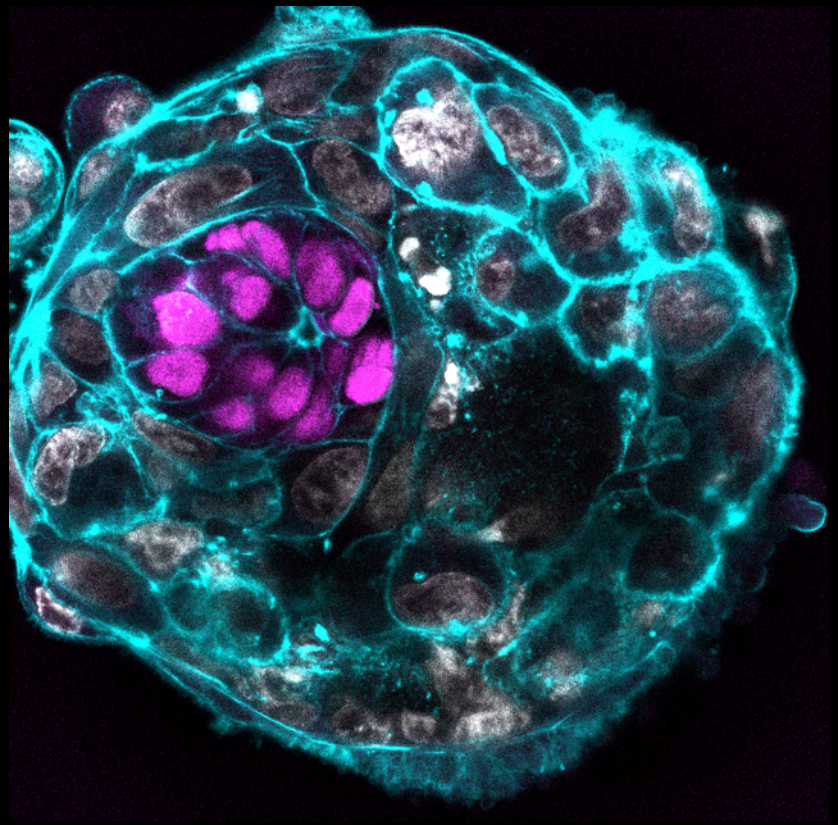
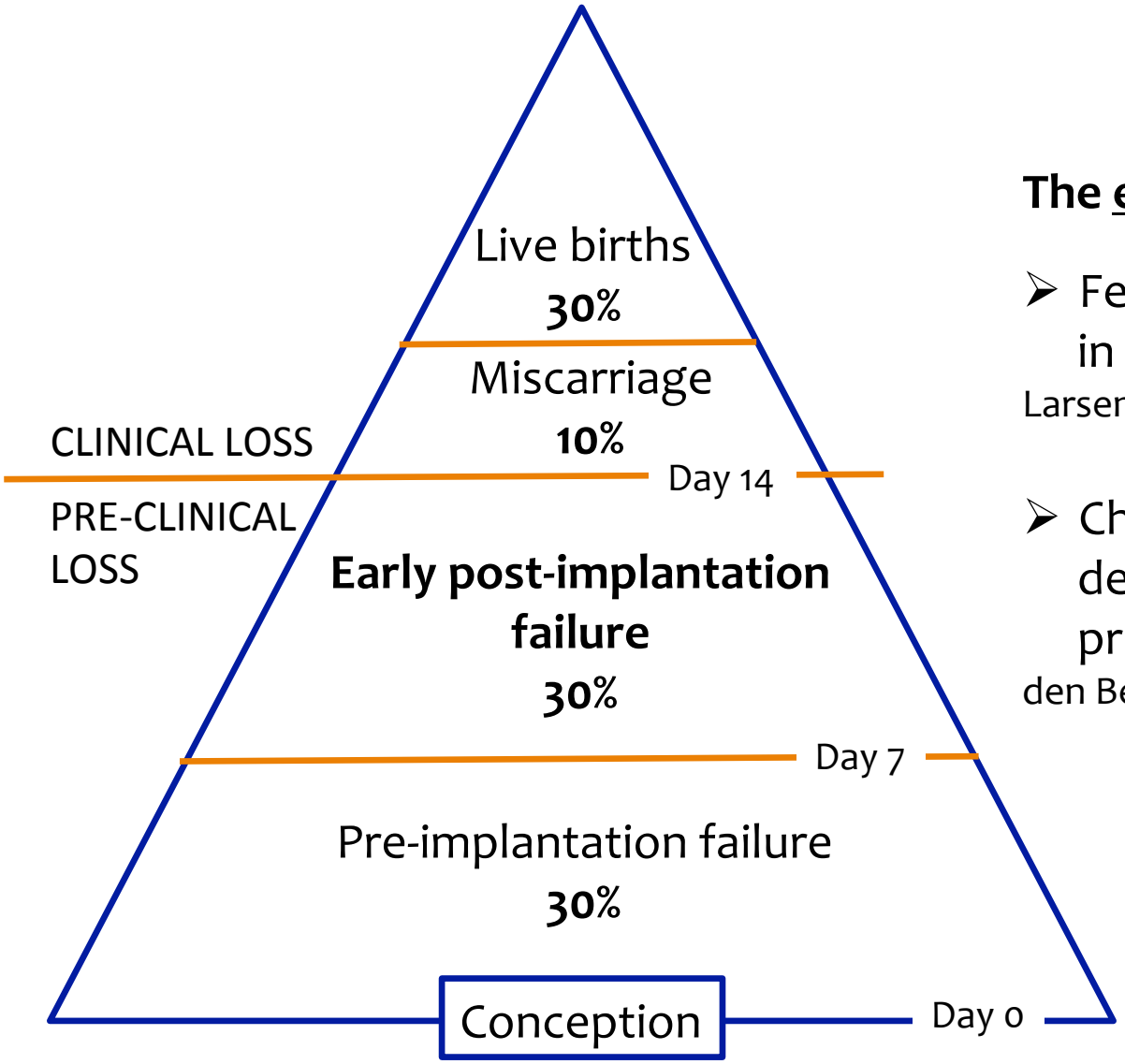
In vitro culture of the human embryo until day 14



Marta Shahbazi PhD
Magdalena Zernicka-Goetz group
University of Cambridge, UK

BSRM Autumn Meeting
23rd-24th November 2018, Brussels, Belgium

Human pregnancy loss: When? Why?



The pregnancy loss iceberg
(modified from Macklon et al (2002) Human Reproduction)

The <u>embryo</u> or the endometrium?

- Fetal abnormalities are present in 85% of early pregnancy losses

Larsen et al (2013) BMC Medicine

- Chromosomal abnormalities are detected in 50% of early pregnancy losses

den Berge et al (2012) Biochim Biophy Acta

Studying the causes of early post-implantation loss

1. *In vitro* culture of human embryo

- A model system to study early post-implantation

2. Mechanisms of development

- The first transformation of the embryonic tissue upon implantation: formation of the amniotic cavity

