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ZUM SCHUTZ VON
VERSUCHSTIEREN

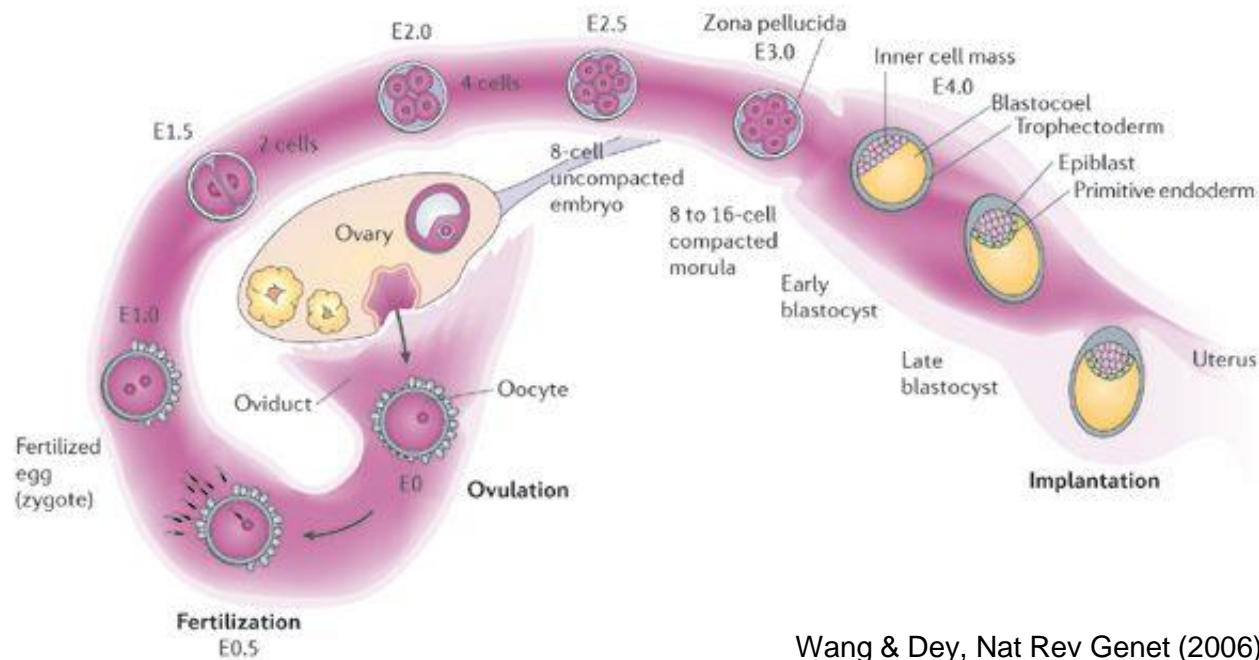


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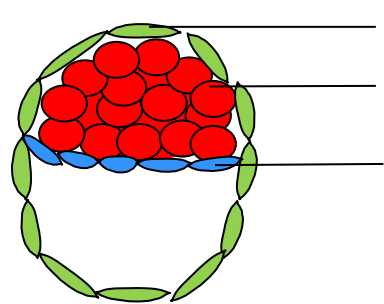
Improved embryoid bodies for studying development, embryotoxicity, and placental function *in vitro*

Marlon Schneider

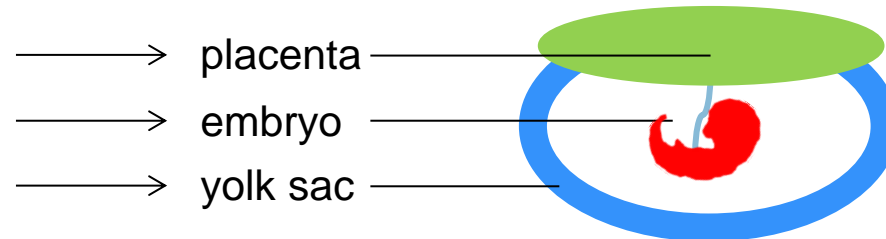
Developmental biology – pre-implantational stages



