embryogenesis; Trophectoderm Specification; CDX2 Signalling; Morphogenetic Self-Organization; Developmental Engineering.

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I. INTRODUCTION

Mammalian embryogenesis is a coordinated process in which cell-fate specification, lineage segregation and tissue morphogenesis occur together. During early development,

embryonic and extraembryonic lineages become progressively specified through changes in cell adhesion, polarity and cytoskeletal organization. After implantation, primitive-streak formation, gastrulation and germ-layer specification establish the basic body plan under the control

of WNT- β -catenin, FGF-ERK, BMP-NODAL and Activin-TGF- β signalling. Because these events are difficult to study directly in mammalian embryos, stem-cell-based embryo models (SCBEMs) have emerged as experimental systems for investigating self-organization, symmetry breaking, axis formation and tissue patterning (Fu et al., 2021; ten Berge et al., 2008).

SCBEM development has progressed from embryoid bodies and gastruloids to multilineage ETS, ETX, ETiX and EiTiX models. Embryoid bodies demonstrated spontaneous three-dimensional differentiation, whereas gastruloids provided greater control over axial organization. The incorporation of embryonic and extraembryonic stem-cell populations enabled reconstruction of trophoblastic and endodermal interactions and highlighted the importance of interlineage signalling and cellular self-organization (Harrison et al., 2017; Sozen et al., 2018). However, embryo-like morphology does not necessarily indicate developmental equivalence. Differences in transcriptional and chromatin states, together with incomplete trophoblast and placental maturation, restricted extraembryonic mesoderm formation and limited embryo-maternal communication continue to affect developmental fidelity (Posfai et al., 2021).

Recent SCBEM research is therefore moving toward programmable and mechanistically defined developmental systems. CRISPR-based transcriptional regulation, transgene-free approaches and chemically induced pluripotent or totipotent-like states allow greater control over developmental competence. Single-cell and spatial transcriptomics, chromatin profiling and multi-omics further resolve lineage transitions and developmental trajectories. These studies indicate that developmental potential depends not only on signalling pathways but also on metabolic state, genome integrity, cell-cycle dynamics and chromatin plasticity. Key mechanisms including CDX2-mediated trophoblast specification, Hippo-YAP-TEAD4 signalling, chromatin-state transitions, morphogen dynamics, mechanotransduction and extracellular-matrix remodelling further connect cell state with tissue organization (Posfai et al., 2021; Bao et al., 2022).

II. DEVELOPMENTAL AND MOLECULAR ARCHITECTURE OF EARLY MAMMALIAN EMBRYOGENESIS

Mammalian embryogenesis involves totipotency, lineage segregation, gastrulation and morphogenesis regulated by Hippo-YAP-TEAD4, WNT- β -catenin, NODAL, FGF-ERK and BMP signalling. SCBEMs progressively reconstruct embryonic-extraembryonic interactions, yet trophoblast maturation, placental specification and embryo-maternal integration remain major fidelity constraints.

➤ Early Mammalian Embryogenesis and Lineage Specification

Mammalian embryogenesis begins with a brief totipotent state after fertilization. The zygote can initially generate both embryonic and extraembryonic lineages (Fig 1).

It then passes through successive cleavage stages and reaches the morula, where E-cadherin-mediated adhesion drives compaction and creates distinct outer and inner cell populations. Outer cells acquire trophoblastic identity through the Hippo-YAP-TEAD4 pathway. Reduced Hippo activity allows nuclear YAP to activate TEAD4-dependent transcription, including *Cdx2* (Nishioka et al., 2009; Rayon et al., 2014). Meanwhile, stronger Hippo activity in inner cells supports pluripotency. Blastocyst formation then involves trophoblast epithelialization and blastocoel formation, followed by separation of the inner cell mass into epiblast and primitive endoderm. OCT4 and NANOG support epiblast identity while GATA6 and SOX17 contribute to primitive-endoderm specification (Niwa et al., 2005). Thus, early lineage segregation results from the combined effects of cell position, polarity, adhesion and transcriptional regulation.

➤ Post-Implantation Development, Gastrulation and Tissue Patterning

Following implantation, the epiblast forms the embryonic disc while the primitive endoderm contributes to the yolk-sac endoderm. In the mouse, the embryo develops an egg-cylinder architecture with coordinated formation of amniotic and extraembryonic compartments. Lumenogenesis and epithelial polarization accompany this tissue reorganization. Gastrulation begins with primitive-streak formation and EMT, during which epiblast cells undergo transcriptional and cytoskeletal changes before migrating through the streak to generate mesoderm and definitive endoderm (Arnold & Robertson, 2009). WNT- β -catenin and NODAL signalling are central to primitive-streak specification, while FGF-ERK and BMP pathways support lineage competence and tissue patterning (Kelly et al., 2004). Signals from the anterior visceral endoderm help establish the anteroposterior axis, followed by paraxial mesoderm specification, somitogenesis, neural induction, neurulation, cardiovascular differentiation, extraembryonic mesoderm development and primordial germ-cell specification. These events depend on coordinated signalling between embryonic and extraembryonic compartments (Arnold & Robertson, 2009).