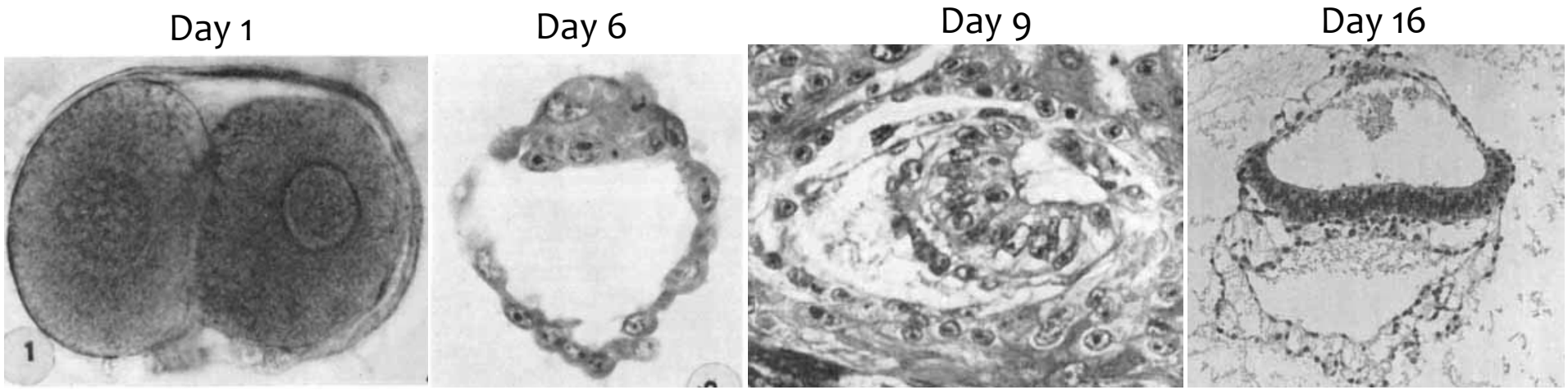
3. Aneuploid human embryos

- Chromosomal abnormalities and early pregnancy loss: When? How?

Human embryo development at implantation: methods to explore the black box of development

- Analysis of *in vivo* developing embryos (Carnegie Institution):

A collection of more than 10,000 human embryos



Hertig et al, Am J Anat (1956)

- Co-culture of human embryos with endometrial cells:

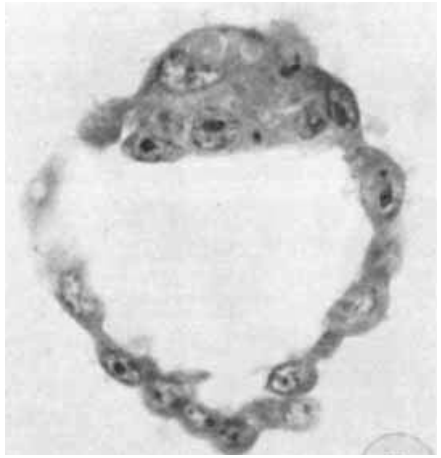
Uterine- embryo crosstalk and attachment. Morphogenesis?

Lindenberg et al, Human Reprod (1986); Weimar et al, Reprod Biomed Online (2013)

Human embryo development at implantation

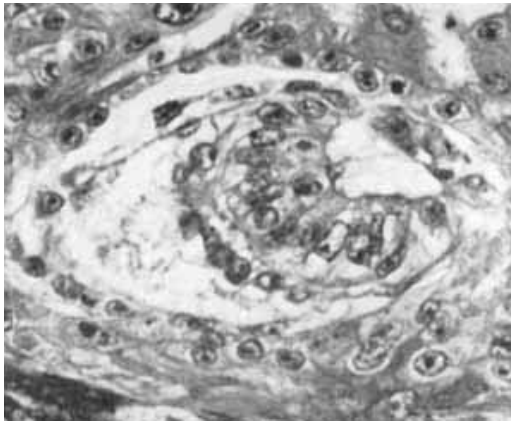
A global **morphogenetic transformation** takes place at the time of **implantation**

Day 6



Day 7-8 ↓ *Implantation*

Day 9



Hertig et al (1956) Am J Anat

Hallmarks of human post-implantation embryos

- Lineage specification:
  - Embryonic tissue: epiblast
  - Extra-embryonic tissues: hypoblast and trophoblast

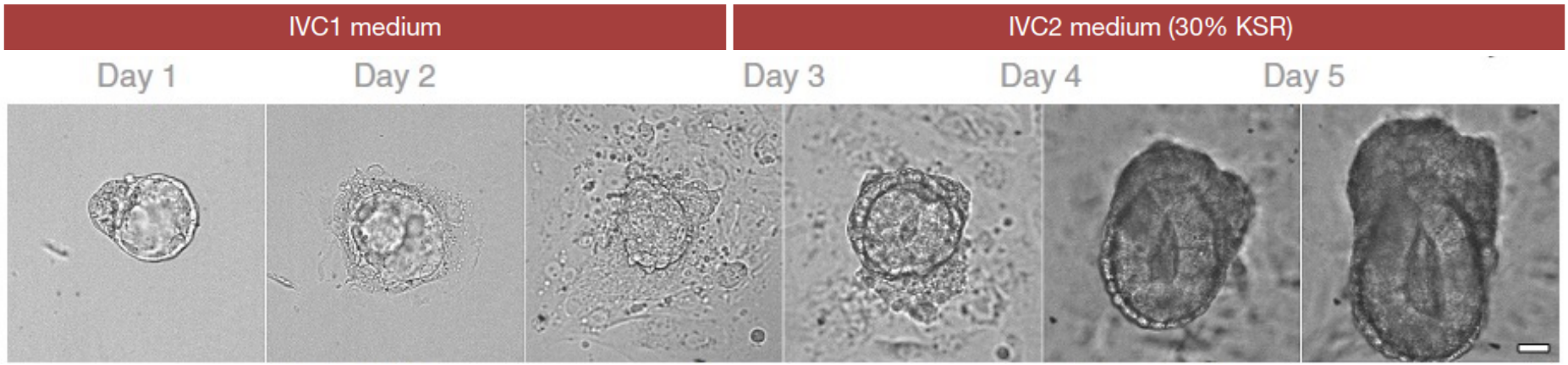
- Morphogenetic transformations:
  - Epiblast: amniotic cavity
  - Hypoblast: yolk sac
  - Trophoblast: cytotrophoblast and syncytiotrophoblast

Can we develop a system to culture human embryos beyond day 7 *in vitro*?

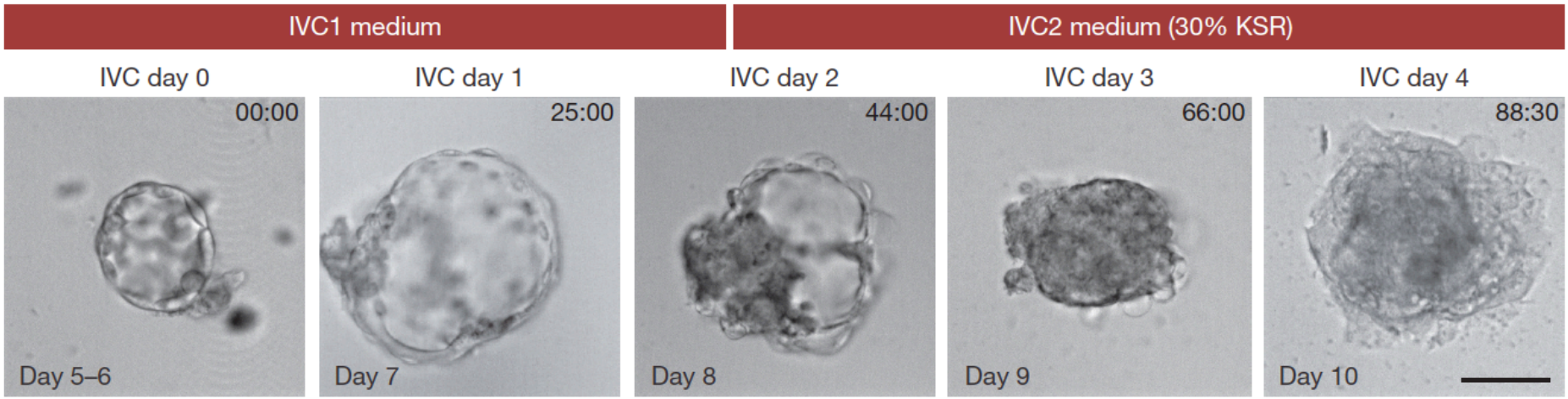
What are the self-organizing capabilities of human embryos?