Wang & Dey, Nat Rev Genet (2006)

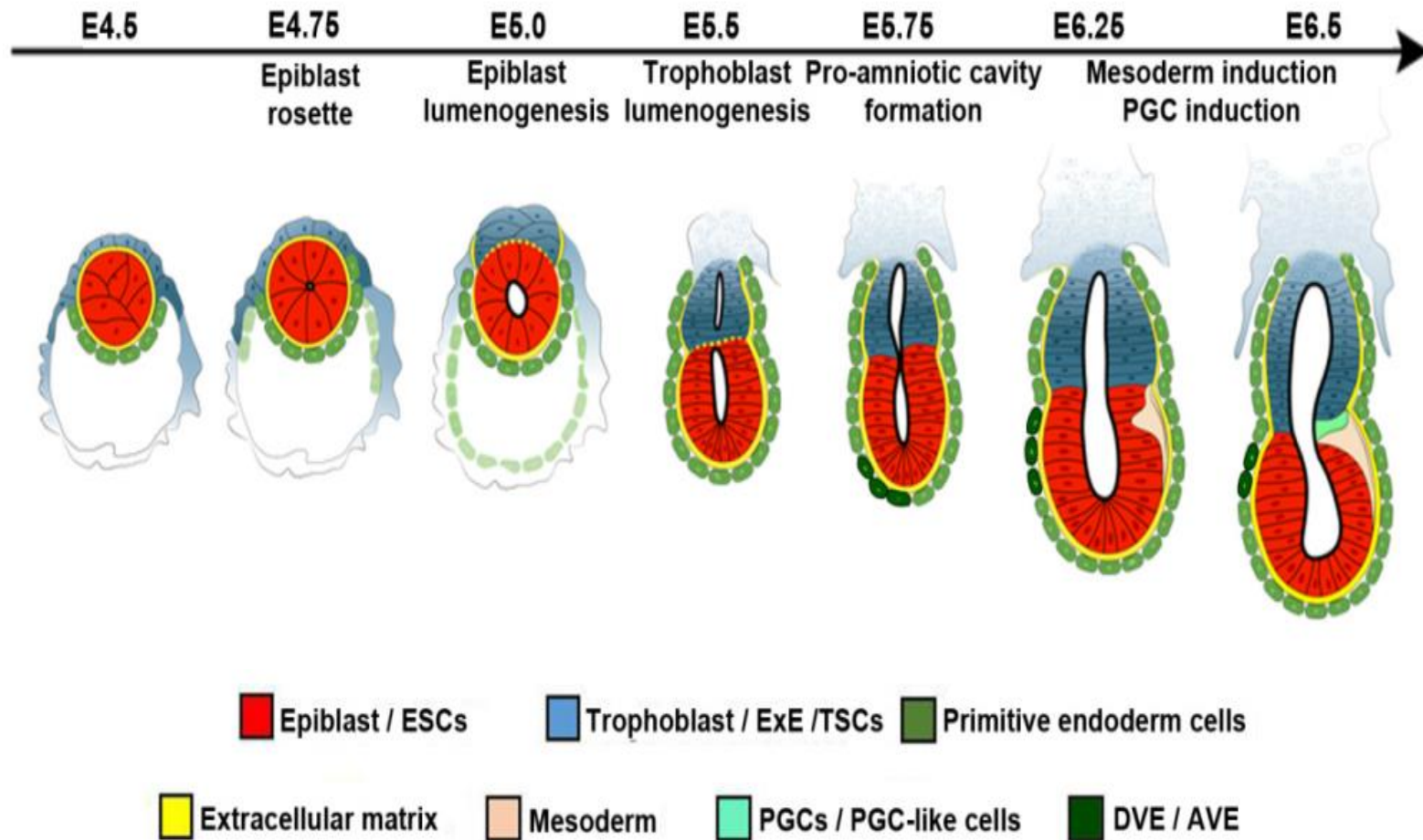


trophectoderm (TE)
epiblast
primitive endoderm (PE)



