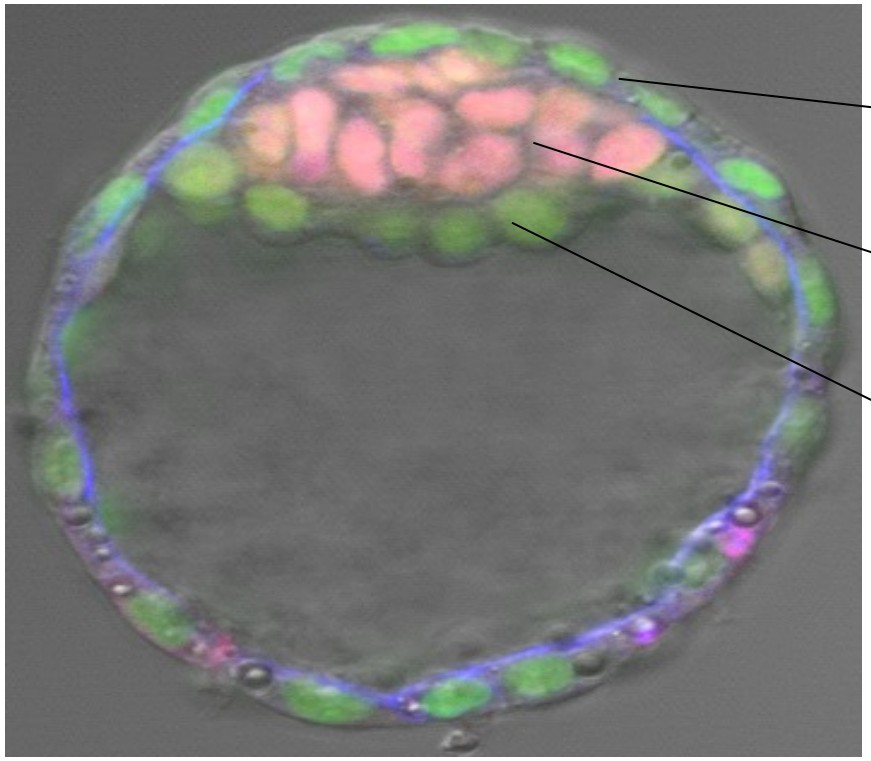
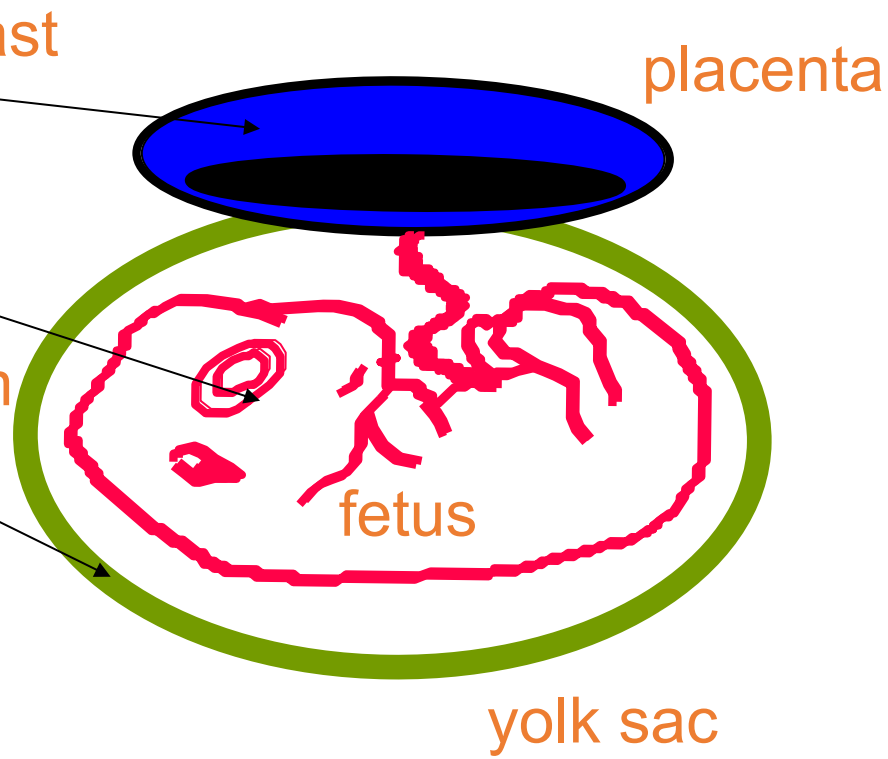
Stem-cell based embryo models:

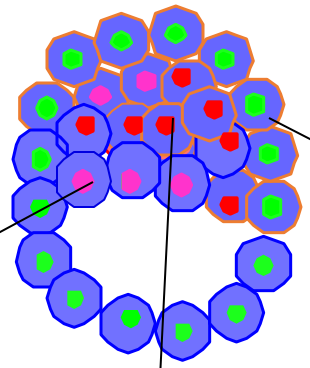
In vitro 3D cultures of pluripotent stem cells +/- other cell lines that reproducibly and robustly generate organized structures that model specific stages or structures of the in vivo embryo.