In vitro culture of mouse embryos beyond implantation

Pre-implantation blastocyst → *In vitro* culture → Post-implantation mouse embryo

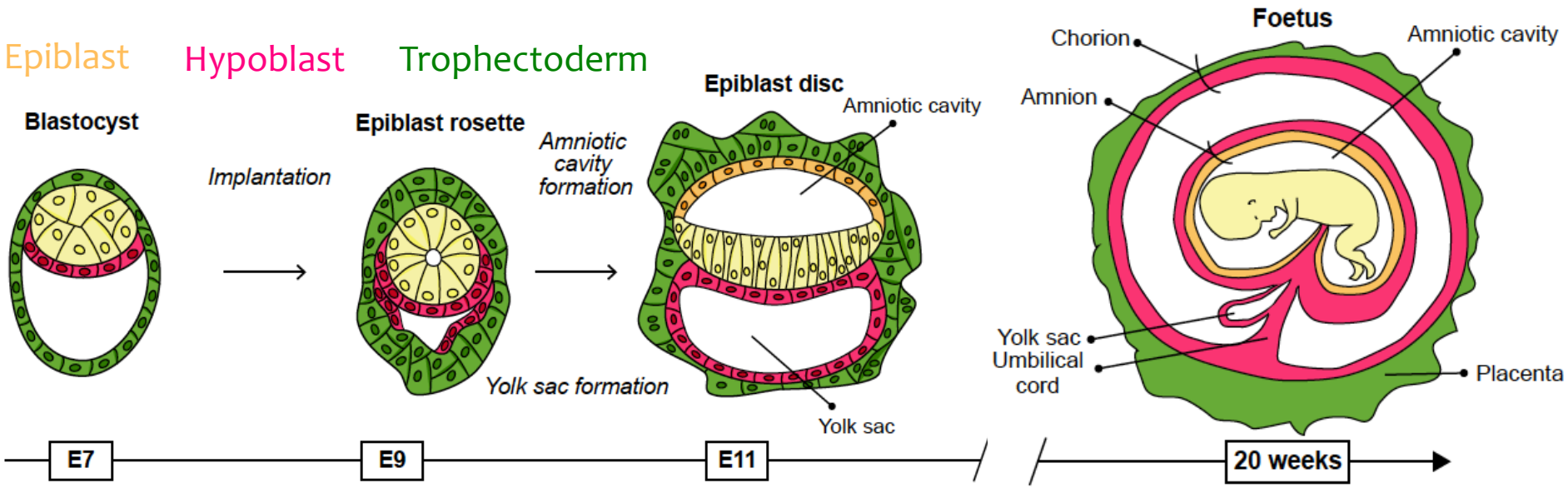


Morris et al (2012) Nat Comm; Bedzhov et al (2014) Nat Prot; Bedzhov et al (2014) Cell



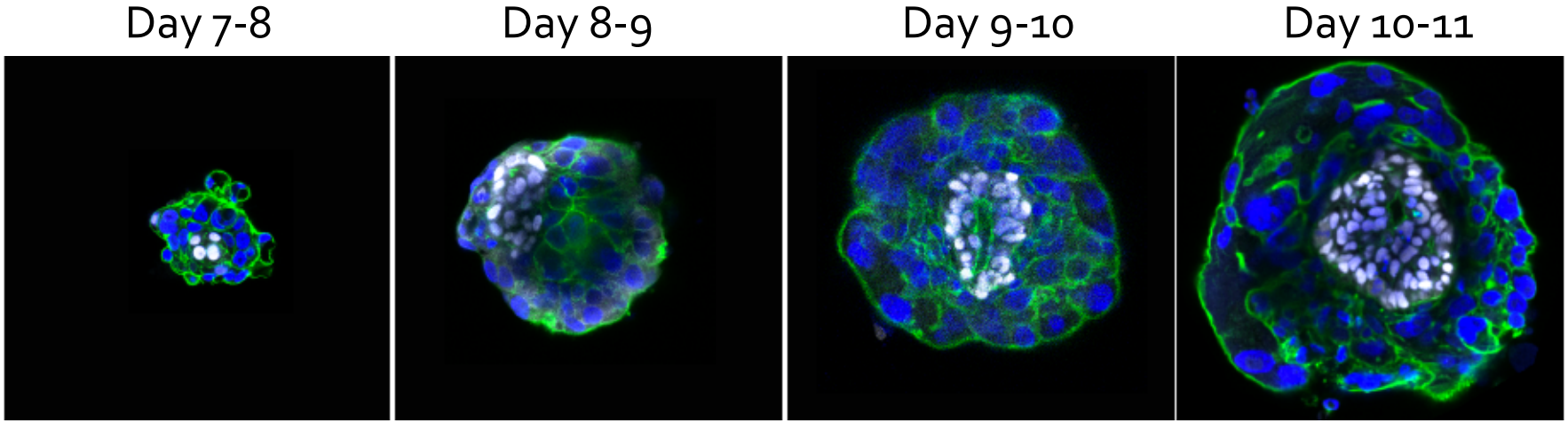
Shahbazi et al (2016) Nat Cell Biol; Deglincerti et al (2016) Nature

