Unique Aspects of Human Embryology and Opportunities and Challenges with Stem Cell-based Embryo Models

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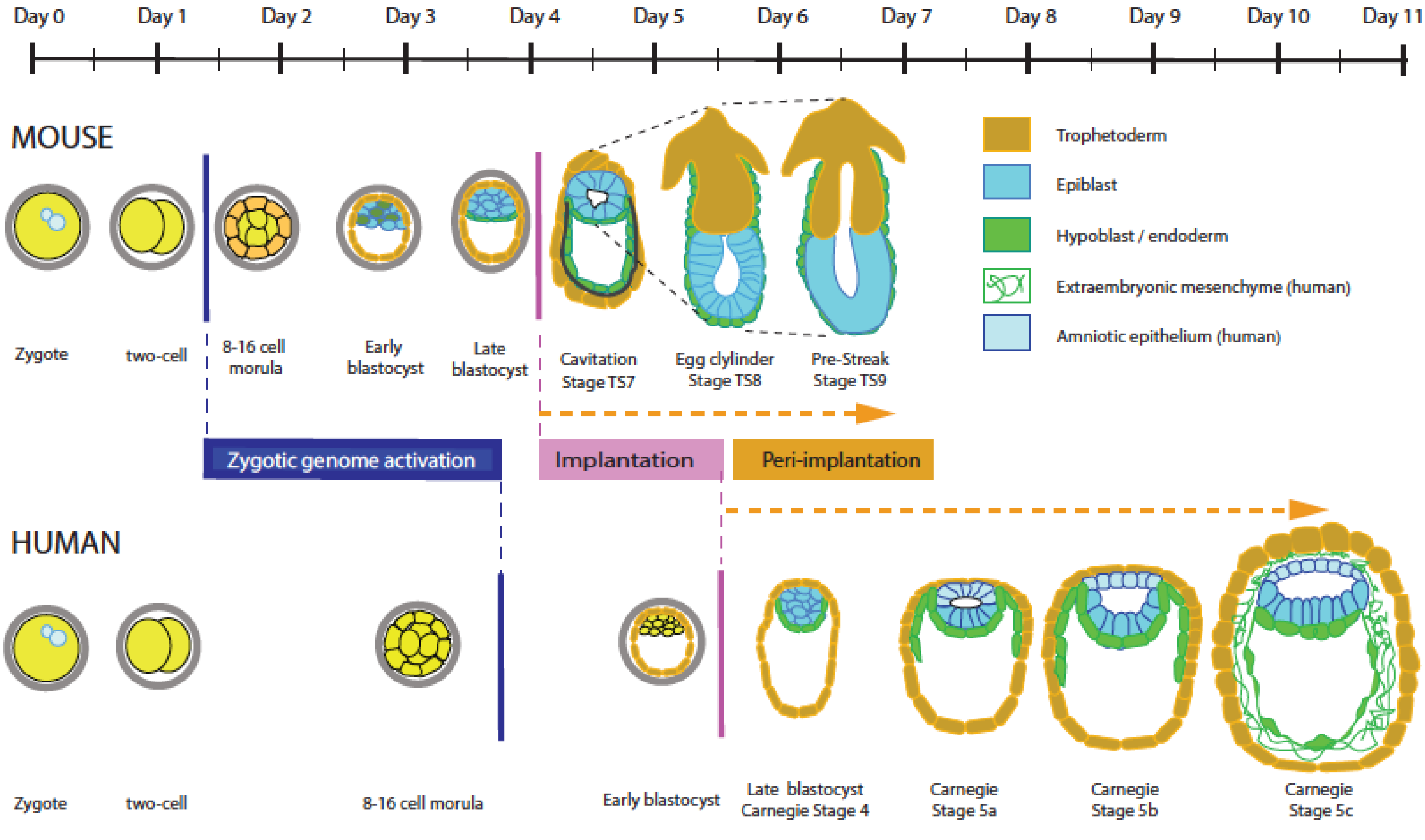
Why study early human development?

- Fundamental understanding of a key stage of human development inaccessible in any other way
- Better understanding of how mouse and human embryos and stem cells differ
- Improve IVF technologies
- Understand origin of germ cells and infertility
- Model the implantation process in 3D to understand placental formation and the reasons for high early embryo loss and placental anomalies
- Using CRISPR gene editing to study genetics of early developmental defects

Why not just study mouse?