➤ Stem-Cell-Based Embryo Models and Developmental Reconstruction

The need to reproduce coordinated embryonic and extraembryonic interactions has driven the development of increasingly sophisticated stem-cell-based embryo models. ETS models combine embryonic stem cells (ESCs) with trophoblast stem cells (TSCs), while ETX models additionally incorporate XEN-derived primitive-endoderm populations (Harrison et al., 2017; Sozen et al., 2018). More recent EiTiX and ETiX systems introduce induced trophoblast and primitive-endoderm states to increase developmental complexity. These models have reproduced selected features of gastrulation and post-gastrulation development, including axis formation, neurulation, somitogenesis, beating cardiac structures and yolk-sac- and chorionic-like components (Amadei et al., 2022). However, important limitations remain in trophoblast maturation and placental lineage specification. Structures corresponding to the ectoplacental cone and trophoblast giant-cell populations are still poorly represented.

These limitations indicate that developmental fidelity depends on the appropriate integration of cellular composition, transcriptional competence, spatial organization and tissue-level signalling Langkabel et al. (2021). Consequently, current

embryo-model research increasingly views embryogenesis as an interconnected gene-regulatory and mechanochemical system rather than as a simple sequence of independent differentiation events.

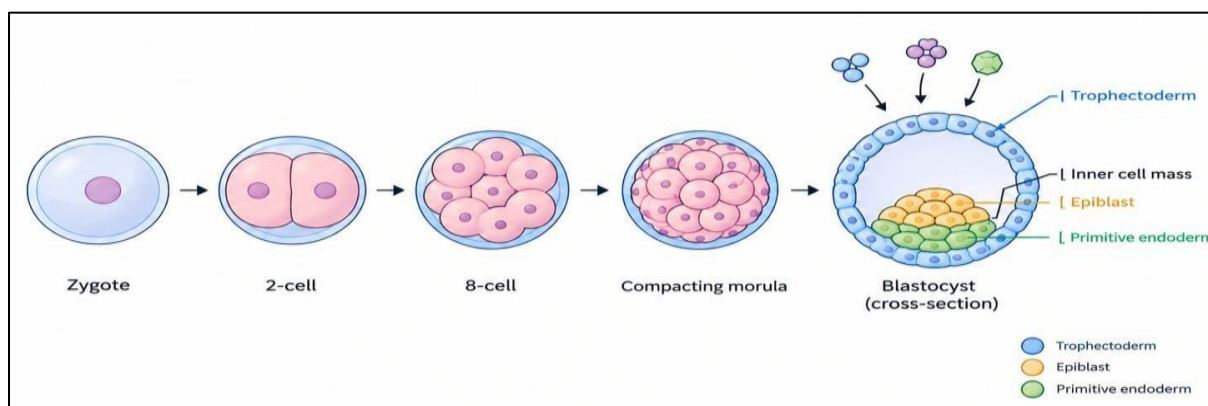


Fig 1 Lineage Decision Schematic from Zygote to Blastocyst, Highlighting Trophectoderm-ICM Segregation.

A. Pluripotency Continuum and Embryonic-Extraembryonic Cellular Building Blocks

Mammalian embryogenesis begins with a totipotent zygote and proceeds through progressive lineage restriction and tissue specification. Cleavage and compaction establish the first spatial organization, followed by Hippo-YAP-TEAD4-mediated trophectoderm specification and segregation of the inner cell mass into epiblast and primitive endoderm. Implantation then initiates extensive morphogenetic remodelling that establishes embryonic and extraembryonic architecture and primes the conceptus for gastrulation (Arnold & Robertson, 2009; Nishioka et al., 2009).

➤ Early Embryogenesis, Compaction and Blastocyst Lineage Specification

Early mammalian development involves progressive cell organization and lineage restriction. After fertilization, cleavage produces the 2-cell, 4-cell, 8-cell and morula stages without a major increase in embryo size. At the morula stage, E-cadherin-mediated adhesion drives compaction and separates the cells into outer and inner populations. Outer cells acquire trophectoderm identity through the Hippo-YAP-TEAD4 pathway, with CDX2 supporting trophoblast specification (Nishioka et al., 2009; Rayon et al., 2014), while stronger Hippo activity in inner cells helps maintain pluripotency. Blastocyst formation then involves trophectoderm epithelialization and segregation of the inner cell mass into epiblast and primitive endoderm. OCT4 and NANOG are associated with epiblast identity, whereas GATA6 and SOX17 contribute to primitive-endoderm specification. Overall, early embryogenesis represents a coordinated transition from broad developmental potential toward spatially organized lineage specification.

➤ Post-Implantation Development, Gastrulation and Tissue Patterning

Following implantation, extensive morphogenetic remodelling reorganizes embryonic and extraembryonic compartments. Epiblast expansion establishes the embryonic disc while primitive endoderm contributes to yolk-sac

endoderm. In the mouse, these changes generate the characteristic egg-cylinder architecture accompanied by epithelial polarization and lumenogenesis. Gastrulation begins with primitive-streak formation and EMT-driven epiblast delamination. WNT- β -catenin and NODAL signalling establish streak identity while FGF-ERK and BMP pathways regulate lineage competence and tissue patterning (Arnold & Robertson, 2009; Kelly et al., 2004). Subsequent axial patterning drives mesodermal diversification, somitogenesis, neurulation and cardiovascular specification. Reciprocal signalling between embryonic and extraembryonic tissues coordinates these processes and establishes the spatial framework required for later organogenesis.