Lineages from the blastocyst





Mouse blastocyst-derived stem cells reflect in vivo lineage restriction



trophectoderm

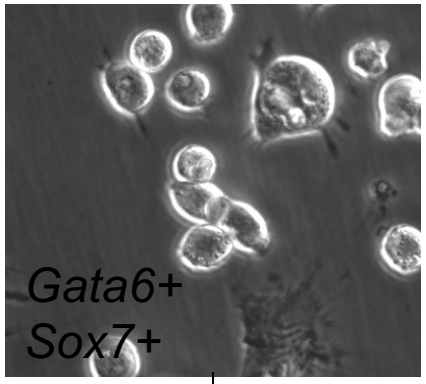
epiblast progenitor

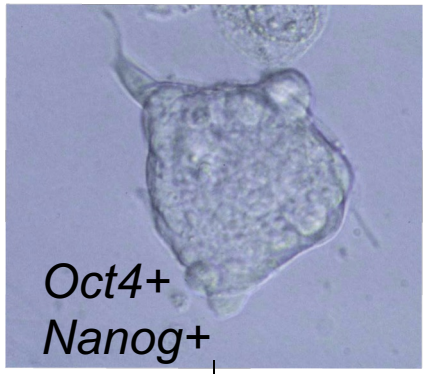
primitive endoderm progenitor

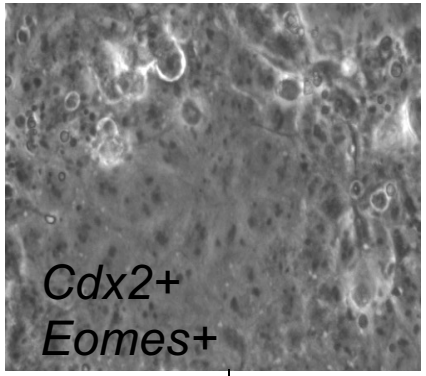
XEN

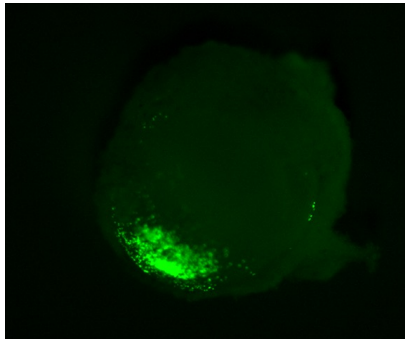
ES

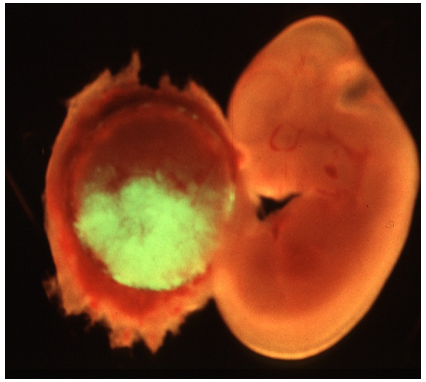
TS



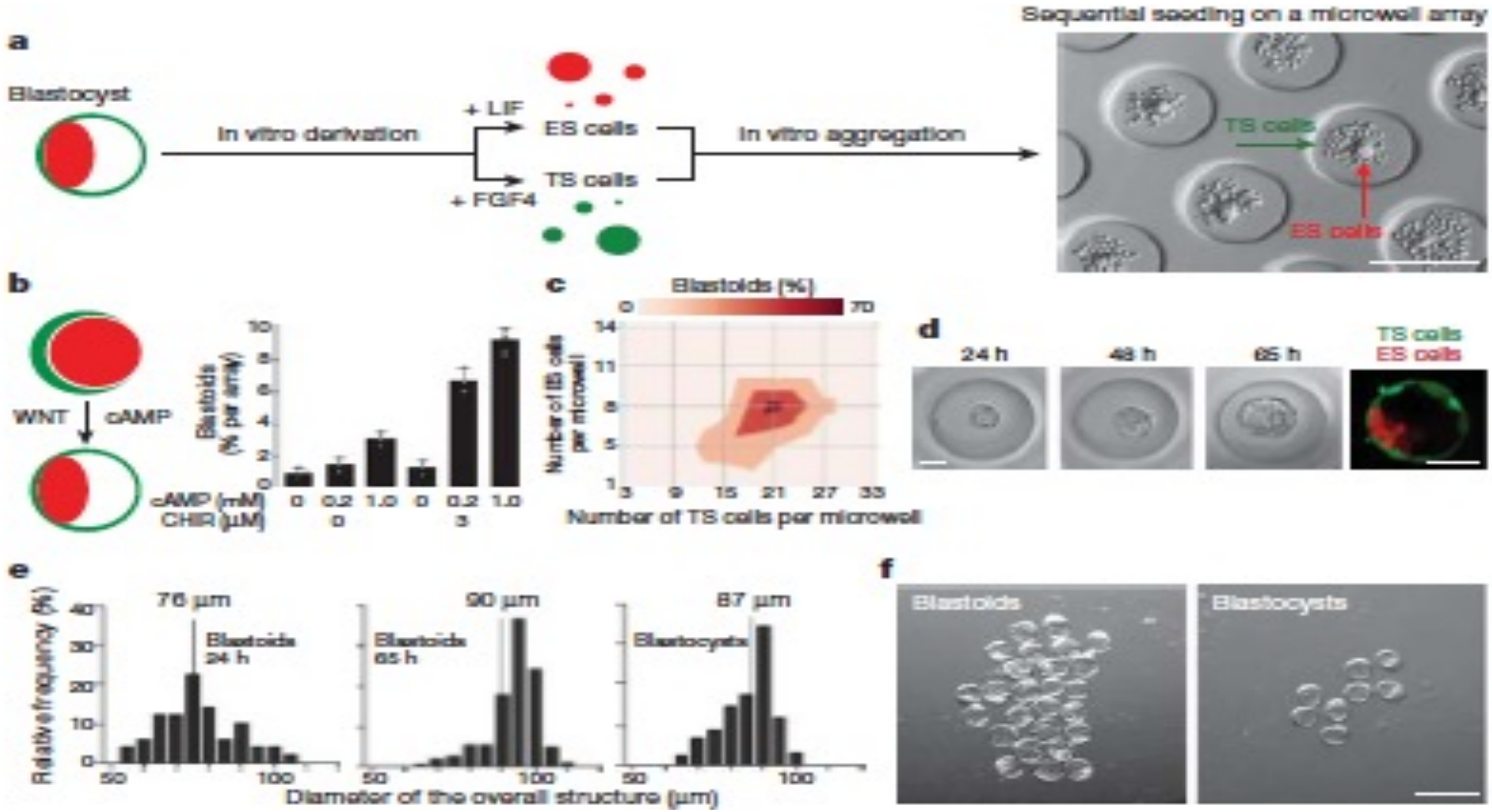