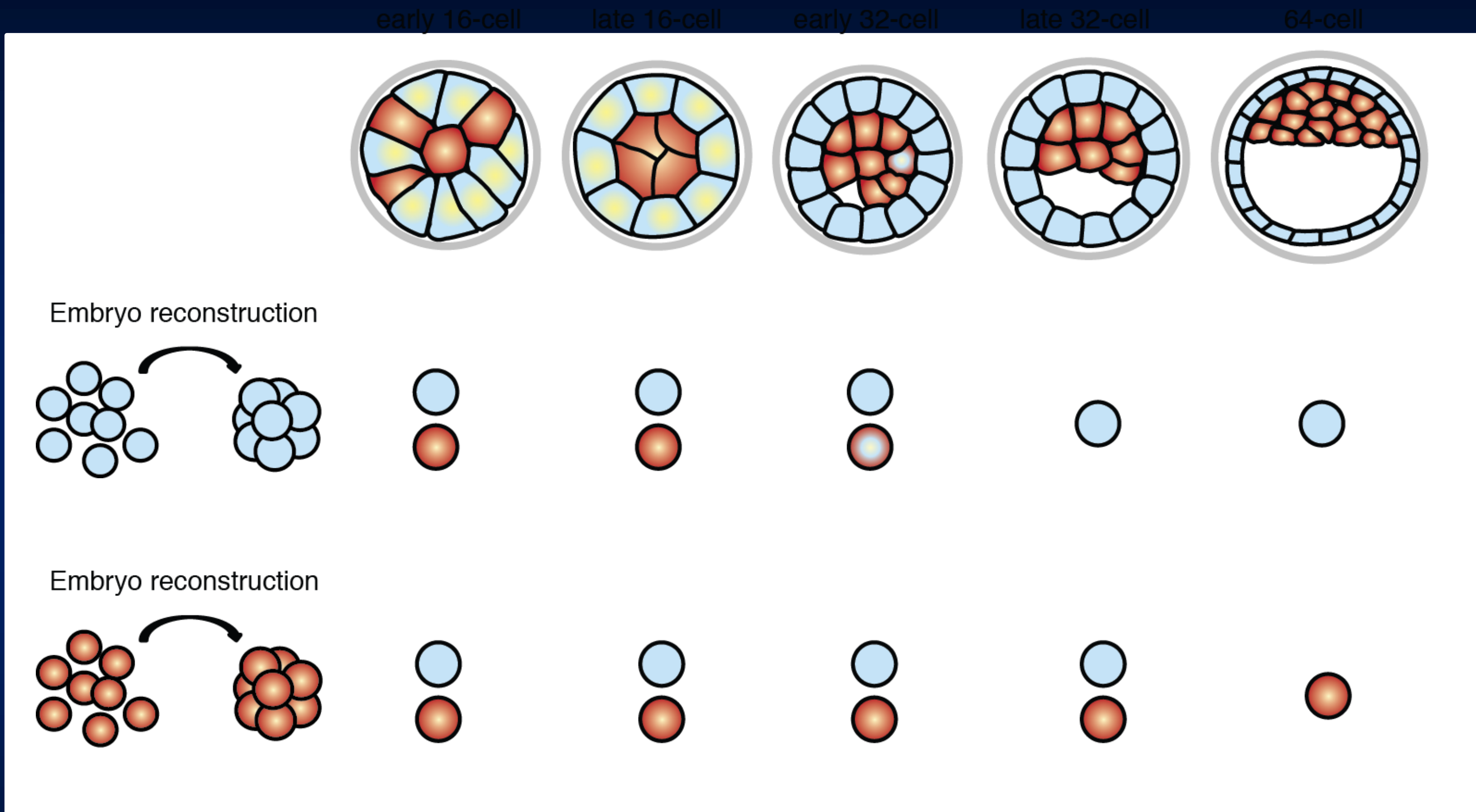
- *Many similarities but morphological and molecular differences exist*



Rossant and Tam (2017) Cell Stem Cell

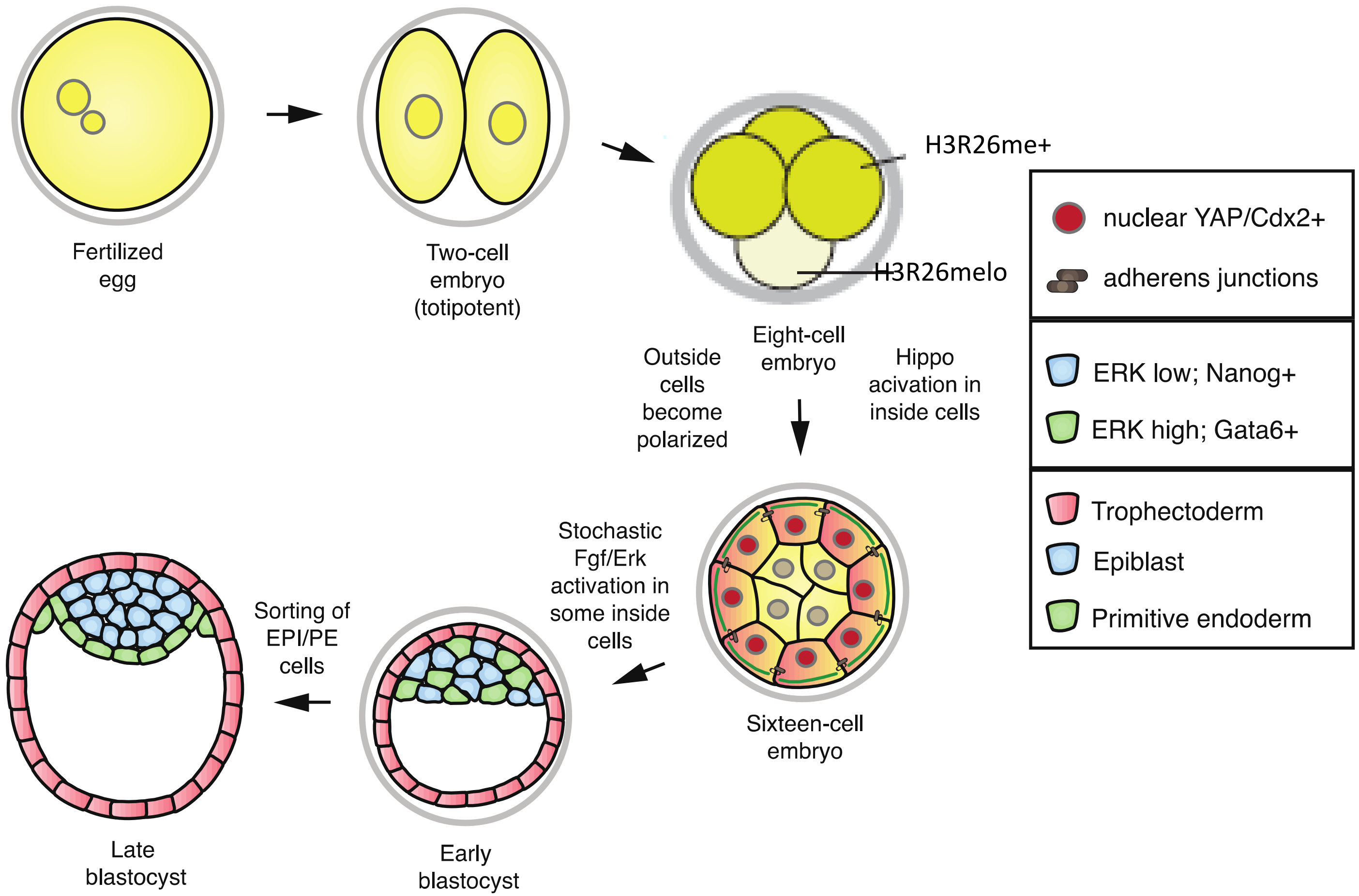
- Is the timing of blastocyst lineage commitment different in mouse and human?