Post-implantation morphogenesis of the human embryo

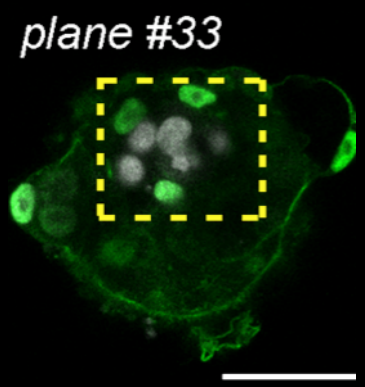


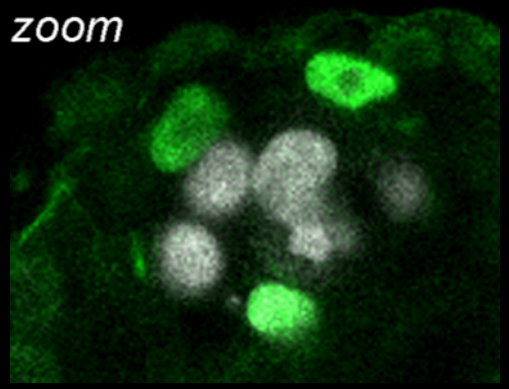
Shahbazi et al (2018) Nat Cell Biol

Embryo growth



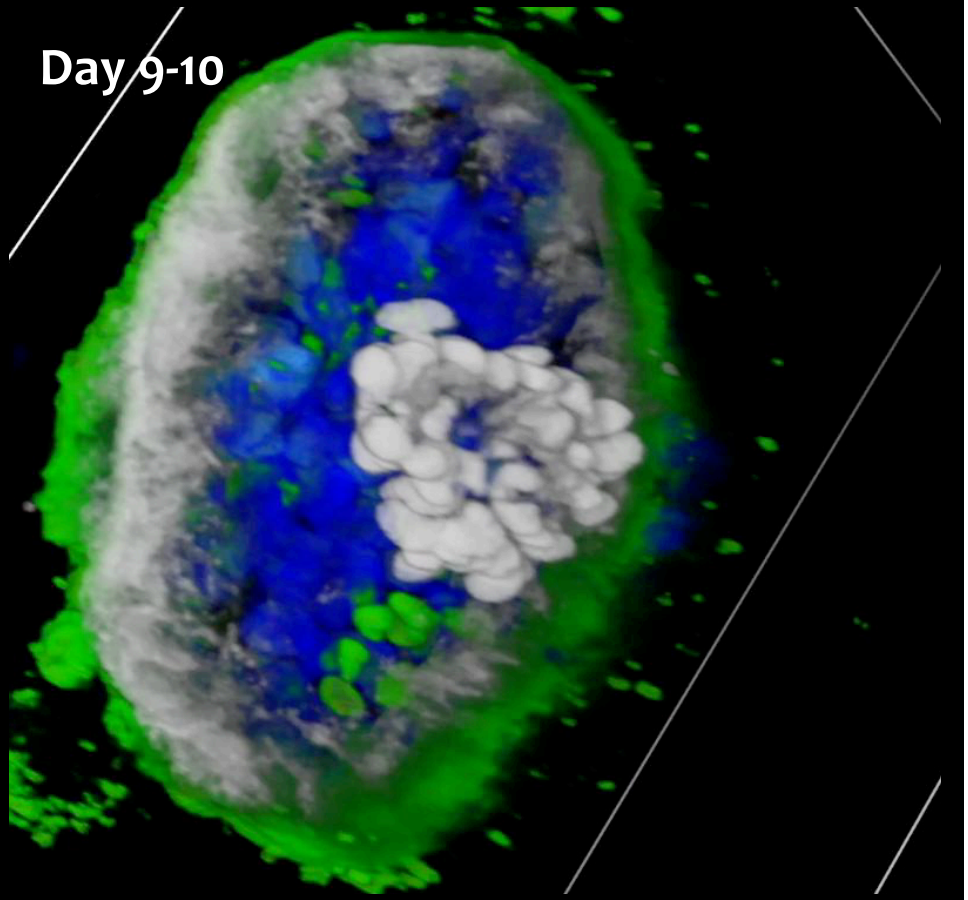
Day 7-8: Segregation of epiblast and hypoblast





Epiblast (OCT4)
Hypoblast (GATA6)

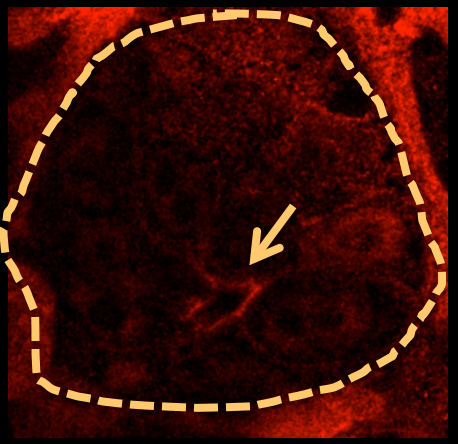
Inner cell mass

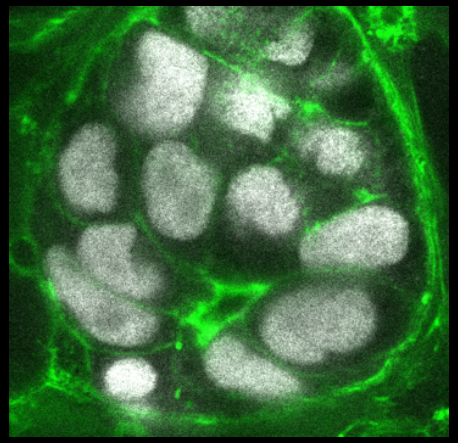


OCT4/GATA6/DAPI

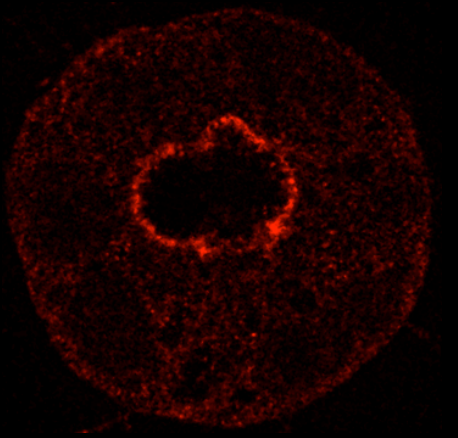
Day 8-9: Amniotic cavity formation

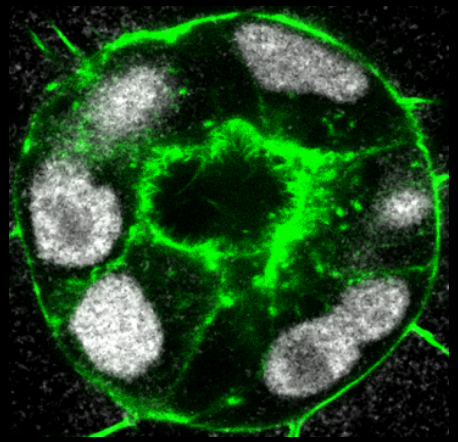
Day 8-9 human embryo





Human ESCs (3D matrix)