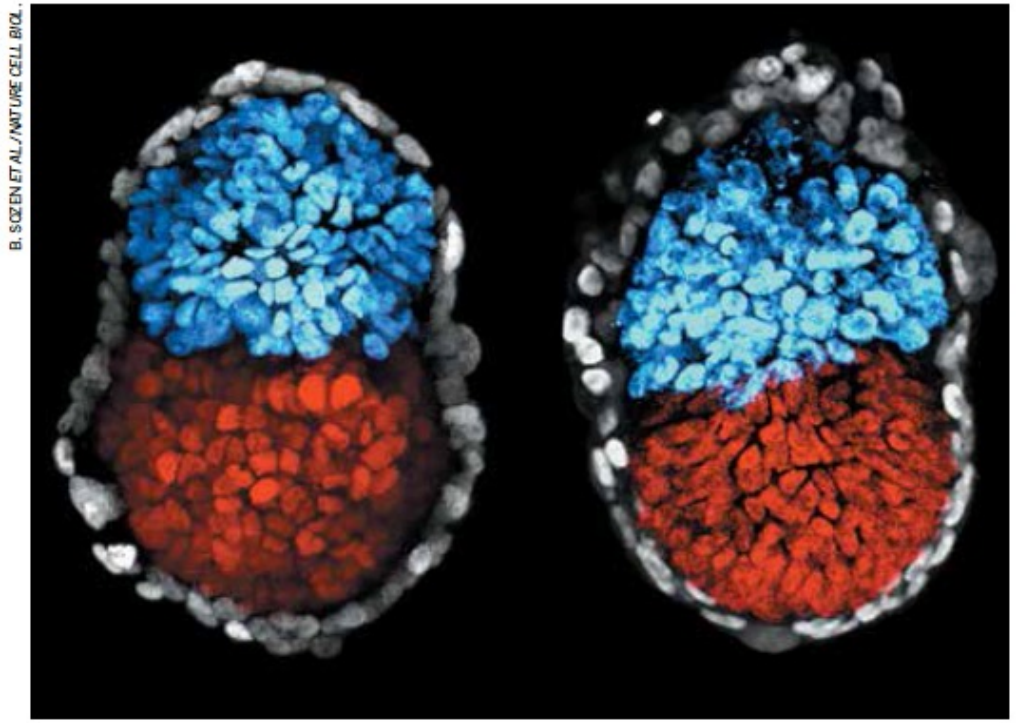
Blastoids mimic blastocyst development



Rivron et al (2018) Nature 557: 106

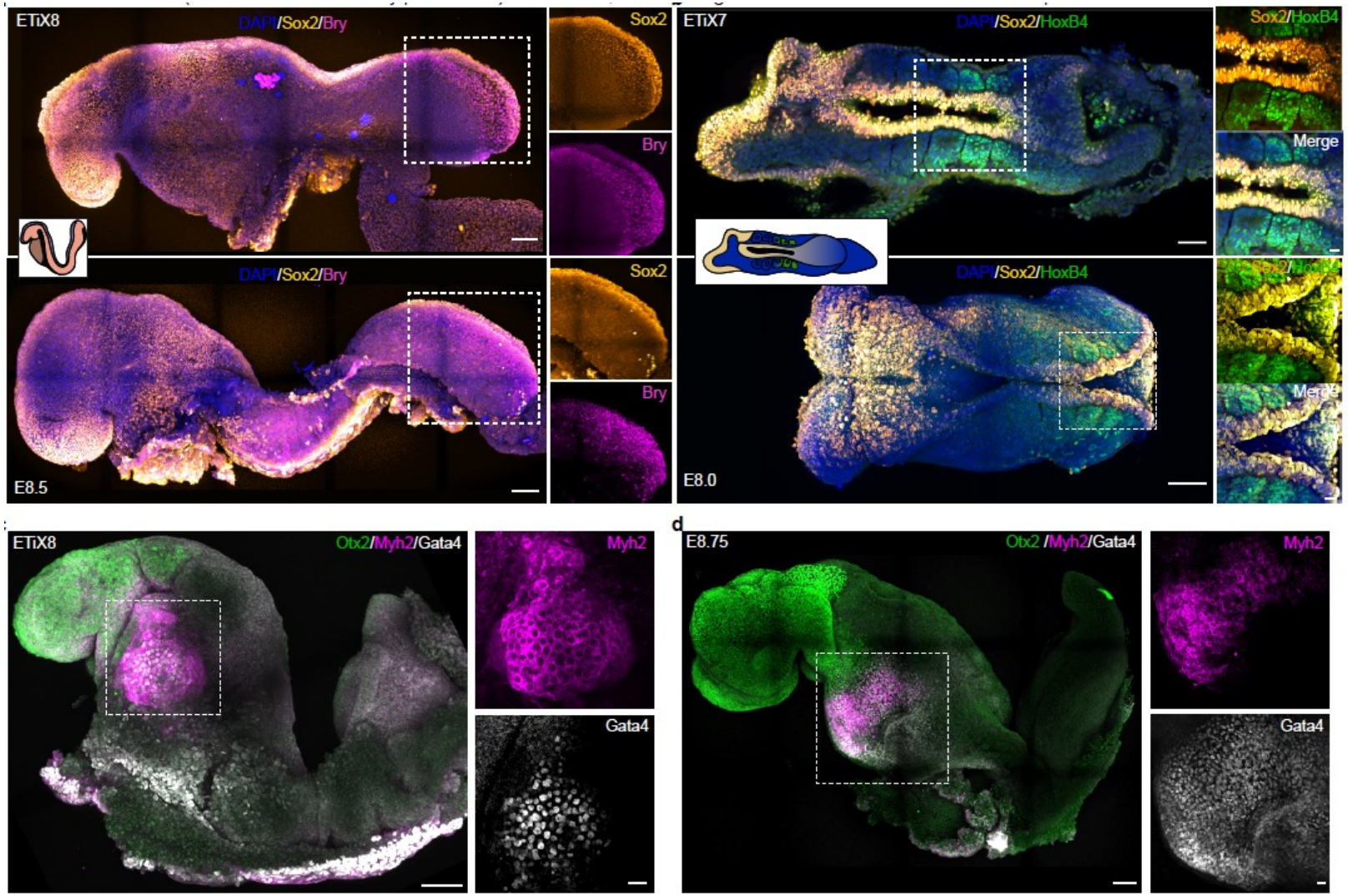
3D embryo models combining ES, TS and XEN cells mimic early postimplantation development