Late blastocyst
>100 cells
E4.5

Developmental biology – post-implantational development

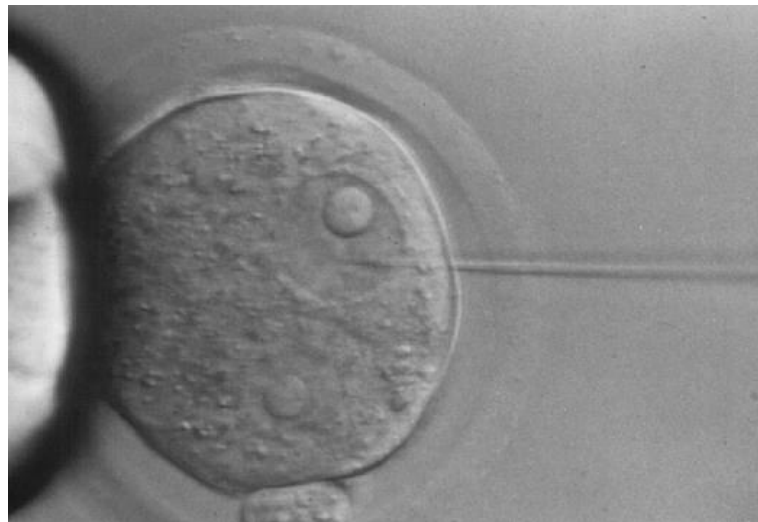


Harrison et al., Science (2017)

Background

Basic and applied research

- Identification of molecular mechanisms and principles of self-organization during embryonic development
- ~20-50% early pregnancy failure (mostly embryo-uterus communication defects)
→ time of implantation is a developmental “black box”
- Largely based on animal studies (rodents, rabbits)



Background

Embryotoxicity

Prenatal development toxicity study (OECD TG 414)

- test substance is administered to pregnant animals (time span: from implantation to as close as possible to the normal day of delivery)
- females are killed before delivery and fetuses are evaluated for soft tissue and skeletal changes

1. Whole embryo culture (WEC) assay

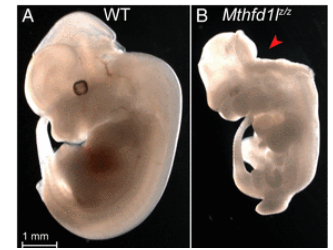
- embryos at different stages are dissected from maternal tissue
- less expensive and more rapid compared to *in vivo* testing
- does not involve experimentation on adult animals

2. Limb bud micromass (MM) assay

- *ex vivo* culture of limb bud cells of mid-organogenesis embryos
- single cell suspension → differentiation into chondrocytes and neurons
- observation of alcian blue staining (cartilage)
- does not involve experimentation on adult animals