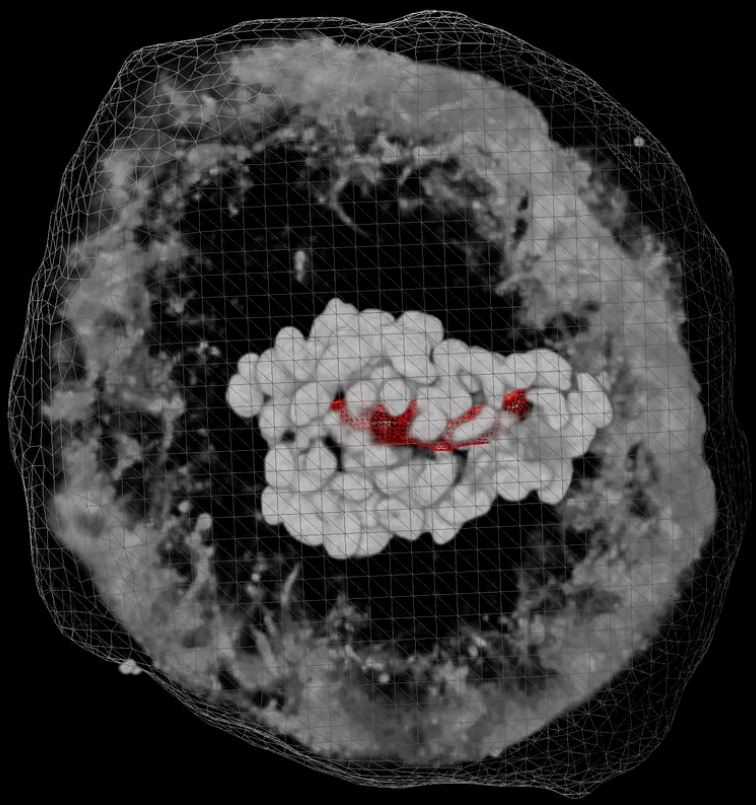




aPKC: apical polarity complex

OCT4/F-actin

Day 9-10



OCT4

Amniotic cavity

Mouse vs human post-implantation development

Development at implantation

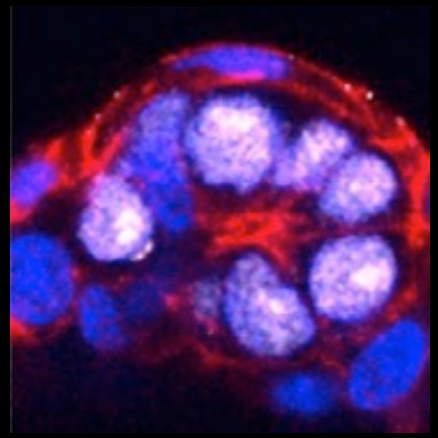
Development at early post-implantation

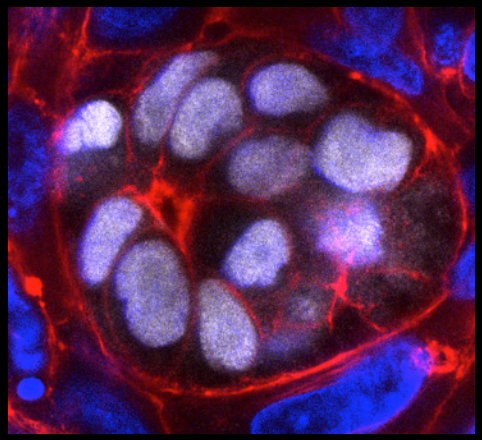
E4.75 mouse embryo

E8-9 human embryo

E5.5 mouse embryo

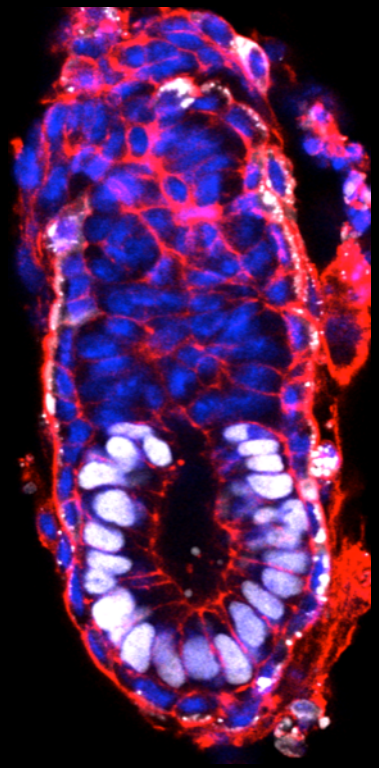
E10-11 human embryo

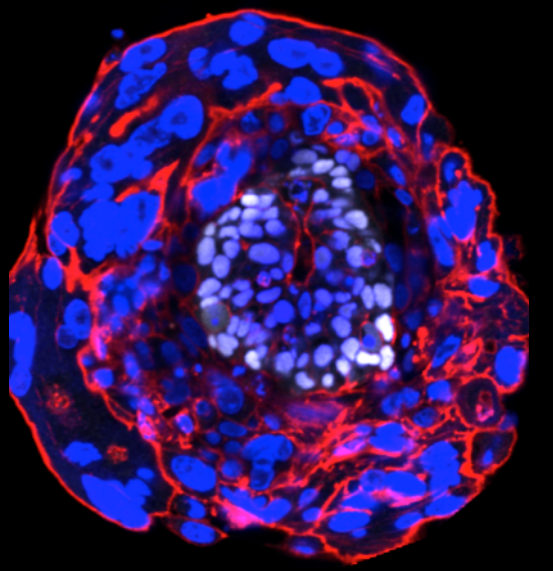




OCT4/F-actin/DAPI

Rosette





OCT4/F-actin/DAPI

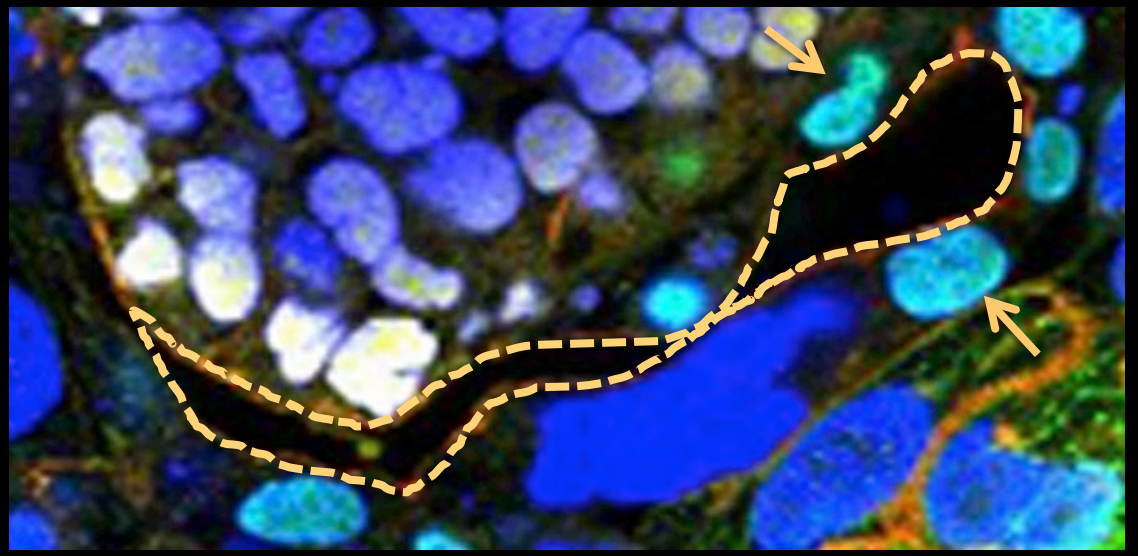
Cylinder

Disc

Day 10-11: Differentiation of extra-embryonic tissues

Hypoblast

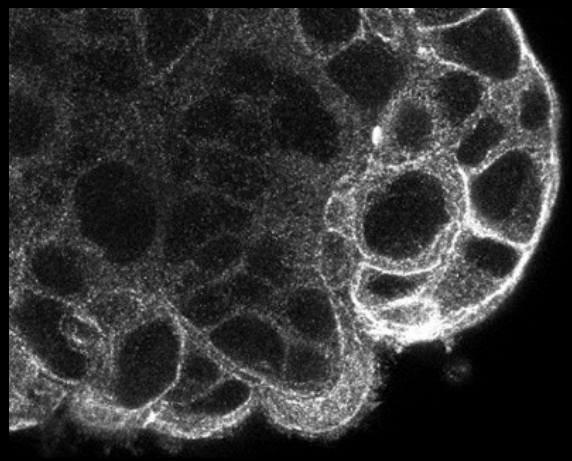
Formation of the prospective yolk sac



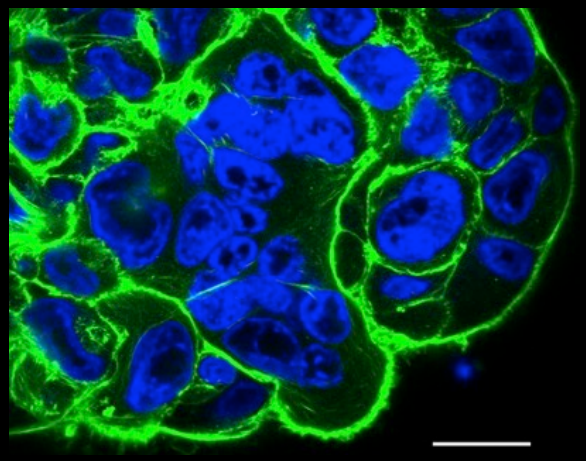
DAPI/GATA6/aPKC/OCT4

Trophoblast

Differentiation into cytotrophoblast and syncytiotrophoblast



Cytokeratin 7: Pan-trophoblast marker

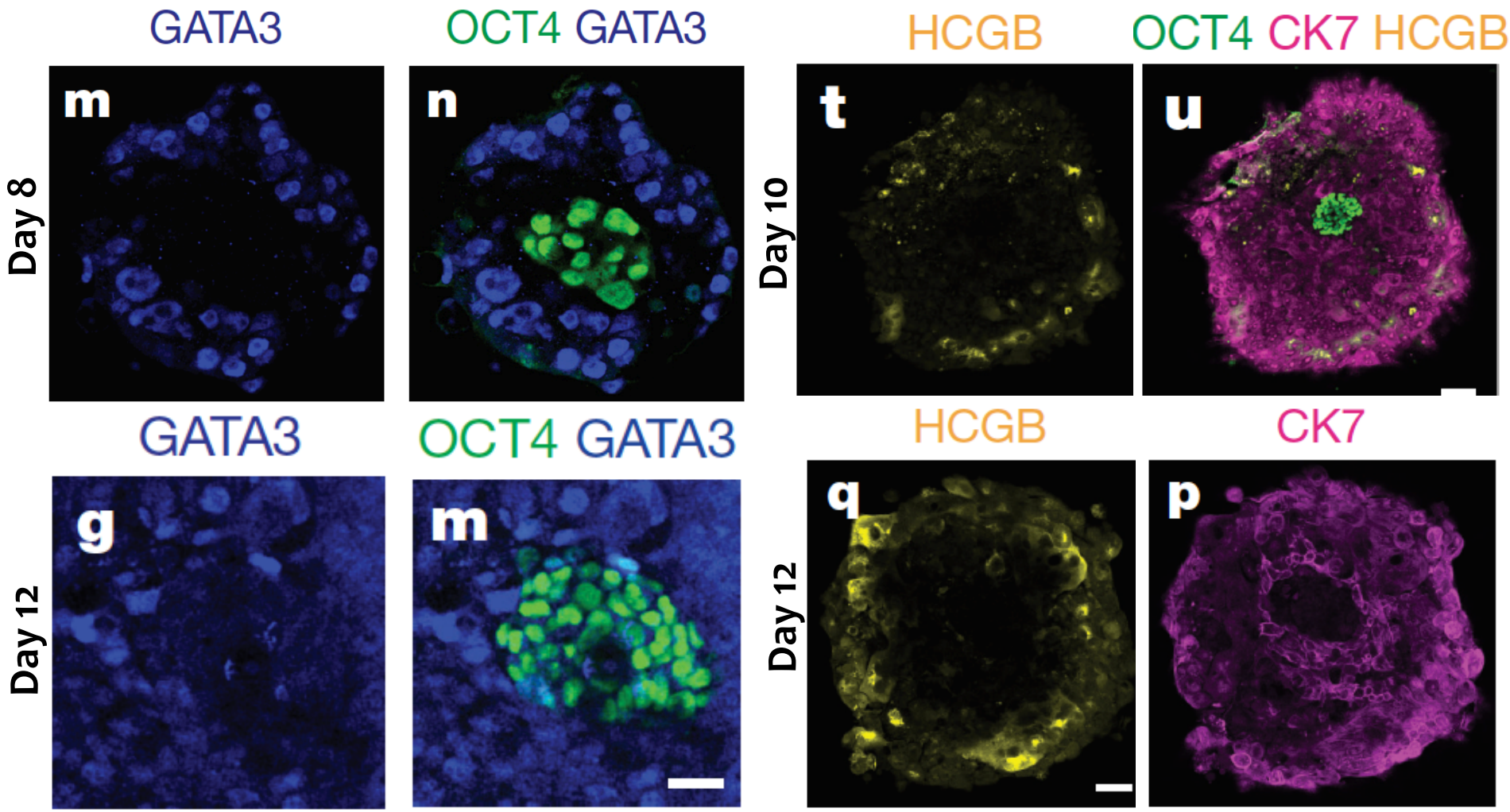


F-actin/DAPI

Day 10-11: Differentiation of extra-embryonic tissues

GATA3: expressed specifically by cytotrophoblast cells

hCG: expressed specifically by syncytiotrophoblast cells



Deglincerti et al (2016) Nature