Mouse lineage commitment occurs in TE before ICM but complete by blastocyst



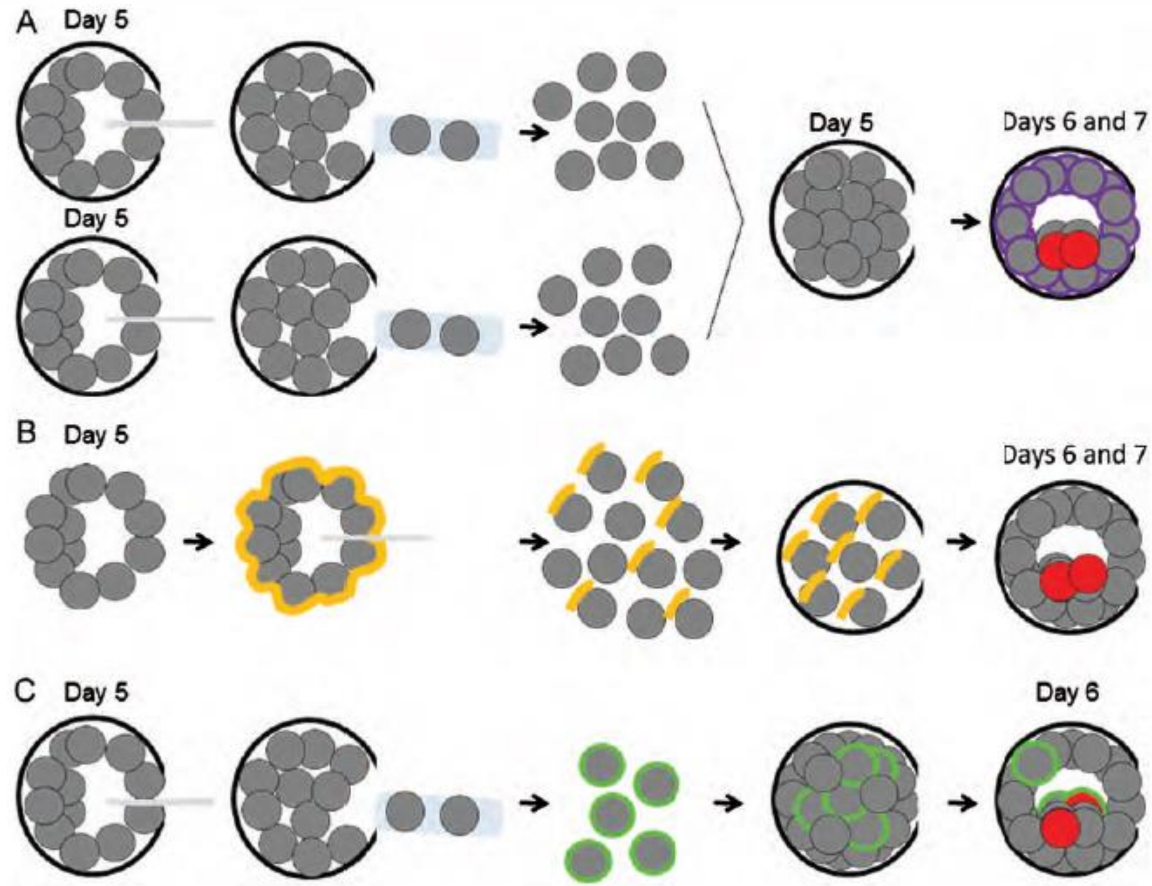
Posfai et al 2017 eLife
Wiggers et al 2017 Sci Reports

Hippo/Yap and FGF signaling are key to final cell fate specification in mouse blastocyst