➤ Stem-Cell-Based Embryo Models and Reconstruction of Developmental

The interdependence of embryonic and extraembryonic tissues has driven the development of increasingly advanced stem-cell-based embryo models (SCBEMs). ETS models combine embryonic stem cells (ESCs) with trophoblast stem cells (TSCs) to reproduce interactions between embryonic and trophoblast lineages. ETX models further incorporate XEN-derived primitive-endoderm-like cells and therefore provide a broader representation of extraembryonic signalling (Harrison et al., 2017; Sozen et al., 2018). More recent ETiX and EiTiX systems introduce induced trophoblast and primitive-endoderm states and can reproduce developmental features such as axis formation, neurulation, somitogenesis and cardiac activity together with yolk-sac-like and chorionic structures (Amadei et al., 2022). However, trophoblast maturation remains incomplete. Ectoplacental-cone derivatives and trophoblast giant cells are poorly represented in many models. These limitations show that faithful embryo modelling requires more than the correct cell types. It also depends on cellular composition, developmental competence, spatial organization and tissue-level signalling. Current SCBEM research therefore increasingly considers embryogenesis as an integrated gene-regulatory and mechanochemical process in which biochemical signalling, adhesion, polarity, mechanical forces and self-organization

jointly shape developmental trajectories.

B. Evolution of Stem Cell-Based Embryo Models: From Embryoid Bodies to Multilineage Morphogenetic Systems

The evolution of SCBEMs has moved from simple pluripotent stem-cell aggregates toward increasingly organized and programmable developmental systems. Embryoid bodies first showed that pluripotent cells can self-organize in three dimensions. Gastruloids added greater control over symmetry breaking and axial patterning but lacked major extraembryonic compartments (Beccari et al., 2018; van den Brink et al., 2014). The development of ETS and ETX models then introduced trophoblast and primitive-endoderm components and improved the reconstruction of embryonic-extraembryonic interactions (Sozen et al., 2018; Harrison et al., 2017). Later ETiX and EiTiX systems enabled more controlled generation of trophoblast-like and primitive-endoderm-like populations through transcription-factor-mediated reprogramming. These models reproduced several

post-implantation features, including primitive-streak formation, EMT, axis establishment, somite formation and cardiac activity, but still showed incomplete trophoblast and placental maturation (Amadei et al., 2022; Lau et al., 2022).

Current SCBEM research is moving beyond embryo-like morphology toward programmable developmental states. CRISPR-based gene regulation, transgene-free approaches and chemically induced pluripotent or totipotent-like states provide greater control over developmental competence. At the same time, bioengineering approaches are being used to influence ECM interactions, morphogen distribution, tissue geometry and mechanical inputs. The overall direction is therefore shifting toward models that reproduce not only embryonic structure but also the underlying gene-regulatory networks, interlineage signalling, spatial organization and mechanobiological mechanisms that drive mammalian development.

Table 1 Comparative Overview of SCBEMs, Summarizing Cellular Composition, Developmental Output, and Principal Fidelity Limitations Across the Embryoid body-to ETiX/EiTiX Progression.

SCBE Models	Cellular Composition	Key Developmental output	Principal Fidelity bottleneck	Reference(s)
Embryoid Body	Pluripotent ESC/EC aggregates (Undirected)	Spontaneous three germ layer differentiation; no defined axis	Absent extraembryonic lineages; no reproducible spatial organization	Ten Berge et al., 2008
Gastruloid	Pluripotent ESC aggregates (Wnt-pulsed)	Axial elongation, symmetry breaking and germ-layer specification	Limited extraembryonic integration	Beccari et al., 2018; van den Brink et al., 2014
ETS	ESC + TSC	Embryonic-trophoblast compartmentalization and early gastrulation-like activity	Primitive-endoderm deficiency	Harrison et al., 2017
ETX	ESC + TSC + XEN	Lumenogenesis, gastrulation, anteroposterior patterning	Incomplete extraembryonic (trophoblast/endoderm) maturation	Sozen et al., 2018; Zheng et al., 2022
ETiX / EiTiX	ESC + induced TSC/XEN (iTSC/iXEN)	Programmable lineage specification; neurulation; somitogenesis; cardiac activity	Incomplete placental lineage specification	Amadei et al., 2022; Lau et al., 2022

III. ETS AND ETX EMBRYOIDS

ETS embryoids represented an important step from simple stem-cell aggregates toward integrated embryonic-extraembryonic models. They combine ESCs with TSCs, which can self-organize into spatially distinct compartments under extracellular-matrix-supported conditions. These

models reproduce features of early post-implantation development, including cell sorting, epithelialization and proamniotic cavity formation. They also show that extraembryonic tissues actively participate in developmental signalling through reciprocal interactions between ESCs and TSCs (Harrison et al., 2017).