Culture of human embryos beyond implantation

HUMAN EMBRYOLOGY

Implantation barrier overcome

The early stages of human development are normally hidden within the womb, but improved techniques for culturing embryos from the blastocyst stage promise to make these steps easier to investigate.

Shahbazi et al (2016) Nat Cell Biol
Deglincerti et al (2016) Nature

What have we learned?

- Human embryos can self-organize *in vitro* in the absence of maternal tissues
- In both mouse and human embryos the epiblast undergoes a process of epithelial tissue formation through a rosette-intermediate step

What can we do with this system?

- Explore the mechanisms behind early post-implantation human development and the consequences of early pregnancy loss

Studying the causes of early post-implantation loss

1. *In vitro* culture of human embryo

- A model system to study early post-implantation

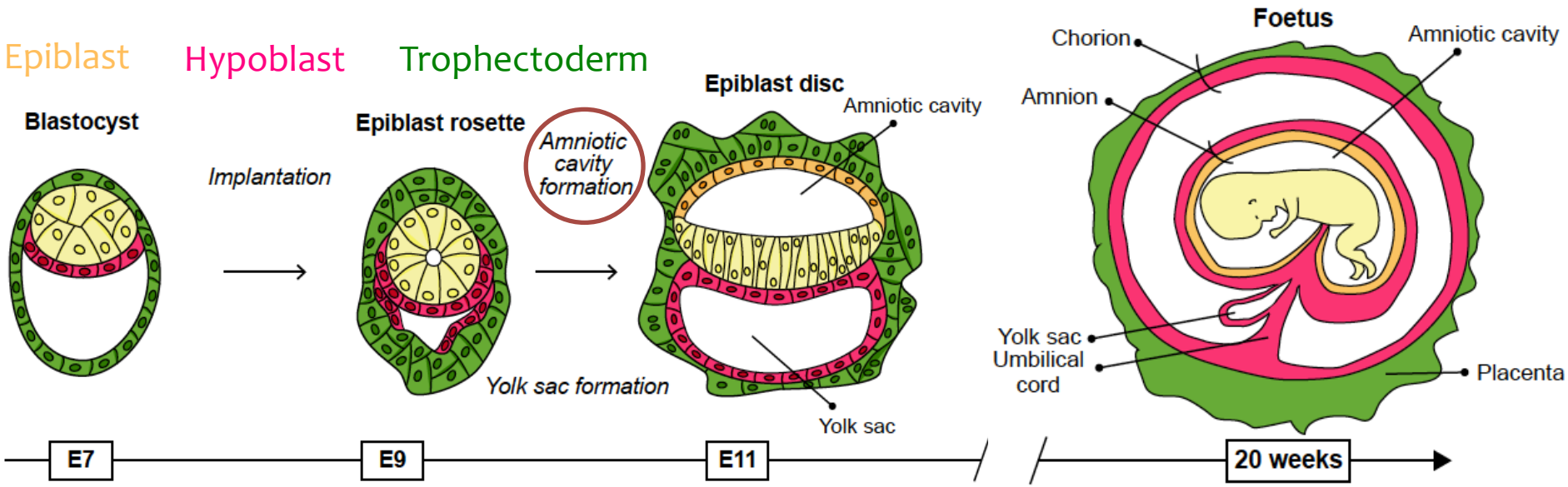
2. Mechanisms of development

- The first transformation of the embryonic tissue upon implantation: formation of the amniotic cavity

3. Aneuploid human embryos

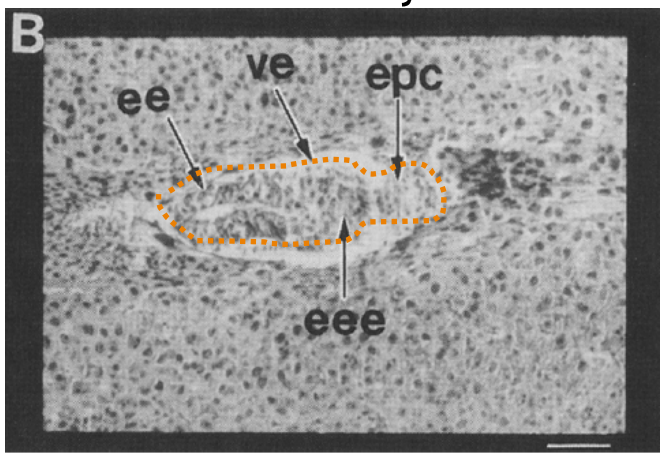
- Chromosomal abnormalities and early pregnancy loss: When? How?

