Embryo culture beyond implantation: past, present and future

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The pregnancy loss iceberg

Human pregnancy loss: When? Why?

The embryo 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

What are the morphogenetic transformations that a human embryo undergoes at implantation?

The pregnancy loss iceberg

- Conception
- Day 0
- Pre-implantation failure 30%
- Day 7
- Early post-implantation failure 30%
- Miscarriage 10%
- Live births 30%
- CLINICAL LOSS
- PRE-CLINICAL LOSS
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

  - Day 1
  - Day 6
  - Day 9
  - Day 16

  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**

**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: cytотrophoblast 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


Early post-implantation morphogenesis of the human embryo

Epiblast | Hypoblast | Trophectoderm

Blastocyst → Epiblast rosette → Epiblast disc → Foetus

Implantation → Amniotic cavity formation → Yolk sac formation → Yolk sac

E7 → E9 → E11 → 20 weeks


Embryo growth

Day 7-8 → Day 8-9 → Day 9-10 → Day 10-11
DAY 7-8: Segregation of epiblast and hypoblast

Epiblast (OCT4)
Hypoblast (GATA6)
Inner cell mass

OCT4/GATA6/DAPI
DAY 8-9: Amniotic cavity formation

OCT4/F-actin

aPKC: apical polarity complex

OCT4
Amniotic cavity
Mouse versus human post-implantation development

<table>
<thead>
<tr>
<th>Development at implantation</th>
<th>Development at early post-implantation</th>
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<tbody>
<tr>
<td>E4.75 mouse embryo</td>
<td>E5.5 mouse embryo</td>
</tr>
<tr>
<td>E8-9 human embryo</td>
<td>E10-11 human embryo</td>
</tr>
<tr>
<td>OCT4/F-actin/DAPI Rosette</td>
<td>OCT4/F-actin/DAPI Disc</td>
</tr>
</tbody>
</table>

Cylinder
DAY 10-11: Differentiation of extra-embryonic tissues

**Hypoblast**

Formation of the prospective yolk sac

**Trophoblast**

Differentiation into cytотrophoblast and syncytiotrophoblast

Cytokeratin 7: Pan-trophoblast marker

F-actin/DAPI

DAPI/GATA6/aPKC/OCT4
DAY 10-11: Differentiation of extra-embryonic tissues

**GATA3**: expressed specifically by cytотrophoblast cells

hCG: expressed specifically by syncytiotrophoblast cells

Culture of human embryos beyond implantation

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.

What have we learned?

- Human embryos 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 pregnancy loss:
  - Mechanisms of embryonic morphogenesis
  - Aneuploidy
What directs amniotic cavity formation in the human embryo?

Alterations in amniotic cavitation may contribute to pregnancy loss at implantation.

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

Epiblast morphogenesis and amniotic cavity formation

Integrins: critical for attachment to the substrate and to provide directionality to the cells (inside versus outside)

Day 9 human embryo

Unpublished results

β₁-INTEGRIN
Epiblast (OCT4)

β₁-INTEGRIN/OCT4/F-ACTIN
What directs amniotic cavity formation in the human embryo?

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

Pluripotent state transitions

Amniotic cavity formation
Epiblast morphogenesis and naïve pluripotency exit

Addition of 5i (MEKi, GSK3i, RAFi, SRCi, ROCKi) + human LIF + Activin-A + bFGF2 blocks human ESCs in the naïve state (Theunissen et al, 2014, Cell Stem Cell)

KLF17/GATA6/PODXL/DAPI

KLF17: naïve pluripotent epiblast
GATA6: hypoblast
PODXL: amniotic cavity

IVC control
N=4/9

IVC +5i/LAF
N=0/11

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

3D human ESC cultures

3D mouse ESC cultures

Control siRNA

Oct4 siRNA

F-actin/PODXL/DAPI

F-actin/PODXL/DAPI

Par6/F-actin/Oct4
Pluripotent state transitions coordinate epiblast morphogenesis

What are the molecular mechanisms of early post-implantation failure?

The pregnancy loss iceberg

- Amniotic cavity formation at implantation is fundamental for developmental progression.
- This process is directed by integrins and a pluripotency state transition.
- Mutations in any of these machineries would lead to early post-implantation failure.

Aneuploidy: How do specific chromosomal alterations affect amniotic cavity formation and developmental progression?
In vitro culture of monosomic and trisomic human embryos

Do embryos with different chromosomal abnormalities show different developmental potential in vitro?

Extended culture of monosomic and trisomic embryos (based on the PGT-A result)

Trisomy 16

Monosomy 21

Day 9

Day 9

Unpublished results

Oct4/Gata6/F-actin/DAPI
**In vitro culture of monosomic and trisomic human embryos**

- Monosomic embryos die/arrest in culture significantly more than trisomic embryos.
- The monosomic embryos that do develop up to day 9 are slightly smaller and have an altered ICM/TE ratio compared to trisomic embryos.
- Our *in vitro* culture method is a bona fide platform to assess developmental competency.

*Unpublished results*
Magdalena Zernicka-Goetz group

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