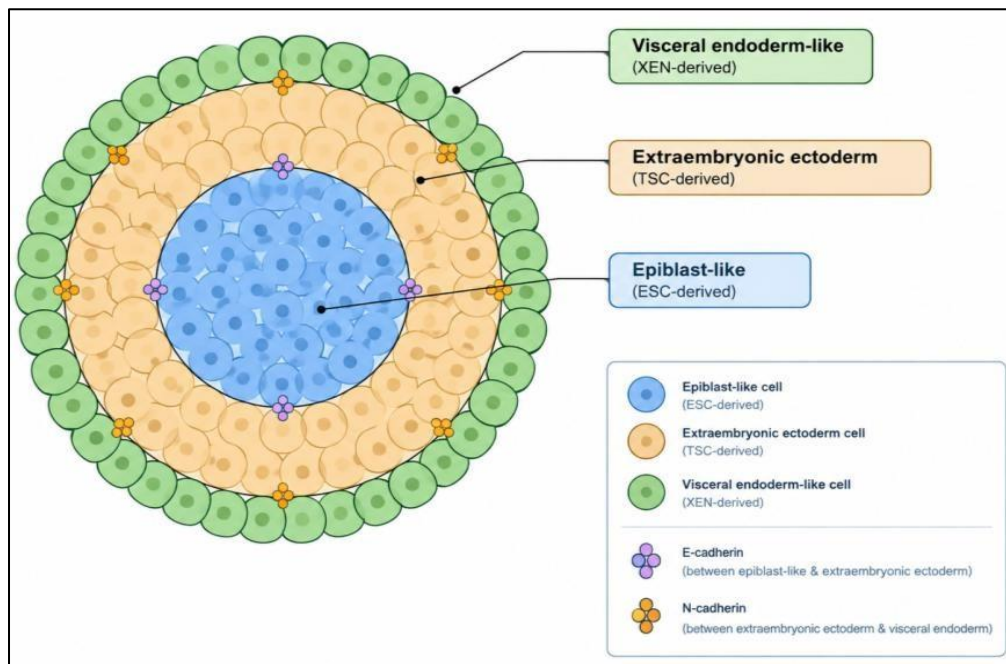


Fig 2 Three-Lineage Compartmental Organization of The ETX Embryoid Model.

ETS models can further show gastrulation-like activity, with Brachyury expression indicating mesoderm specification and populations expressing TFAP2C, OCT4 and STELLA resembling primordial germ-cell-like states. ETS-blastoid systems can also form blastocyst-like structures under defined aggregation conditions (Rivron et al., 2018). However, the absence of a genuine primitive-endoderm compartment limits visceral-endoderm signalling and therefore restricts robust axis formation and sustained gastrulation (Harrison et al., 2017; Sozen et al., 2018).

The development of ETX models addressed this limitation by incorporating XEN-derived primitive-endoderm-like cells. This produced a three-lineage architecture containing ESC-derived epiblast-like cells, TSC-derived extraembryonic ectoderm and an XEN-derived visceral-endoderm-like layer (Fig 2). The additional compartment improves lumenogenesis, epithelial polarization, tissue organization and interlineage signalling and supports developmental features including symmetry breaking, mesoderm and definitive-endoderm specification and primordial germ-cell-like states (Sozen et al., 2018; Amadei et al., 2022; Zheng et al., 2022).

ETX development also showed that embryoid fidelity depends on cell adhesion, spatial organization and tissue mechanics in addition to lineage composition. Differential E-cadherin, P-cadherin and K-cadherin expression contributes to compartmentalization, while XENC-derived endoderm reduces dependence on exogenous extracellular matrix and supports alternative culture approaches (Bao et al., 2022). Nevertheless, incomplete primitive-endoderm lineage diversity, deficient parietal yolk-sac development and limited trophoblast maturation remain important limitations. Overall, these findings established that accurate SCBEM development requires coordinated lineage identity, spatial positioning, interlineage signalling, differential adhesion and tissue-level

organization, leading toward the more programmable ETiX and EiTiX systems (Sozen et al., 2018; Zheng et al., 2022).

A. EiTiX/ETiX Embryoids

EiTiX and ETiX embryoids represent a further advance toward programmable SCBEMs by integrating controlled lineage specification with autonomous tissue organization. The progression from ETS to ETX and then ETiX/EiTiX has increased control over embryonic and extraembryonic lineage composition. ETX models demonstrated that coordinated ESC-TSC-XEN interactions can support EMT, definitive-endoderm specification and anteroposterior organization. Subsequent GATA4- and CDX2-mediated induction enabled programmable generation of primitive-endoderm-like and trophoblast-like populations (Amadei et al., 2022; Lau et al., 2022). These systems provide tractable platforms for investigating lineage specification, inter-tissue signalling and self-organization while still showing limitations in trophoblast differentiation and placental maturation.