Zernicka-Goetz Lab



Sozen et al (2018) Nat Cell Biol 20: 1229

Neural patterning and heart formation in ETiX embryos



Amadei et al. 2022

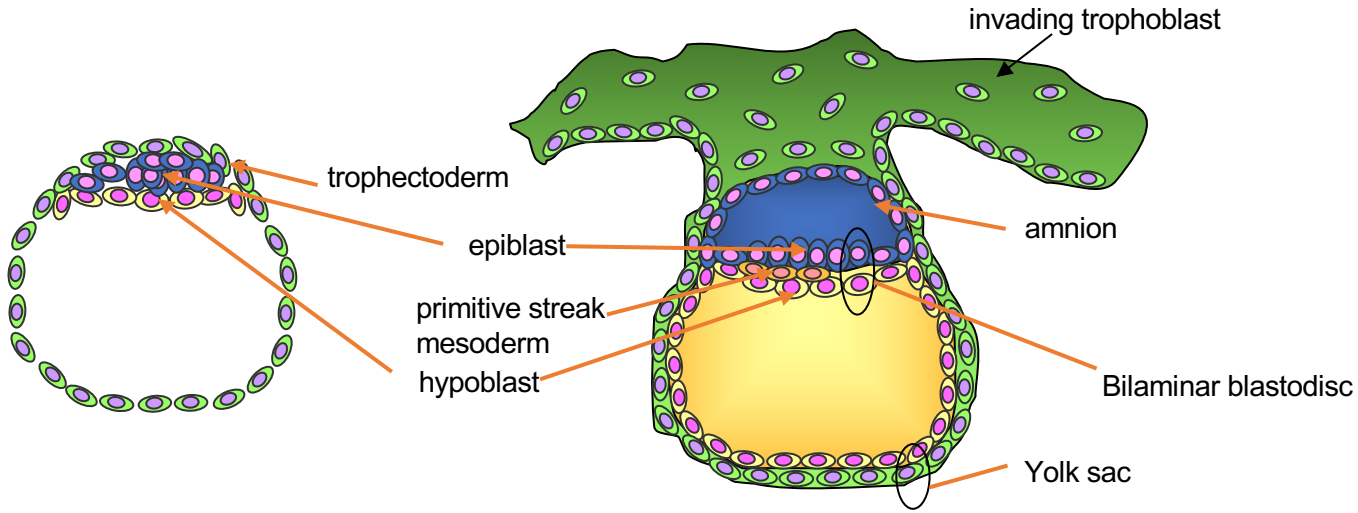
Can similar approaches be used to generate human stem cell-derived embryo models?

How far can they develop?

Why study early human development with stem cell models?

- Fundamental understanding of key stages of human development inaccessible in any other way
- Live imaging and lineage analysis to understand dynamics of development
- Assessing and improving the pluripotent stem cell state.
- Improve IVF technologies
- Model the implantation process in 3D to understand placental formation and the reasons for high early embryo loss and placental anomalies
- Assessing embryotoxicity of drugs and environmental toxins
- Assessing safety of novel reproductive technologies, eg gametes from stem cells
- Understand origin of germ cells and infertility
- Study initiation of early developmental defects
- Developmental origins of health and disease (DoHAD)

Human peri-implantation embryos and their 3D stem cell models



Blastocyst, day 5-7

Post-implantation conceptus, day 10-14

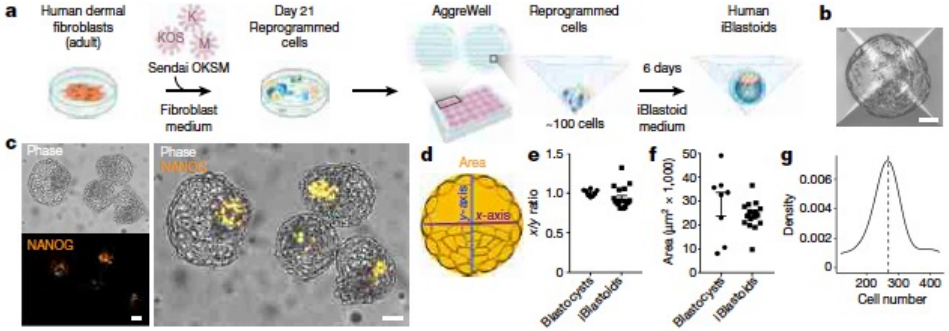
Blastoids:
epiblast, hypoblast
and trophectoderm

Gastruloids:
epiblast alone

Perigastruloids, etc:
epiblast + hypoblast

Integrated embryo models:
epiblast + hypoblast + trophoblast

Making human blastoids directly from ES cells



Article

Modelling human blastocysts by reprogramming fibroblasts into iBlastoids

https://doi.org/10.1038/s41586-021-03372-y

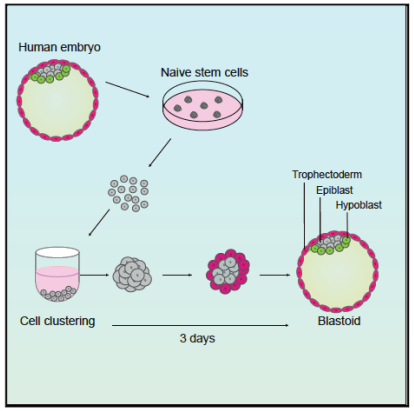
Received: 24 August 2020

Accepted: 18 February 2021

Xiaodong Liu[1,2,3,11], Jia Ping Tan[1,2,3,11], Jan Schröder[1,2,3], Asma Aberkane[3], John F. Ouyang[4], Monika Mohenska[1,2,3], Sue Mei Lim[1,2,3], Yu B. Y. Sun[1,2,3], Joseph Chen[1,2,3], Guizhi Sun[1,2,3], Yichen Zhou[1,2,3], Daniel Poppe[5,6], Ryan Lister[5,6], Amander T. Clark[7,8,9,10], Owen J. L. Rackham[4], Jennifer Zenker[3] & Jose M. Polo[1,2,3]✉