Amniotic cavity formation in the human embryo

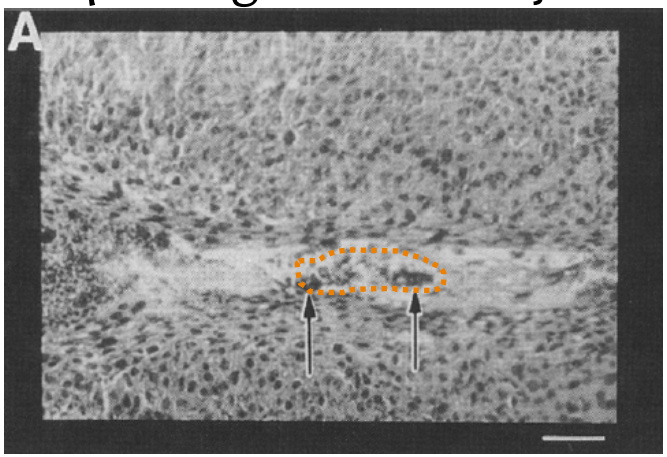


Alterations in amniotic cavitation may contribute to pregnancy loss at implantation

WT embryo



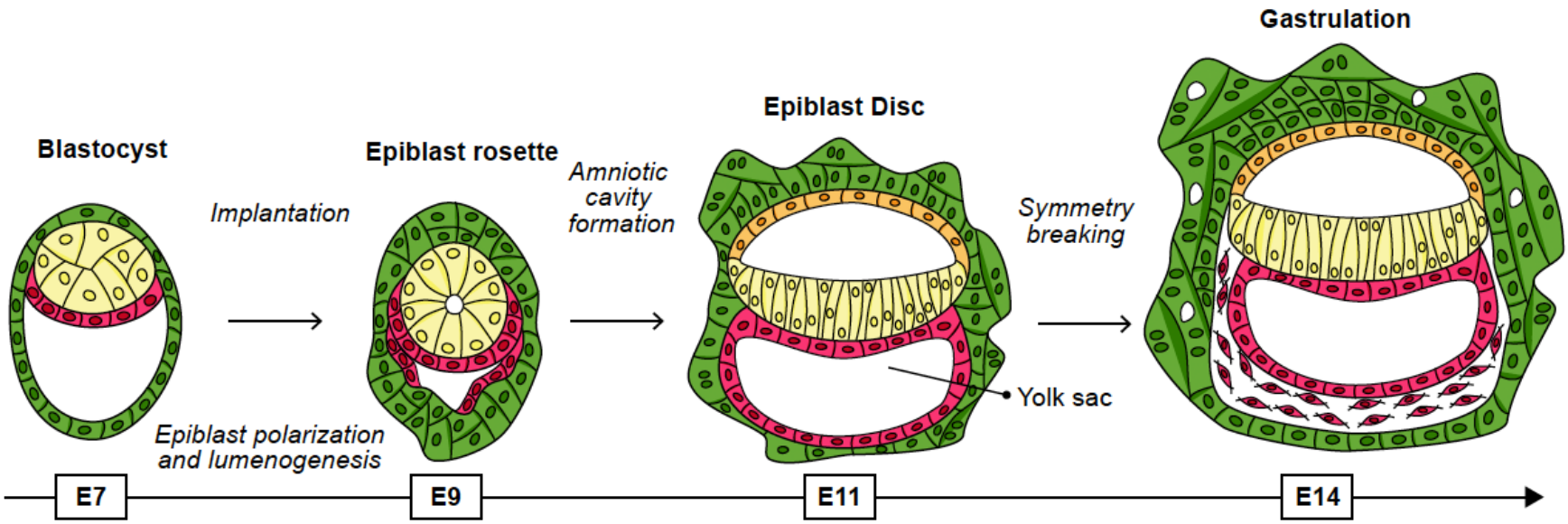
β1 integrin KO embryo



In mouse embryos lack of amniotic cavity formation leads to embryo loss at implantation

Stephens et al (1995) Genes&Dev

Pluripotency and amniotic cavity formation



Pluripotency (Oct4+)

Naïve (unrestricted) — Primed (lineage-biased)

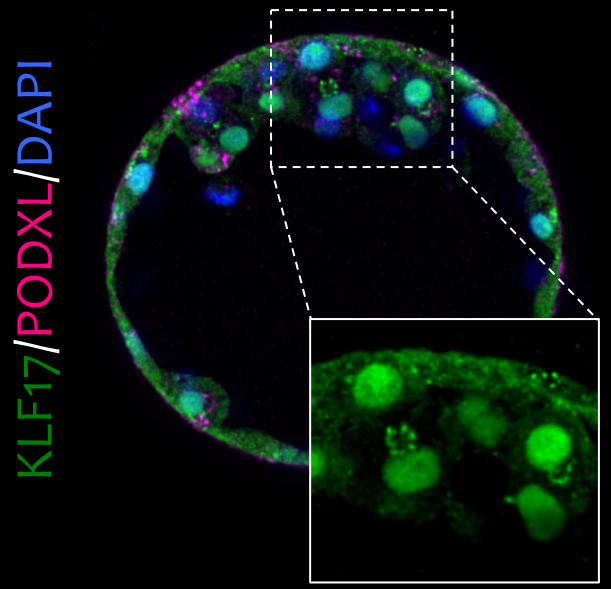
Lineage specification Differentiation

Hypothesis: loss of naïve pluripotency is necessary for amniotic cavity formation at the time of embryo implantation

Nakamura et al (2016) Nature; Smith et al (2017) Development

Epiblast morphogenesis and naïve pluripotency exit

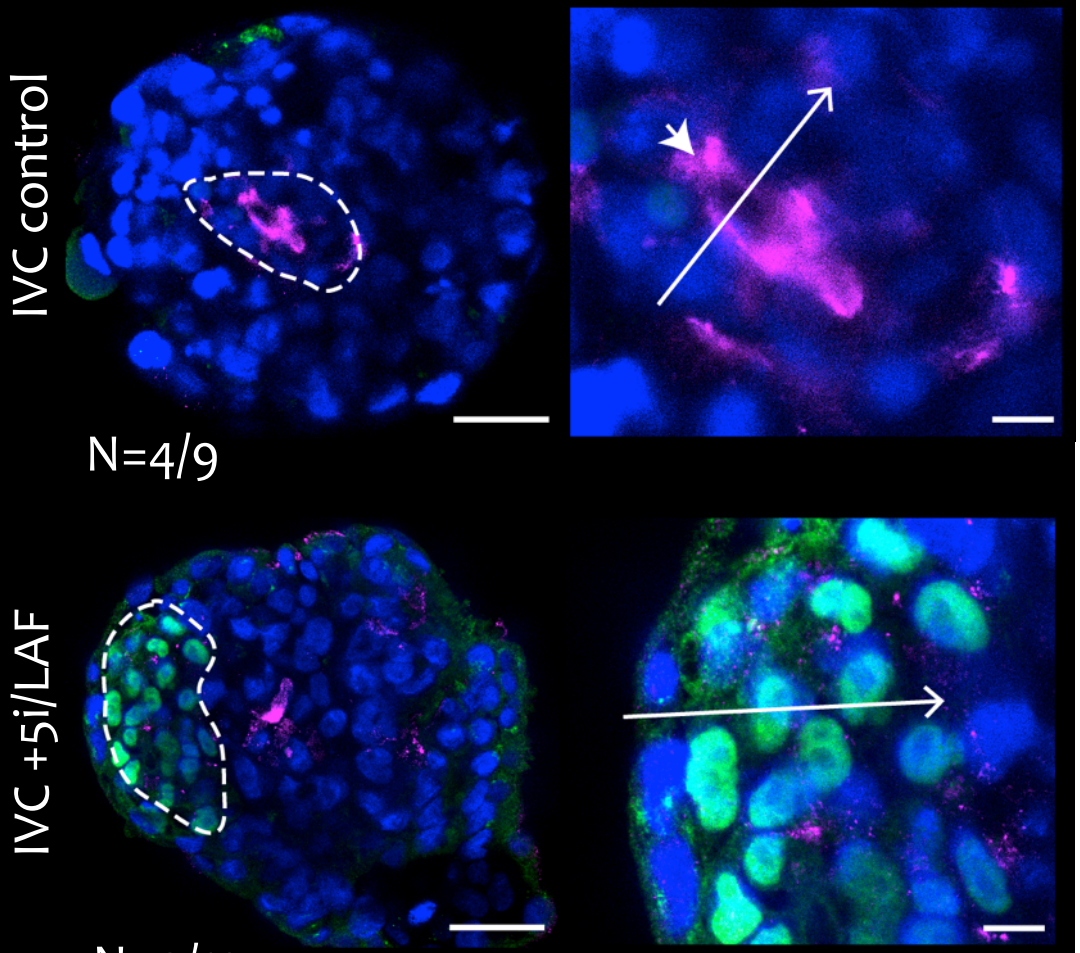
Blastocyst (E6)