➤ Transcription-Factor-Mediated Generation of iTSCs/iXENCs and Multilineage Embryoid Assembly

ETiX and EiTiX systems use transcription-factor-mediated lineage conversion to generate extraembryonic cell states from pluripotent stem cells. GATA4 induction produces primitive-endoderm-like iXEN populations while CDX2 induction generates trophoblast-like iTSC populations. These induced populations can replace naturally derived XEN and TSC cells within multilineage embryo models (Amadei et al., 2022; Lau et al., 2022) (Fig 3). Although they improve control over lineage composition and support embryo-like morphogenesis, induced identity does not always reflect complete developmental equivalence. Developmental history and molecular state can influence extraembryonic lineage diversity and morphogenetic competence (Lau et al., 2022).

➤ *Spatial Self-Organization, Epithelialization, Lumenogenesis and Compartmental Patterning*

Following assembly, ETiX and EiTiX embryoids undergo coordinated epithelialization, compartmental segregation and lumenogenesis that establish the basic architecture required for post-implantation-like morphogenesis (Amadei et al., 2022). GATA4-induced endoderm-like populations further support organized anterior visceral endoderm migration and regional tissue patterning,

➤ *Gastrulation, Symmetry Breaking, Anteroposterior*

highlighting the role of extraembryonic compartments in embryonic organization. These processes are not controlled by transcriptional specification alone. Lineage-specific cadherin expression, cell adhesion and cortical mechanics also influence tissue sorting, cellular positioning and compartment stability. Thus, epithelialization and lumenogenesis emerge through the combined effects of cell identity, polarity, adhesion, mechanical forces and intercellular signalling.

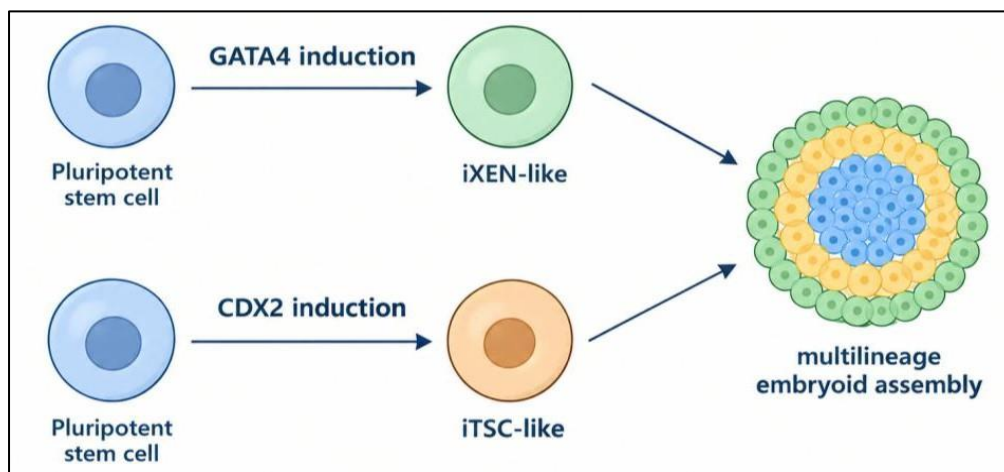


Fig 3 Transcription-Factor-Mediated Reprogramming of iTSCs and iXENCs for Multilineage Embryoid Assembly.

➤ *Axis Establishment and Germ-Layer Patterning*

ETX and ETiX embryoids can undergo gastrulation-associated morphogenesis through coordinated interactions between embryonic and extraembryonic compartments. XEN-containing systems support EMT, mesoderm and definitive-endoderm specification, while GATA4-induced systems enhance anterior visceral endoderm migration and anteroposterior organization. WNT, NODAL, BMP and FGF signalling act together with cellular-state transitions to regulate primitive-streak formation and germ-layer specification. These models therefore capture aspects of the spatial and temporal organization of gastrulation, although their molecular and cellular fidelity remains incomplete (Amadei et al., 2022).

➤ *Neurulation, Somitogenesis, Organ Primordia and Cardiovascular Morphogenesis*

Following gastrulation, ETiX and EiTiX embryoids can reach E8.0-E8.5-like stages with neural structures, somites, cardiac regions and extraembryonic tissues (Fig 4). Ex utero culture further supports neural-tube formation, foregut development, axial organization and beating cardiac structures (Lau et al., 2022). However, morphological similarity alone does not confirm physiological equivalence, making single-cell and spatial transcriptomic benchmarking essential for assessing developmental fidelity.

➤ *Chorionic and Trophoblast Maturation, Placental Lineage Specification and Developmental Fidelity*

Trophoblast maturation and placental lineage specification remain major limitations of ETiX and EiTiX embryoids. Although CDX2-induced cells can contribute to chorion-like structures, they do not fully reproduce ectoplacental-cone, trophoblast giant-cell and spongiotrophoblast populations. This indicates that transient transcription-factor induction alone cannot recapitulate complete trophoblast development. Developmental fidelity therefore depends on lineage history, molecular state, spatial organization and temporal progression rather than marker expression or morphology alone (Amadei et al., 2022). Emerging embryo-founder-cell systems with broader organogenic potential further indicate a shift toward models capable of reconstructing more extensive embryonic and extraembryonic development.