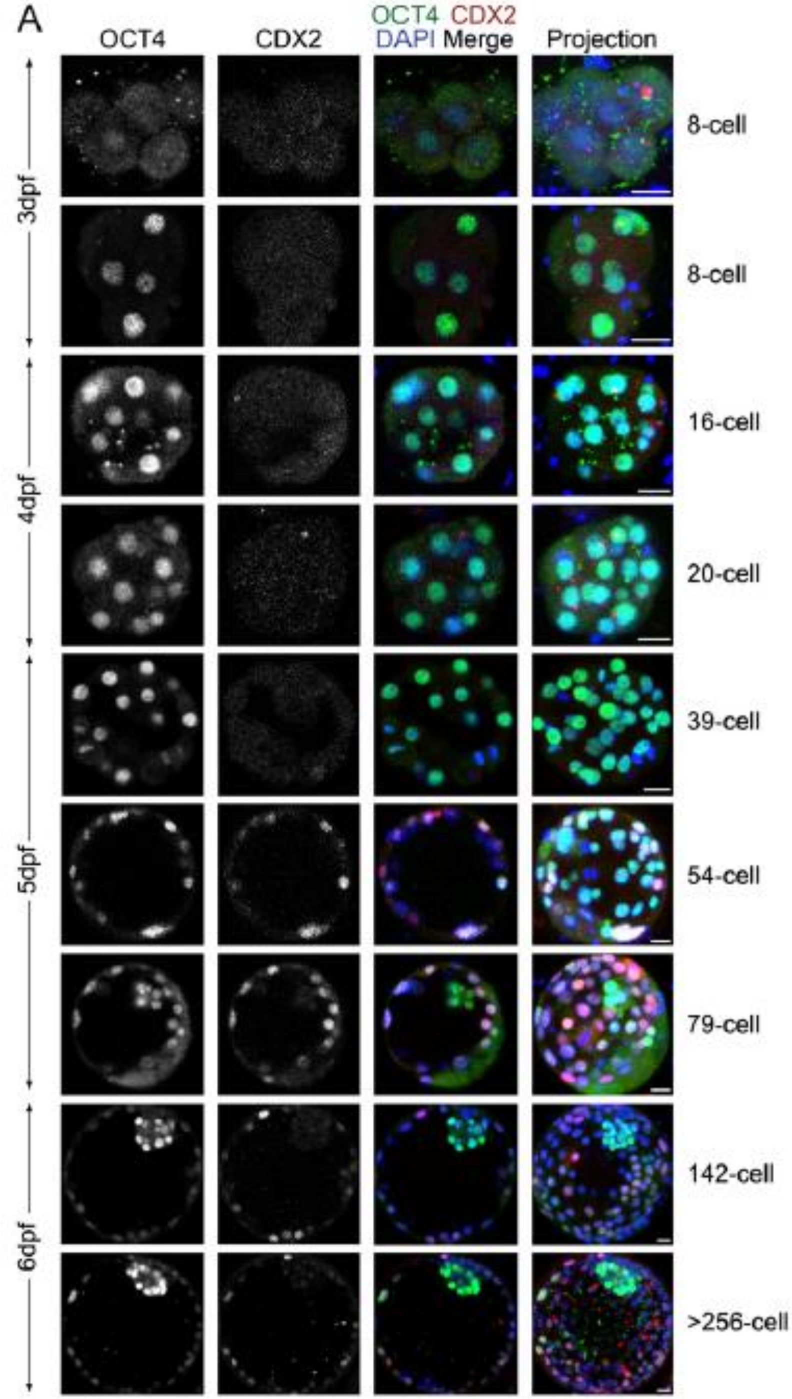


Work of multiple labs

Human early blastocyst cells are uncommitted to lineage



De Paepe et al (2013) Human Reprod. 28: 740-789



Timing of expression of key transcription factors differs and may differ in function

Single cell RNA seq identifies conserved and divergent lineage-specific genes.
Blakeley et al (2015) Development 142: 3151-3165
Petropoulos et al (2016) Cell 165: 1012-1026

Cdx2 does not begin expression until blastocyst stage and Oct4 not restricted to ICM till very late blastocyst.
Niakan and Eggan (2013)Dev Biol 375: 54-64

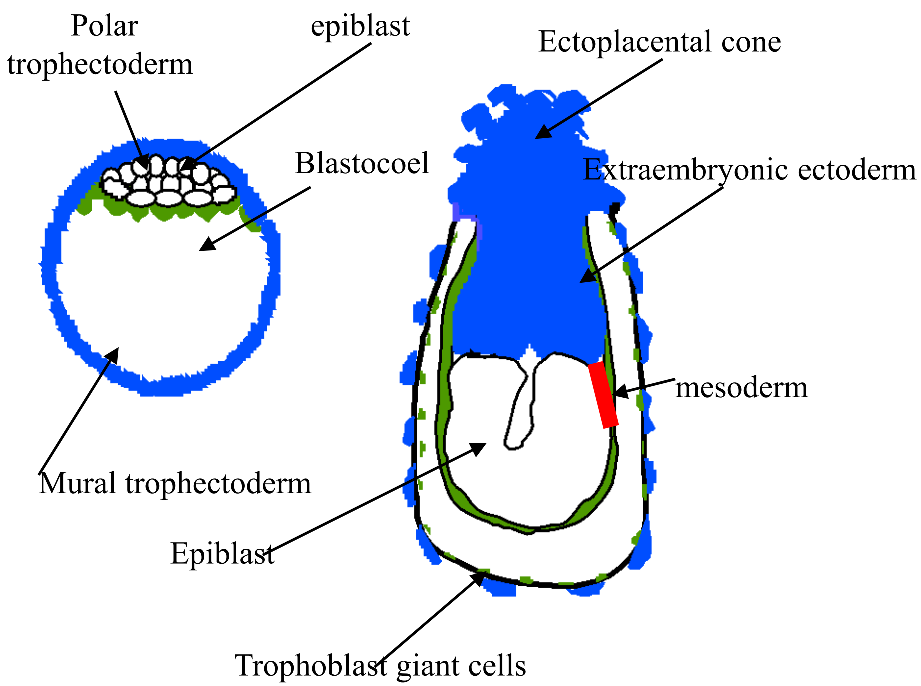
Oct4 may have earlier role in human development
Fogarty et al (2017) Nature 550:67-73

Morphogenesis of human blastocyst precedes lineage specification

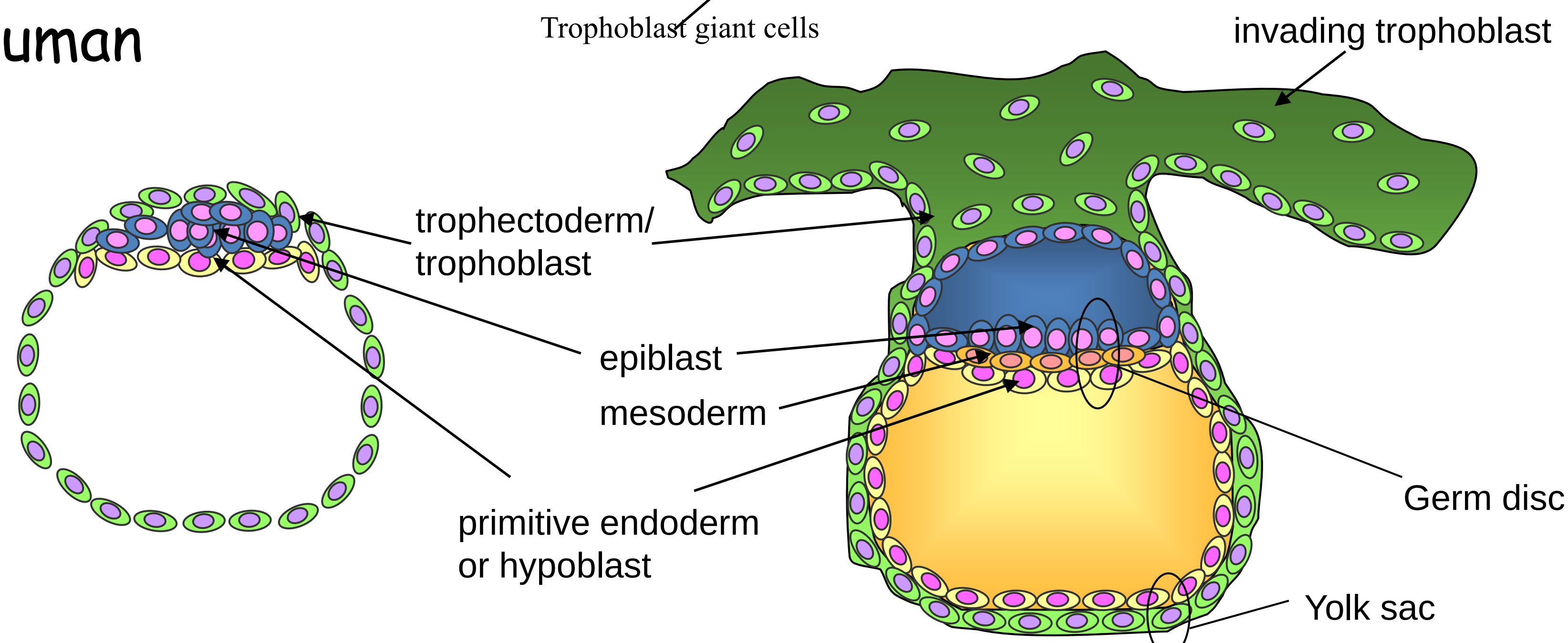
- Is Cdx2 required to specify TE fate and downregulate Oct4 in humans?
- Is HIPPO signaling required and when?
- How does this relate to derivation of human pluripotent stem cells and ‘naïve’ state?