KLF17: naïve pluripotent epiblast
PODXL: amniotic cavity

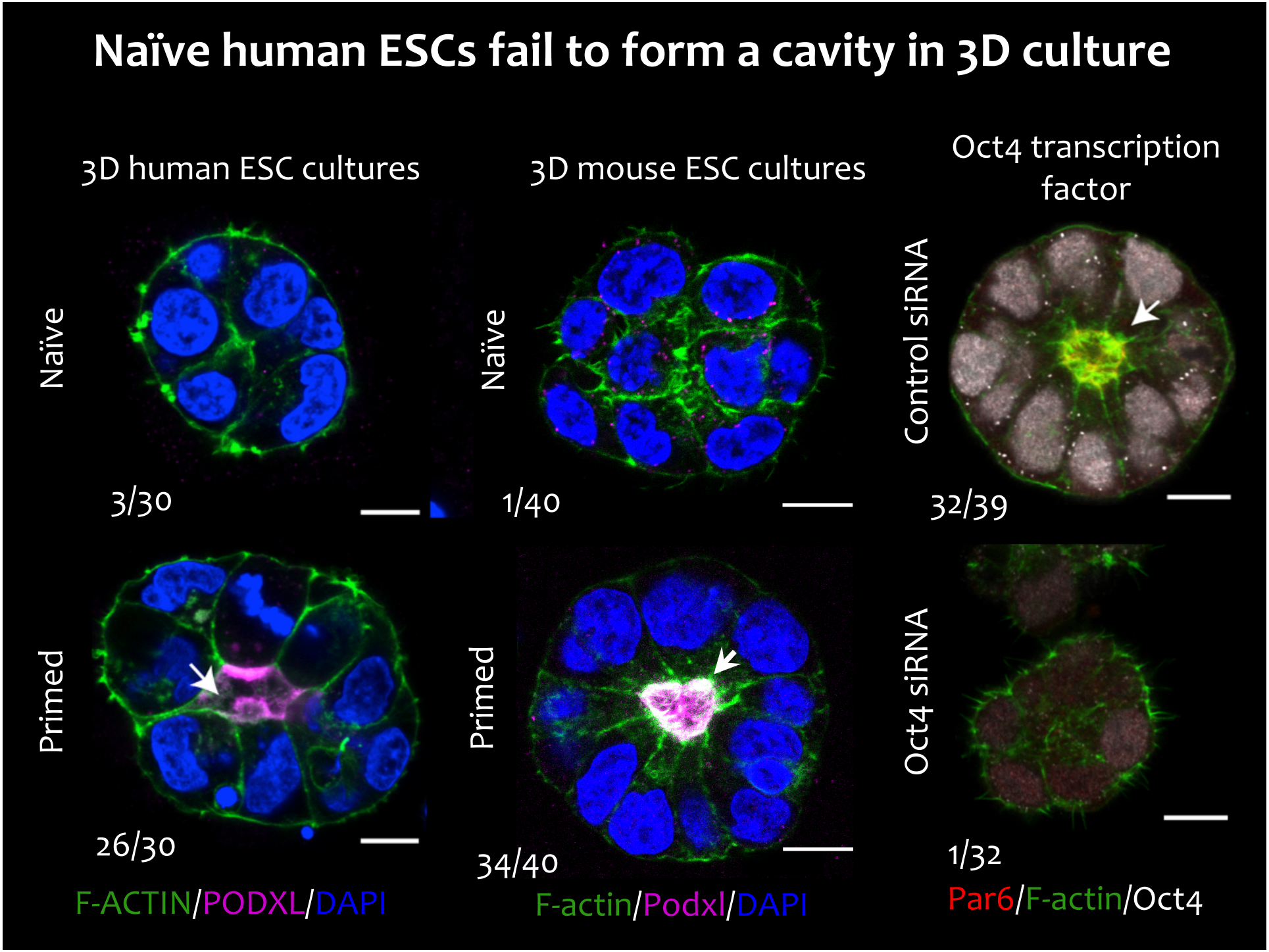
Addition of 5i/LAF blocks human ESCs in the naïve state
(Theunissen et al, 2014, Cell Stem Cell)

Post-implantation embryo (E9-10)



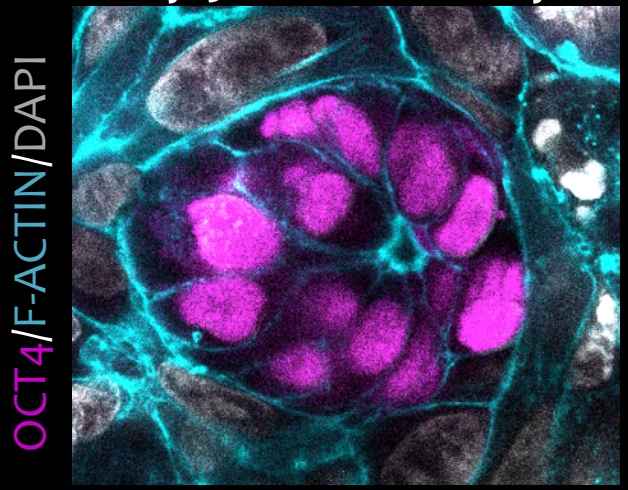
Shahbazi et al (2017) Nature

Naïve human ESCs fail to form a cavity in 3D culture



Molecular regulation of epiblast morphogenesis at implantation

Day 9 human embryo



Extracellular matrix → INTEGRINS → POLARIZATION → AMNIOTIC CAVITY FORMATION

Loss of naïve pluripotency → OCT4 → AMNIOTIC CAVITY FORMATION

Shahbazi et al (2017) Nature

Studying the causes of early post-implantation loss

1. *In vitro* culture of human embryo

- A model system to study early post-implantation

2. Mechanisms of development

- **The embryonic tissue at implantation forms the amniotic cavity**
- **Amniotic cavity formation is directed by integrins and naïve pluripotency exit**
- **Alterations in this process would lead to pregnancy loss**

3. Aneuploid human embryos

- Chromosomal abnormalities and early pregnancy loss:
  When? How?

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Sanger Institute
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