Naive stem cell blastocyst model captures human embryo lineage segregation

Graphical abstract



Authors

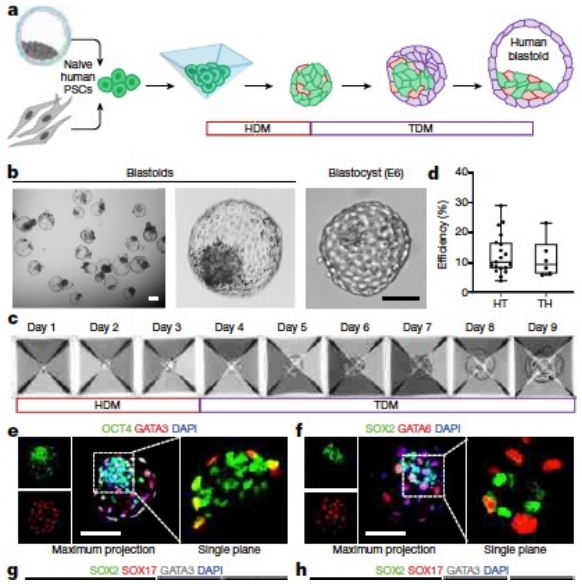
Ayaka Yanagida, Daniel Spindlow, Jennifer Nichols, Anish Dattani, Austin Smith, Ge Guo

Correspondence

austin.smith@exeter.ac.uk (A.S.), g.guo@exeter.ac.uk (G.G.)

In brief

Reliable stem cell models of the early embryo would open new approaches to studying human development and infertility. Yanagida and colleagues used naive stem cells to generate human blastocyst-like structures comprising the three founding tissue layers for the extraembryonic membranes and the embryo.



Article

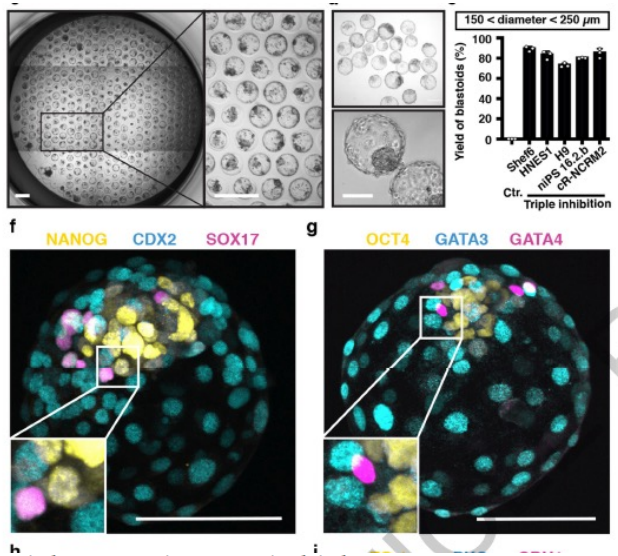
Blastocyst-like structures generated from human pluripotent stem cells

https://doi.org/10.1038/s41586-021-03356-y

Received: 24 May 2020

Accepted: 12 February 2021

Leqian Yu[1,2,10], Yulei Wei[1,3,4,10], Jialei Duan[5,10], Daniel A. Schmitz[1,2], Masahiro Sakurai[1], Lei Wang[5], Kunhua Wang[6], Shuhua Zhao[7], Gary C. Hon[5,8,9]✉ & Jun Wu[1,2]✉