Postimplantation morphogenesis differences: mouse to human

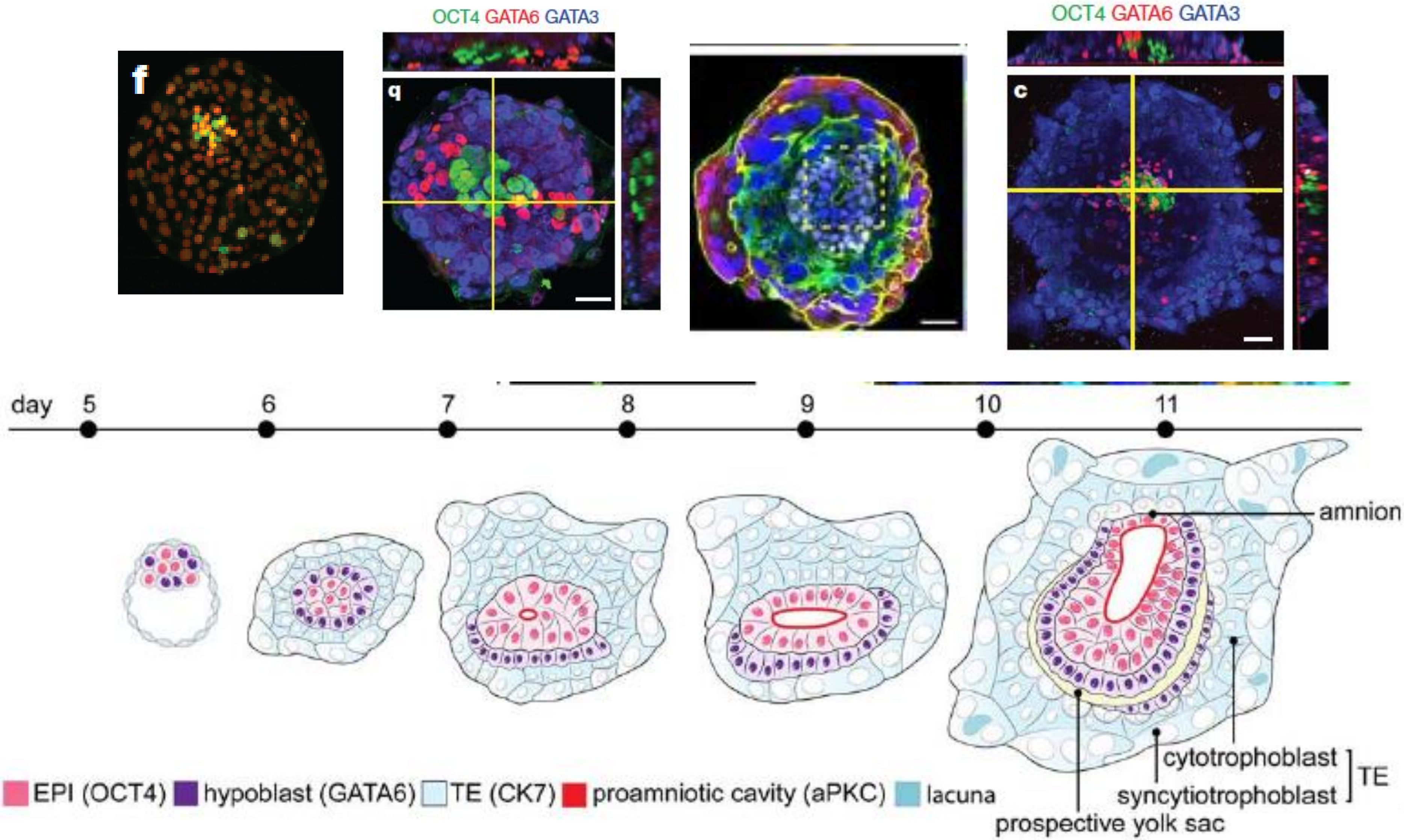
mouse



Human

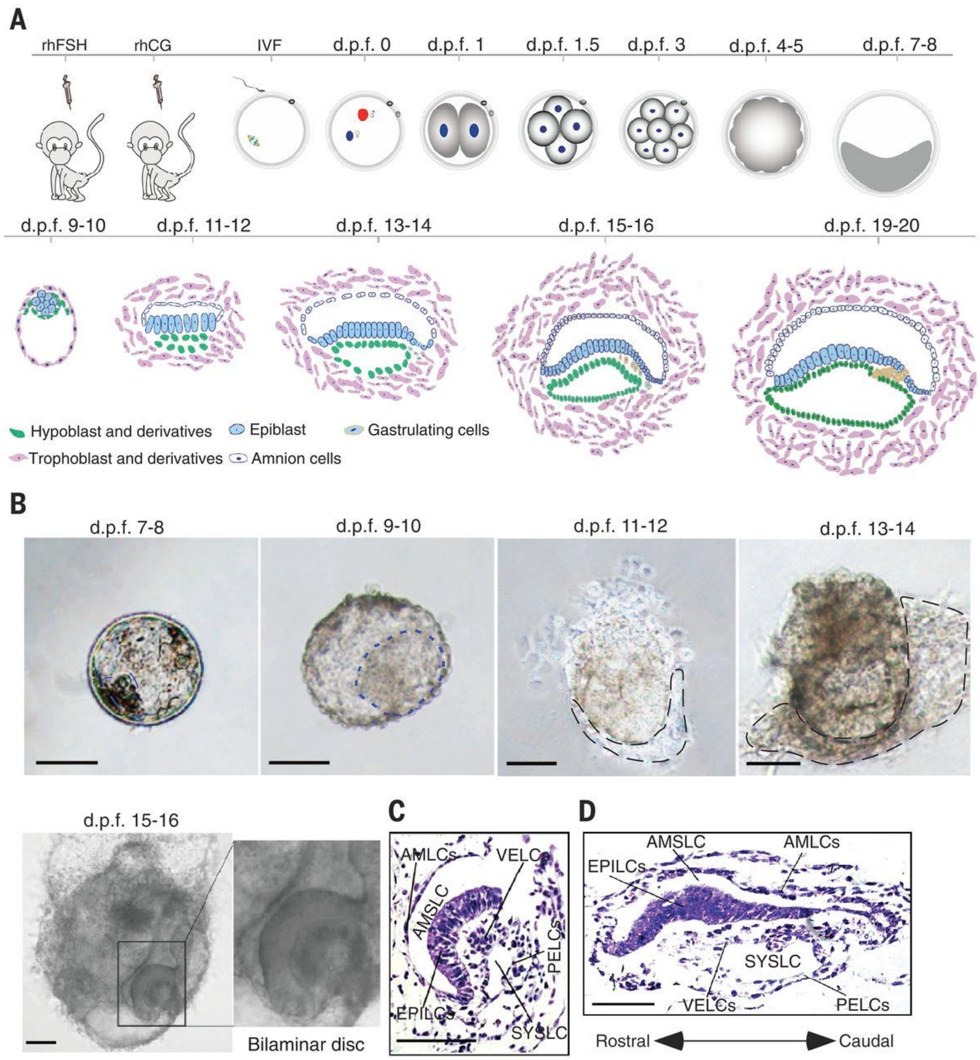


In vitro outgrowths of human embryos across the implantation period



From Deglincerti et al, Shabazi et al, 2016