IV. TRANSCRIPTIONAL CIRCUITS OF TROPHECTODERM SPECIFICATION AND DEVELOPMENTAL CELL-FATE ALLOCATION

Trophectoderm specification is governed by an integrated regulatory network involving CDX2, OCT4, SOX2, NANOG, TEAD4, YAP and TFAP2C together with polarity-associated mechanisms. Rather than functioning as a binary switch, CDX2 expression is dynamically regulated during early.

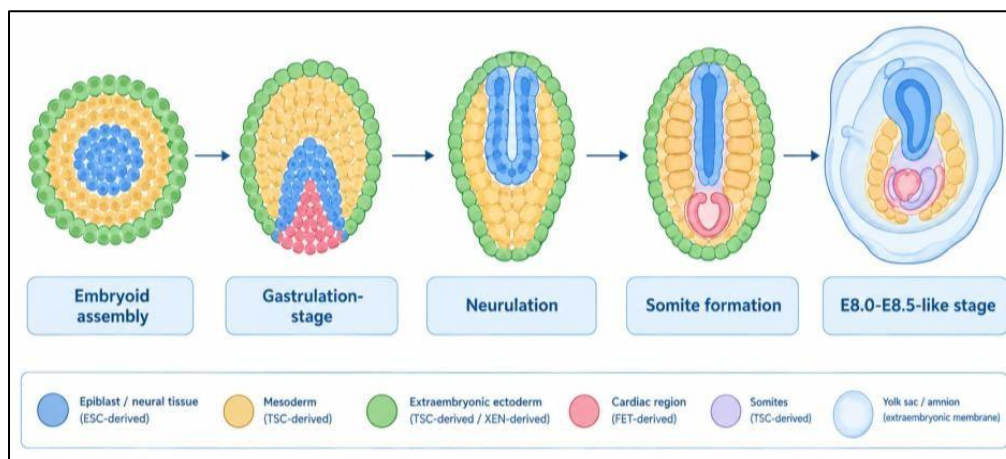


Fig 4 Developmental Timeline of ETiX/EiTIX Morphogenesis from Assembly to Organogenesis-Stage Milestones.

➤ *Extraembryonic Morphogenesis, Yolk-Sac Development, Hemato-Endothelial Specification and Early Hematopoiesis*

Advanced ETiX and EiTiX systems can form extraembryonic structures resembling the yolk sac, allantois and amnion along with blood-island-like regions and early hemato-endothelial development. Some models also show yolk-sac-associated blood circulation suggesting coordination between extraembryonic vascular development and embryonic cardiovascular morphogenesis. However, hematopoietic maturation remains incomplete and the lack of a mature placental-maternal interface limits further physiological development. Differences between endogenous TSC-based ETiX and CDX2-induced EiTiX systems further indicate that extraembryonic progenitor identity strongly influences developmental outcomes. cleavage development (Rayon et al., 2014). Its enrichment in outer blastomeres is influenced by cleavage geometry and cell polarity and contributes to trophectoderm lineage allocation. The Hippo-YAP-TEAD4 pathway provides an important link between positional and mechanical information and transcriptional regulation (Nishioka et al., 2009). Reduced Hippo activity promotes nuclear YAP-TEAD4 activity and supports Cdx2 expression. Notch signalling further contributes to the stabilization of the trophectodermal programme. As trophoblast identity becomes established, CDX2 cooperates with transcription factors including EOMES, GATA3, TFAP2C and ELF5 while progressively opposing the pluripotency-associated OCT4-SOX2-NANOG network (Yagi et al., 2007; Home et al., 2012).

Importantly, CDX2 activity is not uniform throughout early development. Its effects depend on cellular context, developmental stage and signal intensity. Transient CDX2 activity can influence early blastomere behaviour while sustained activity supports trophoblast differentiation (Strumpf et al., 2005; Niwa et al., 2005). Trophectoderm allocation therefore emerges from the coordinated interaction of transcriptional regulation, cell polarity, positional information, signalling networks and tissue mechanics (Wicklow et al., 2014). This regulatory complexity helps explain why CDX2-based SCBEMs can successfully generate trophoblast-like compartments while still showing limitations in achieving complete trophoblast maturation and placental

development (Strumpf et al., 2005; Ralston et al., 2010).

B. Signaling, Adhesion and Mechanobiology of Embryo Self-Organization

Embryonic morphogenesis is governed by the coordinated interplay of biochemical signalling, cell-cell adhesion, cellular polarity, cytoskeletal dynamics and tissue mechanics rather than by transcriptional regulation alone. This coordination is particularly important in stem-cell-based embryo models, where complex developmental structures must emerge through the self-organization of defined cellular populations. Major signalling pathways including WNT/ β -catenin, FGF-ERK, BMP-NODAL and Activin/TGF- β provide positional information and influence lineage competence (Morgani & Hadjantonakis, 2020). These molecular signals are translated into physical changes through adhesion molecules, cortical tension and extracellular matrix interactions. Such mechanisms regulate cell sorting, epithelialization, lumenogenesis, cell migration and tissue deformation during morphogenesis. Evidence from synthetic embryo systems further indicates that self-assembly can be influenced by lineage-specific cadherin expression and cortical tension. This suggests that physical interactions between neighbouring cells are not merely structural consequences of differentiation (Bao et al., 2022). They actively contribute to developmental patterning and tissue organization. Thus, understanding SCBEM development requires consideration of signalling networks together with the adhesive, mechanical and biophysical properties that determine how individual cells collectively generate organized embryonic structures.