Human blastoids model blastocyst development and implantation

Received: 12 February 2021

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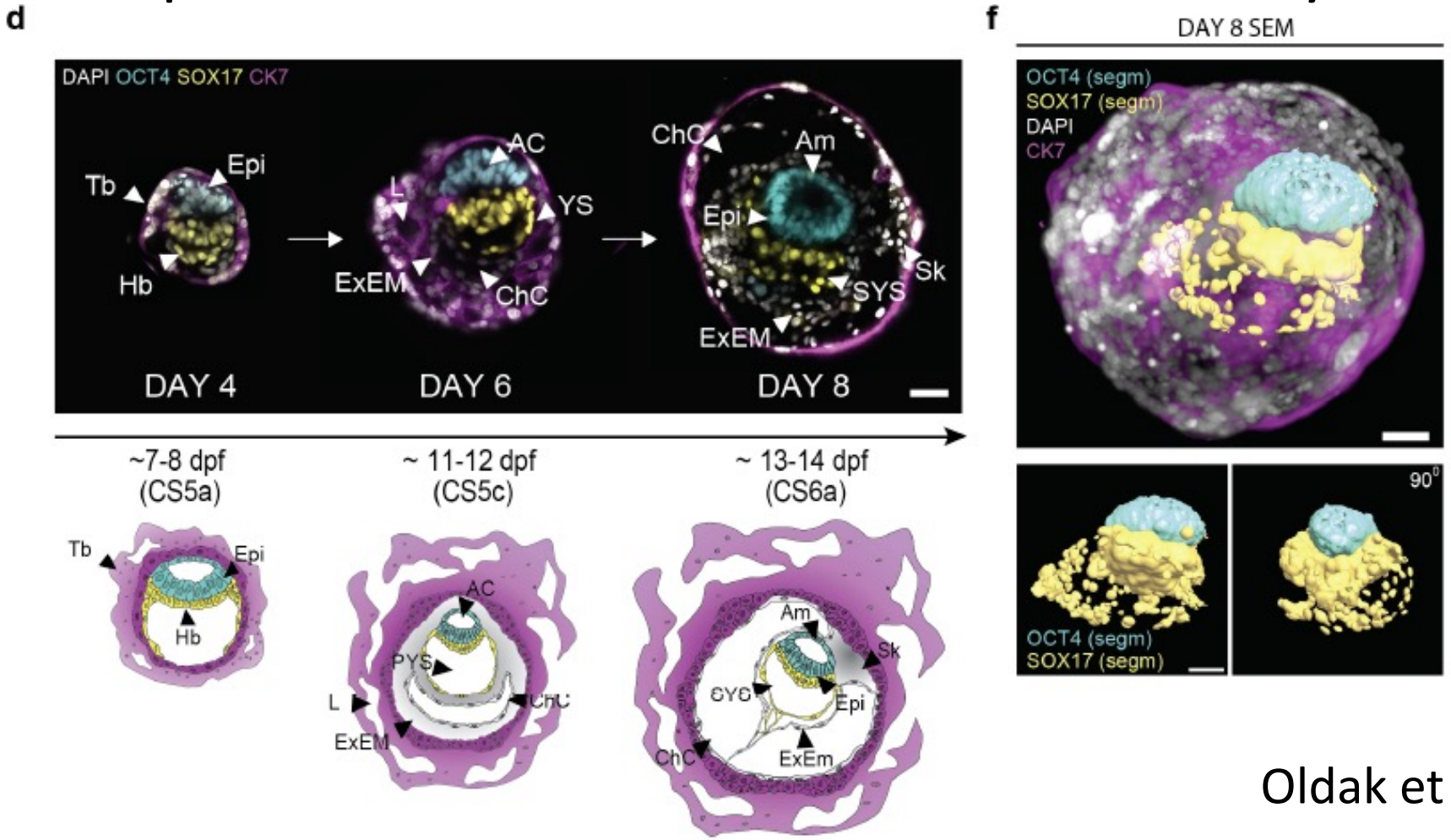
Accelerated Article Preview Published online 2 December 2021

Harunobu Kagawa, Alok Javali, Heidar Heidari Khoei, Theresa Maria Sommer, Giovanni Sestini, Maria Novatchkova, Yvonne Scholte op Reimer, Gaël Castel, Alexandre Bruneau, Nina Maenhoudt, Jenna Lammers, Sophie Loubersac, Thomas Freour, Hugo Vankelecom, Laurent David & Nicolas Rivron

More complex human stem cell-derived embryo models that mimic peri/postimplantation stages

- Weatherbee, B. A. T. et al. 2023. Pluripotent stem cell-derived model of the post-implantation human embryo. Nature. *622*, 584-593.
- Pedroza, M. et al. 2023. Self-patterning of human stem cells into post-implantation lineages. Nature *622*, 574-583.
- Oldak et al 2023. Complete human day 14 post-implantation embryo models from naive ES cells. Nature *622*, 562-573.
- Hislop, J., et al. (2023). Modeling postimplantation human development to yolk sac blood emergence. Nature, in press.
- Liu, L., *et al.* (2023). Modeling post-implantation stages of human development into early organogenesis with stem-cell-derived peri- gastruloids. Cell. *186*, 3776-3792
- And others!

Key features must include organized development similar to intact embryo



Oldak et al 2023

- Some of these models contain derivatives of all three blastocyst cell lineages but none of them is an accurate replica of the in vivo embryo
- Most will form amnion and beginning of primitive streak
- Some are used to study onset of germ cell development
- Some give rise to yolk sac and blood stem cells
- Some make the invasive trophoblast needed for implantation