Extended in vitro culture of Cynomolgus embryos

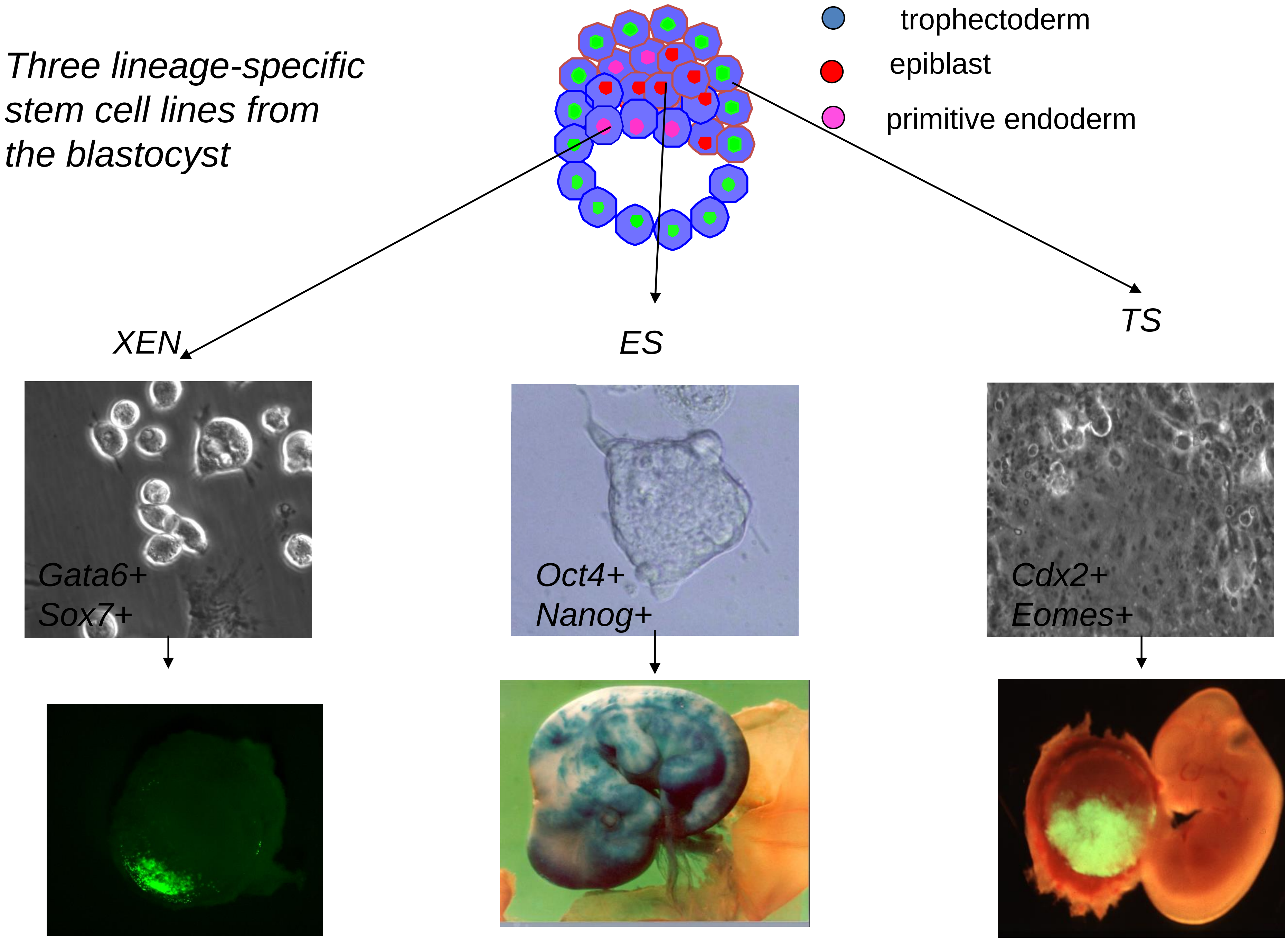


Ma et al, Niu et al (2019) Science 366

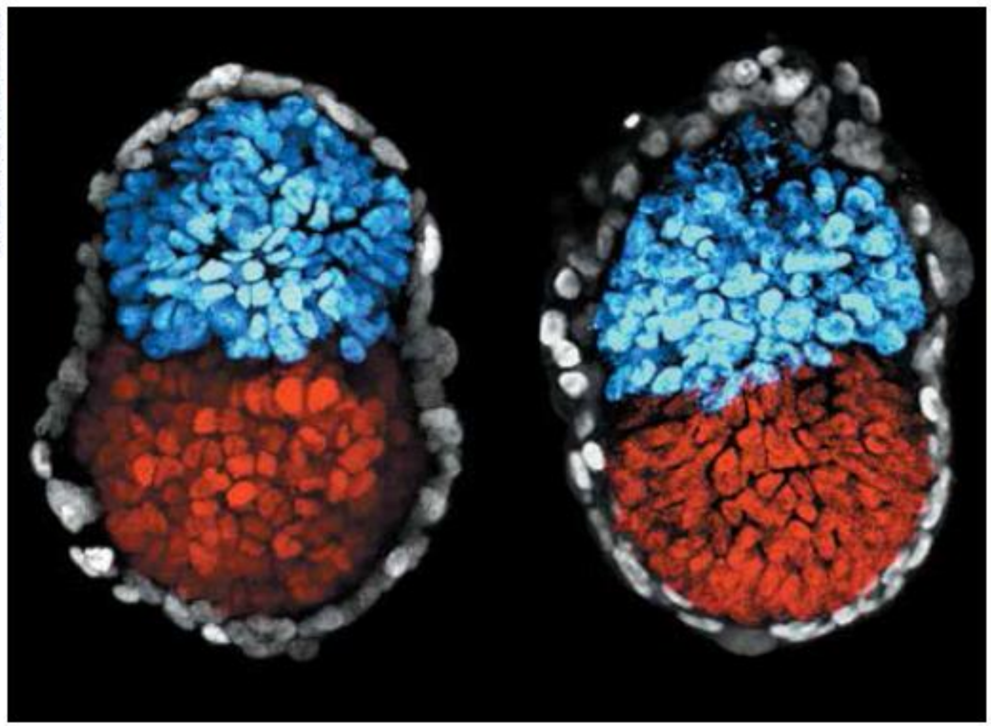
- But access to human embryos is ethically a challenge in many jurisdictions
- NHP embryos are expensive and only available in a few centres