➤ *WNT/ β -Catenin, FGF-ERK, BMP-NODAL and Activin/TGF- β Signalling*

Embryonic development is regulated through the coordinated activity of several interconnected signalling pathways rather than by a single pathway acting independently. WNT/ β -catenin, FGF-ERK, BMP, NODAL and Activin/TGF- β signalling collectively regulate lineage specification, symmetry breaking, gastrulation and germ-layer formation. Their developmental effects depend strongly on the timing, spatial distribution and cellular context in which these signals are received. In SCBEMs, reproducing this precise signalling environment is therefore essential for

obtaining appropriate developmental organization (Tanaka et al., 1998; Murohashi et al., 2010).

Communication between embryonic and extraembryonic tissues also occurs through these signalling networks. Activin/TGF- β signalling can influence trophoblast cavitation while FGF4 signalling contributes to trophoblast development. During gastrulation, coordinated WNT, BMP, NODAL and FGF activity support primitive-streak formation and epithelial-mesenchymal transition (EMT) (Arnold & Robertson, 2009; Morgani & Hadjantonakis, 2020). Thus, developmental signalling cannot be accurately reproduced simply by exposing cells to uniform concentrations of morphogens. The spatial and temporal coordination of signalling is essential because embryonic development depends on localized signalling environments that guide cell fate and tissue organization.

➤ *Cell Adhesion, Polarity and Tissue Mechanics*

Developmental signals are translated into physical changes in tissues through cell adhesion, polarity, actomyosin contractility and extracellular matrix interactions. E-cadherin-mediated adhesion together with cortical tension helps blastomeres become more closely organized during compaction. In synthetic embryo models, differences in cadherin expression between embryonic and extraembryonic populations can also influence their spatial sorting and contribute to proper tissue arrangement (Bao et al., 2022).

These mechanical processes act together with biochemical signalling to regulate cell sorting, epithelialization, lumenogenesis, cell migration and tissue deformation. During gastrulation, changes in actomyosin activity and cellular polarity support epithelial-mesenchymal transition (EMT) and enable cells to migrate through the primitive streak. Embryonic development can therefore be viewed as a dynamic feedback system in which cell identity influences adhesion and mechanics, while tissue organization feeds back into signalling and subsequent cell specification.

V. INTEGRATED BIOCHEMICAL AND MECHANOCHEMICAL REGULATION OF EMBRYONIC MORPHOGENESIS

The coordinated interaction between biochemical signalling and mechanical forces drives embryonic morphogenesis. Developmental signalling through WNT- β -catenin, FGF-ERK, BMP, NODAL and Activin-TGF- β pathways establish positional information and regulates lineage specification. These signals operate together with cell-cell adhesion, extracellular matrix (ECM) interactions and cytoskeletal remodelling to shape developing tissues (Gjorevski & Nelson, 2010). E-cadherin-mediated adhesion links neighbouring cells to the actomyosin cytoskeleton and contributes to changes in cortical tension, cell contractility, and epithelial organization (Lecuit et al., 2011). Such interactions influence cell sorting, tissue compaction, lumen formation and morphogenetic rearrangement. During gastrulation, coordinated changes in polarity, junctional tension and actomyosin dynamics facilitate epithelial-mesenchymal transition and directional cell movement. The

resulting changes in tissue architecture can then modify cellular signalling through mechanochemical feedback, creating a reciprocal relationship between biochemical regulation and tissue mechanics. Consequently, developmental outcome is influenced not only by molecular signalling but also by cell density, tissue geometry, ECM composition and mechanical state. In SCBEMs, these parameters can substantially affect morphogenesis even when major signalling pathways are appropriately activated. A mechanistic understanding of embryonic development therefore requires integration of morphogen gradients, mechanotransduction, ECM remodelling, cytoskeletal dynamics and controlled signalling environments to reproduce the spatial and mechanical conditions that govern mammalian tissue organization (Bao et al., 2022).

➤ *Future Perspectives: Toward Programmable, Predictive, Epigenetically Stable and Physiologically Integrated Embryo Models*

Stem-cell-based embryo modelling is shifting from reproducing embryo-like morphology toward programmable developmental engineering. Transgene-free approaches and chemically induced pluripotent or totipotent-like states suggest that developmental competence can be actively engineered rather than generated only through predefined embryonic and extraembryonic populations (Mazid et al., 2022). Emerging strategies combining CRISPRa/CRISPRi, transient epigenome editing and chemically controlled signalling may provide greater control over lineage trajectories while retaining endogenous developmental feedback (Li et al., 2024). The starting cellular state is also critical because naïve, primed and totipotent-like states differ in developmental competence (Wu & Wang, 2026) (Fig 5). Therefore, SCBEM fidelity should increasingly be evaluated through molecular, cellular and structural benchmarks rather than morphology alone.