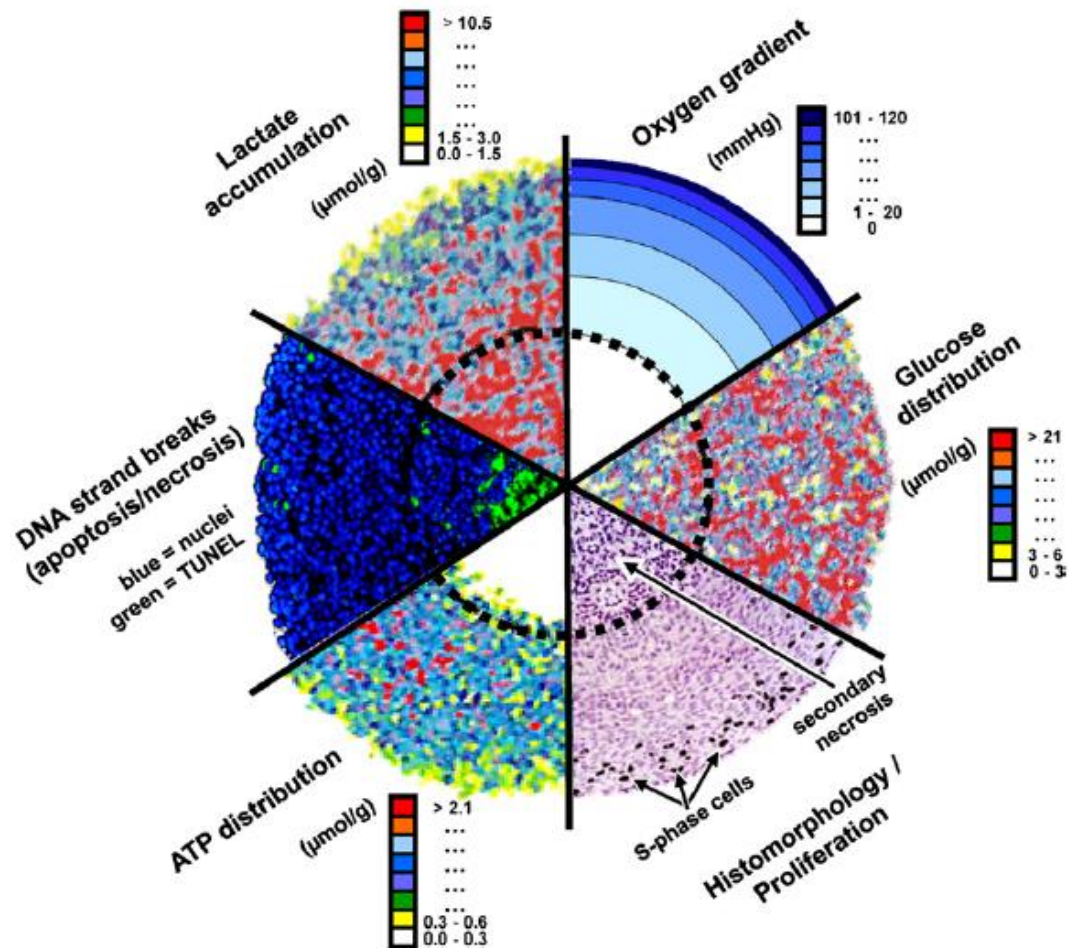
3. Embryonic stem cell test (EST)

- employs embryonic stem cells and 3T3 cells
- composed of two procedures: cytotoxicity (3T3 and D3) and differentiation (D3) assays
- less expensive, no animals needed
- biological relevance?

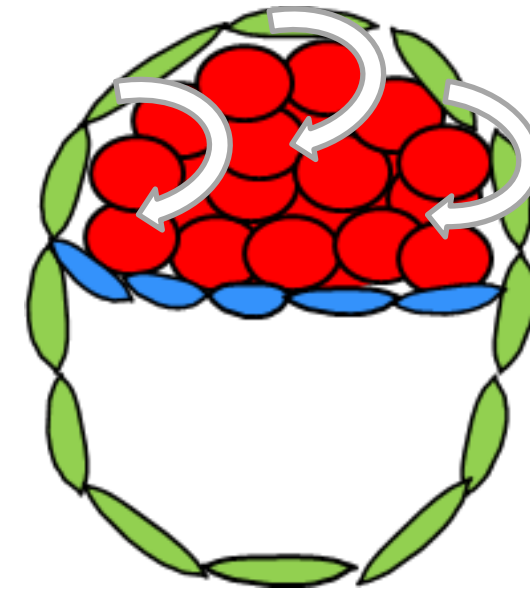


Momb et al. , PNAS (2013)