Can human embryos be replaced by self-organizing stem cell cultures?

Start with mouse

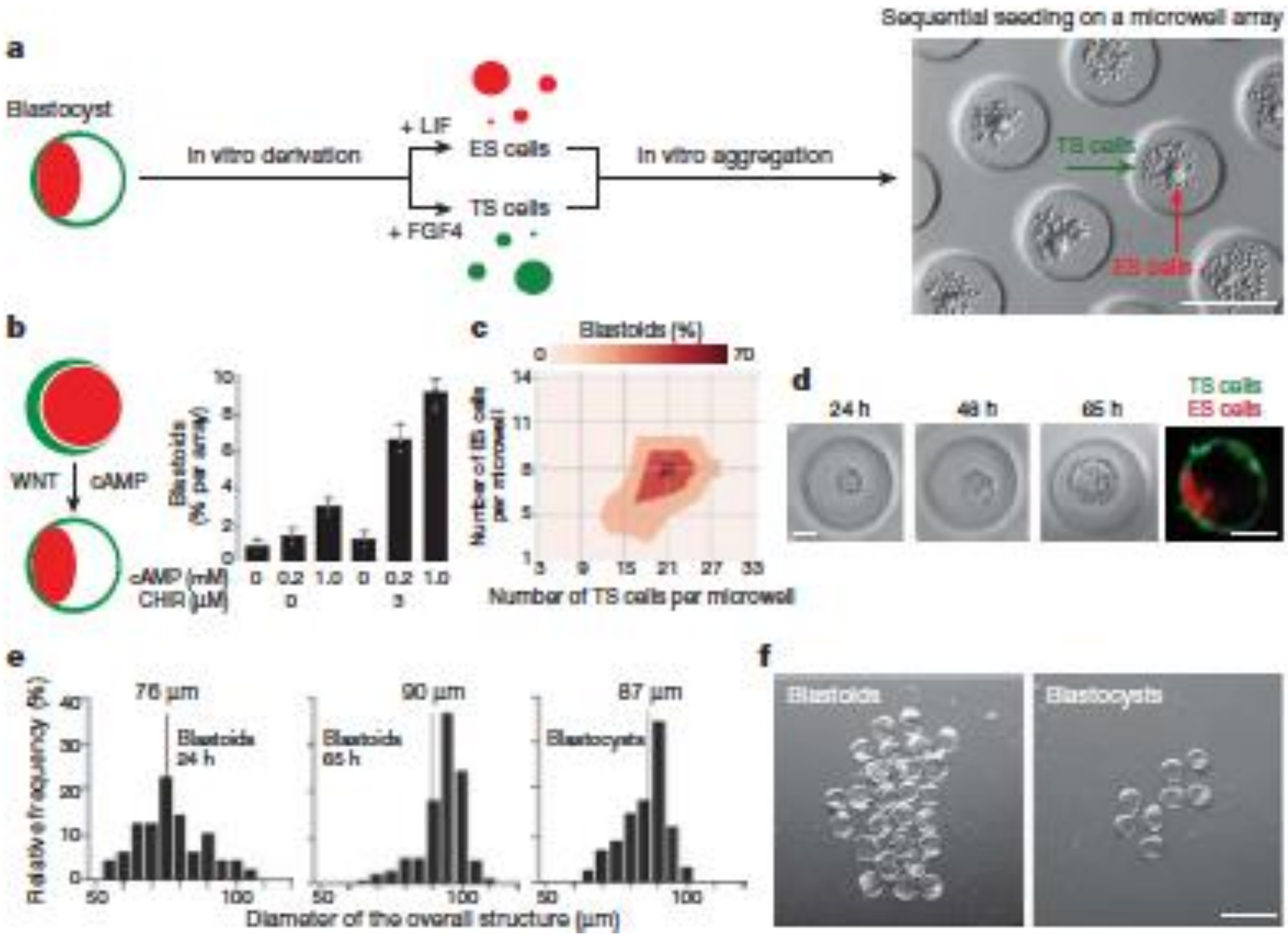

Three lineage-specific stem cell lines from the blastocyst
trophectoderm
epiblast
primitive endoderm
XEN
ES
TS
Gata6+
Sox7+
Oct4+
Nanog+
Cdx2+
Eomes+