A major challenge is establishing quantitative benchmarking frameworks that improve reproducibility across laboratories. Differences in stem-cell genotype, pluripotency state, culture conditions, aggregation geometry and signalling regimes can produce substantial variation between SCBEMs (Fu et al., 2021; Xue et al., 2025). Reference cell lines, transparent protocols, predefined efficiency measures and multi-omics benchmarks can provide more consistent assessment. Integration of live-cell imaging and artificial intelligence may further enable prediction of lineage allocation, morphogenetic progression and developmental arrest before major morphological changes occur (Martinez Arias et al., 2026). Combining single-cell, spatial and imaging datasets could also provide a multiscale view of developmental trajectories and reveal how perturbations in regulators such as CDX2, TEAD4, WNT, NODAL and FGF-ERK propagate from molecular states to tissue organization (Deiningner et al., 2026; Zhao et al., 2025).

Physiological integration represents another important direction. Although ETiX and EiTiX models reproduce several embryonic and extraembryonic features, placental maturation and embryo-maternal interactions remain incompletely represented. Future platforms may incorporate

engineered ECMs, dynamic morphogen gradients, controlled oxygen and metabolic conditions, microfluidics and stage-matched endometrial, stromal and vascular components (Xue et al., 2025). Human SCBEMs should also be benchmarked against human-specific single-cell and spatial datasets because developmental mechanisms identified in mouse models may not directly translate to human development (Weatherbee et al., 2023; Zhao et al., 2025). Increasing model complexity must therefore be accompanied by ethical oversight, transparent reporting and a clear distinction between embryo models and embryos. Ultimately, SCBEM fidelity should be assessed by their ability to reproduce developmental progression, lineage dynamics, spatial organization and environmental interactions in a quantitatively measurable and mechanistically interpretable manner (Martinez Arias et al., 2026).

VI. CONCLUSION

Stem-cell-based embryo models have become valuable experimental systems for figuring out the cellular and molecular processes that govern mammalian embryogenesis in vitro. The development of ETS and ETX models followed by ETiX and EiTiX systems has gradually improved the reconstruction of embryonic and extraembryonic interactions.

The inclusion of trophoblast and extraembryonic endoderm lineages has added further developmental complexity to these models. Advanced SCBEMs can reproduce several important features of post-implantation development, including primitive streak formation, gastrulation, anteroposterior patterning, neural differentiation, somitogenesis and yolk-sac development (Amadei et al., 2022; Lau et al., 2022). Despite these advances, SCBEMs still differ from their in vivo counterparts in several important aspects. Incomplete extraembryonic maturation and the limited representation of the embryo-maternal environment remain major constraints on developmental fidelity. Future development of these models will therefore depend on improving lineage specification, spatial organization, and tissue maturation while more accurately reconstructing developmental signalling networks (Fu et al., 2021; Wu & Wang, 2026). Integrating single-cell and spatial multi-omics, genome engineering, and quantitative morphogenetic approaches may further improve the mechanistic resolution of SCBEMs and their ability to model mammalian development. Overall, SCBEMs are becoming increasingly sophisticated platforms for investigating early developmental mechanisms and for exploring developmental disorders under controlled in vitro conditions.

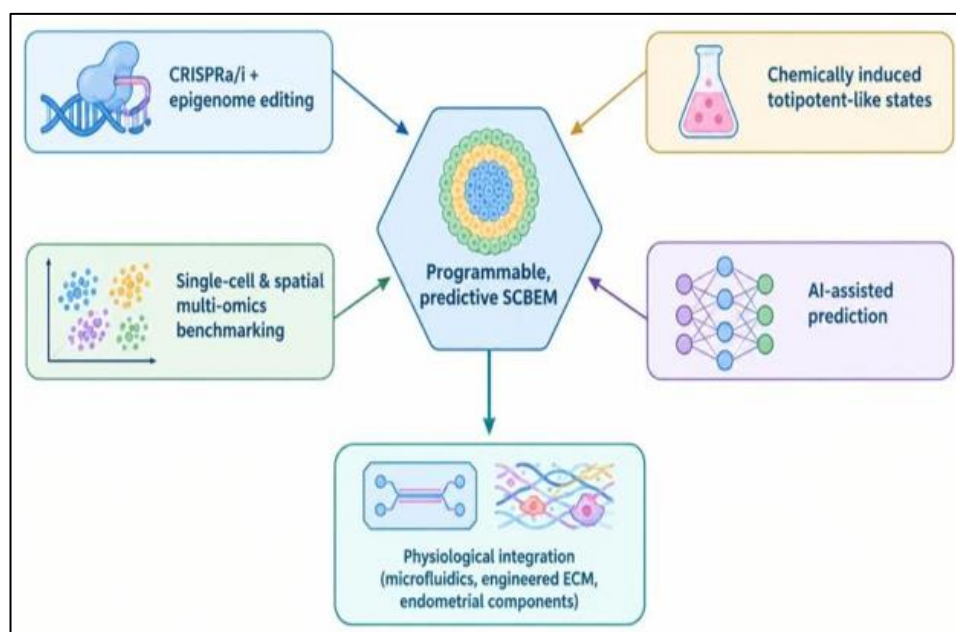


Fig 5 Emerging Strategies Toward Programmable, Predictive Stem Cell-Based Embryo Models.

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