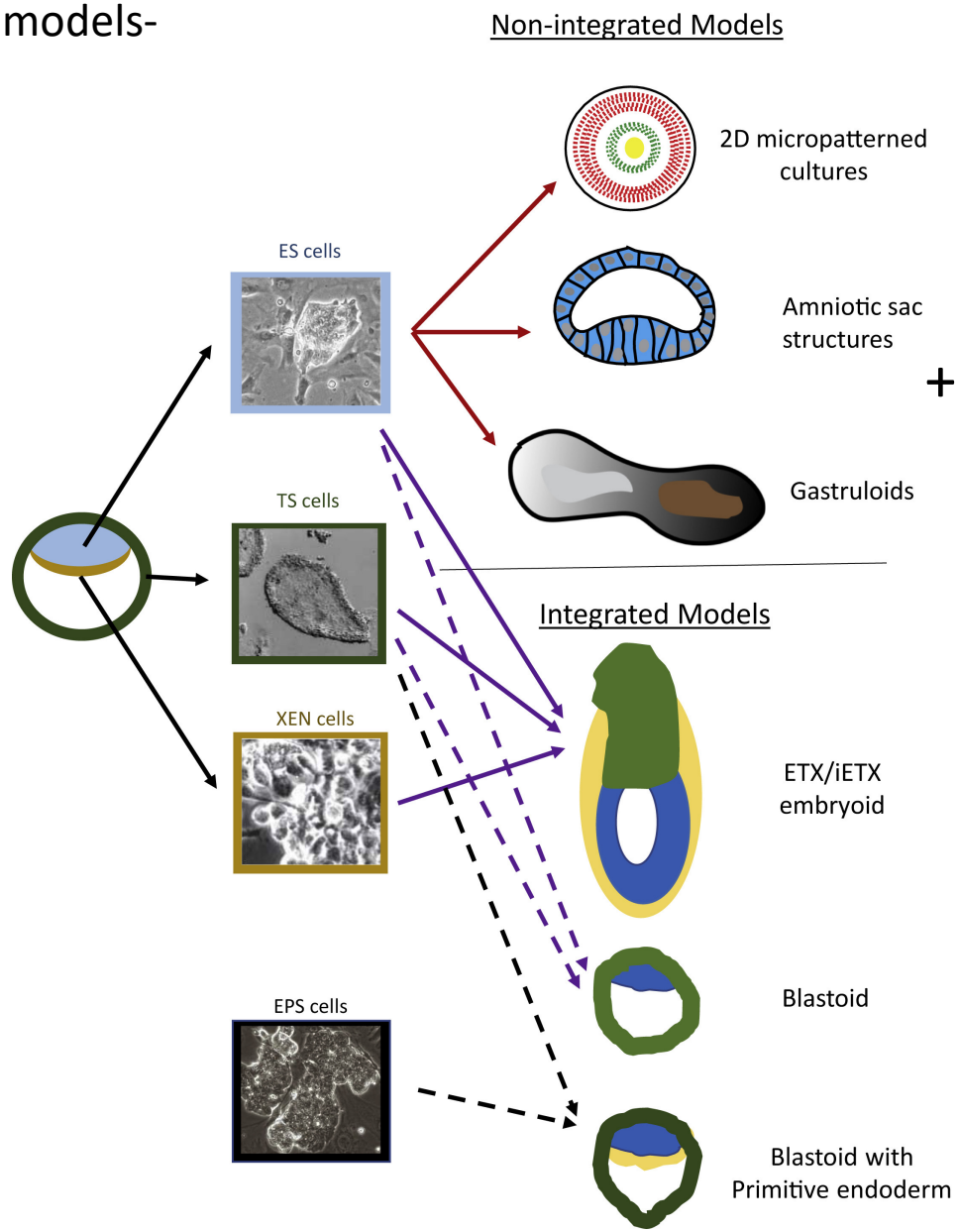
- They are models and not facsimiles of the embryo itself!

If stem cell models do not replicate normal development, are they valid models?

It depends on the scientific question being asked

Many questions in early development can be studied using defined stem cell systems without the need to replicate the entire embryo.

Integrated vs nonintegrated models-
Some examples



+ neuruloids, axoloids, somitoids, etc

Rossant and Tam Stem Cell Reports 2021

Studying phases of development in non-integrated models

- Trophoblast development and invasion at implantation
  - Trophoblastoids from ES cells or primary trophoblast combined with endometrial cells/endometrioids
- Breaking symmetry in epiblast development
  - 2D and 3D patterning of ES cells
  - Amniotic sac/microfluidics
- Epiblast-hypoblast interactions and primitive streak formation
  - Extra-embryoids, assembloids, iDiscoids etc- all approach gastrulation without TE
- Germ cell development
  - Multiple systems
- Neural tube development
  - Neurulation and neural fold formation
  - Patterning the spinal cord
- Axial mesoderm development
  - Axoloids, somitoids, replicate the segmentation clock
- Integrated neural and mesoderm axial models
  - Gastruloids

Multiple authors!

ISSCR Stem Cell Guidelines revisions 2021

- NO human stem cell-based embryo model should be transplanted into a human or animal uterus
- Integrated models should be subject to more rigorous review for scientific rationale and ethical issues
- Length of time in culture for human embryos and stem cell models should be appropriate for the scientific question under study, unless restricted by law

Need for further guidance?

- Stem cell embryo models are not embryos. They are in vitro research tools
- 12/14 day rule should not be applied
- Scientific rationale for all embryo models must be clear- making a better embryo model is not a sufficient justification.
- Experiments should use the most appropriate model for the question being asked
- Length of time in culture needs to be defined ahead of time and not be open-ended
- Use of integrated models that include extraembryonic lineages needs to be well justified
- Definition of subtypes of integrated models
  - Models with all three blastocyst lineages represented
  - Some models with epiblast and hypoblast alone if they show organized embryonic development
- Better definition of appropriate stopping points
  - Is primitive streak still an important stopping point?
  - Formation of the nervous system?
  - What are the criteria used to define key stages?