3D ‘artificial’ mouse embryos combining ES, TS and XEN cells


B. SOZEN ET AL./NATURE CELL BIOL.

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

Blastoids mimic blastocyst development

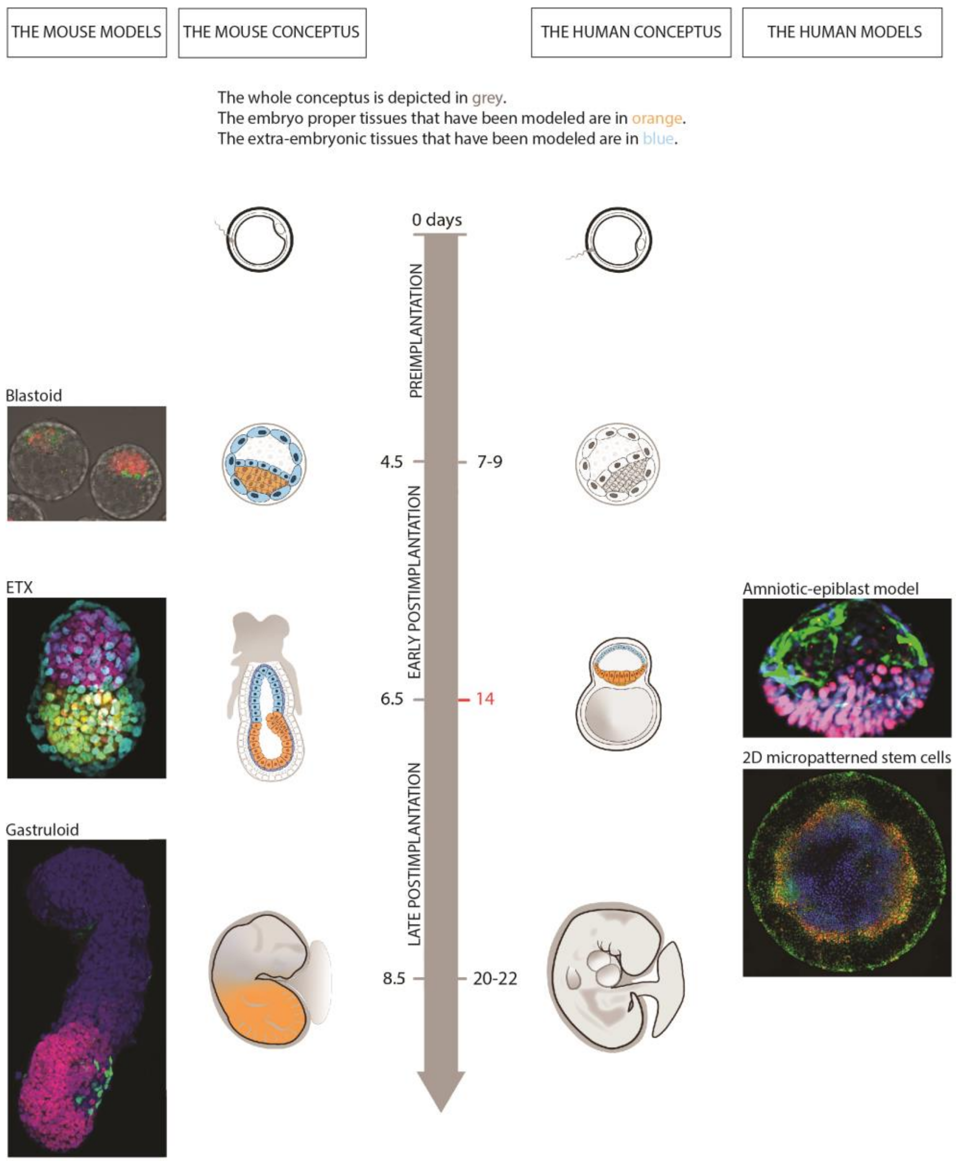


Rivron et al (2018) Nature 557: 106

Updates on mouse stem cells for model generation

- Rivron lab- culture Cdx2hi TS cells more like polar TE (Frias-Aldeguer et al BioRixv)
- Brickman lab- Culture conditions for nEND cells that are closer to PrE than are XEN cells (Anderson et al 2017 Nature Cell Biol 19:1164-1177)
- Extended potential stem cells can generate EPI/PE (Sozen et al 2019 Dev Cell 51:698-712) and possibly also TE-like cells (Li et al 2019 Cell 179:1-16) for blastoid production

From Mouse to Human?



Hyun et al (2020) Stem Cell Reports online

Human stem cells ready for embryo models?

- Human naïve ES cells
  - closest to epiblast of blastocyst
  - can grow in 2iLIF+FGFR inhibitor like mouse naïve ES cells (Anderson et al 2017 Nature Cell Biol 19:1164-1177)
- Human TS cells (Okae et al 2018 Cell Stem Cell 22:50-63)
  - from blastocyst or early villus
  - not dependent on FGF
  - is there still a blastocyst TE-type cell to be found?
- Human XEN cells
  - overexpress Sox7 (Seguin et al 2008 Cell Stem Cell 3:182-195)
  - or culture in same conditions as mouse nEND cells (Linneberg-Agerholm et al. 2019 Development 146)
- Human expanded potential cells (Gao et al 2019 Nat Cell Biol 21:687)
  - similar to mouse culture conditions and similar properties
  - Can they make blastoids?

Some intriguing differences between mouse and human stem cells

- Human naïve ES cells- are they actually not lineage-committed?
  - Expression profiles show similarity to earlier stages as well as to epiblast
    - Stirparo et al (2018) Devt 145
  - Transposon expression and other properties similar to cleavage stages
    - Theunissen et al (2017) Cell Stem Cell 18:502
  - Does this relate to late plasticity of blastocyst cells in human?

- Human expanded potential cells (and naïve cells?)
  - More readily able to generate TS cells than mouse EPSC
    - Gao et al (2019) Nature Cell Biol 21:687-699

- Many different opportunities to generate partial or more complete stem cell-derived human embryo models
- Still need to compare back to mouse system and to align directly with events in the human embryo itself via culture in vitro or comparison with non-human primate models

- More exciting updates to come today!

- Thanks to NAS/NIH staff for organization and to speakers for all their input