Drawbacks of embryoid bodies as an experimental tool



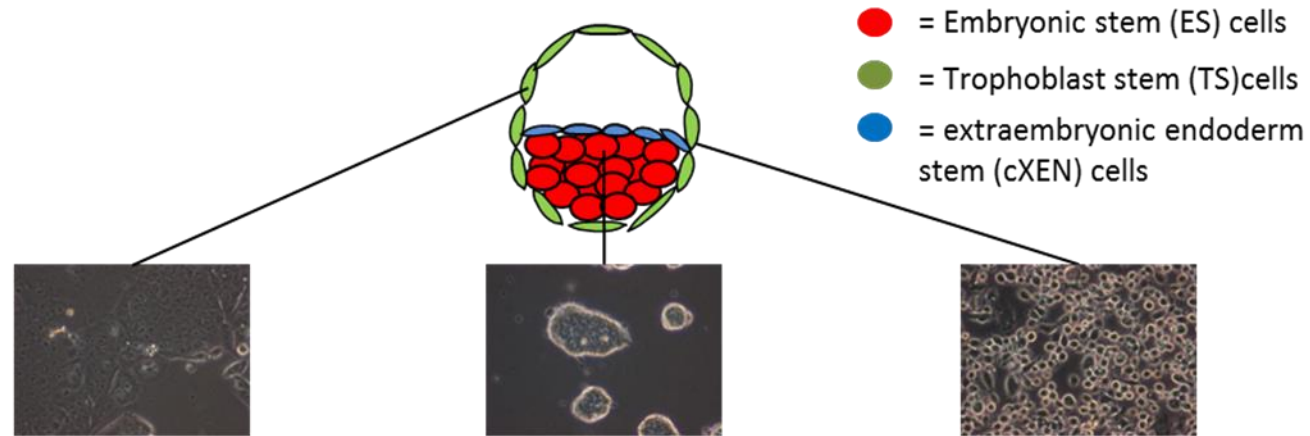
Hirschhäuser et al., J Biotechnol (2010)



Lack of inductive signals

Unphysiological gradients of cell proliferation, viability and metabolism

Approach - Self-assembly of all three cell types



Cell number

650 cells/
embryoid



36 cells/
embryoid

Cell ratio

50% ES
50% TS

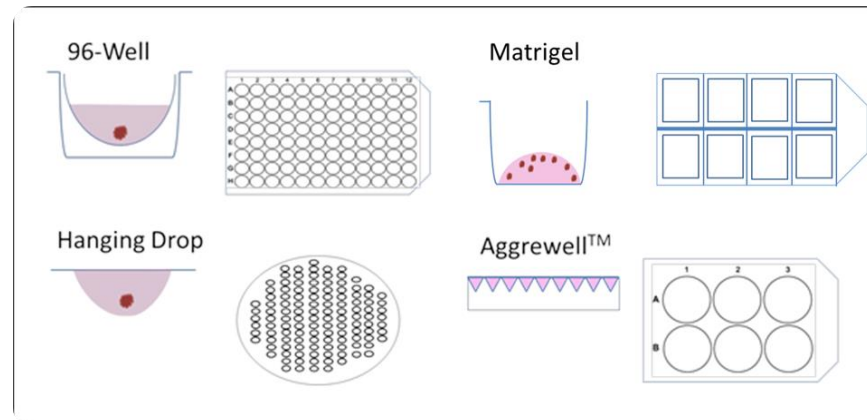
or

30% ES
70% TS

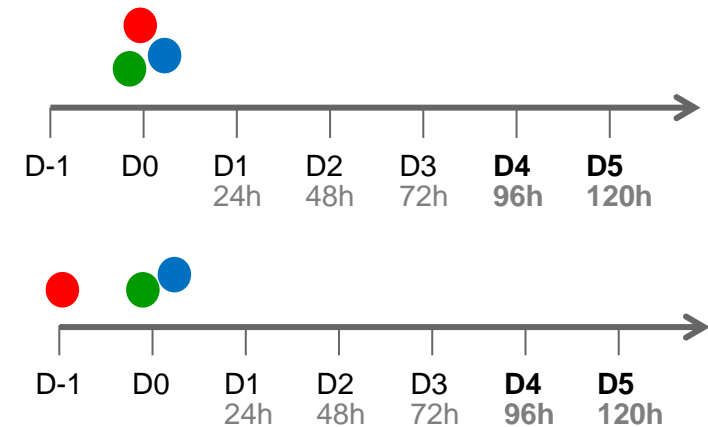
or

8% ES
8% XEN
83% TS

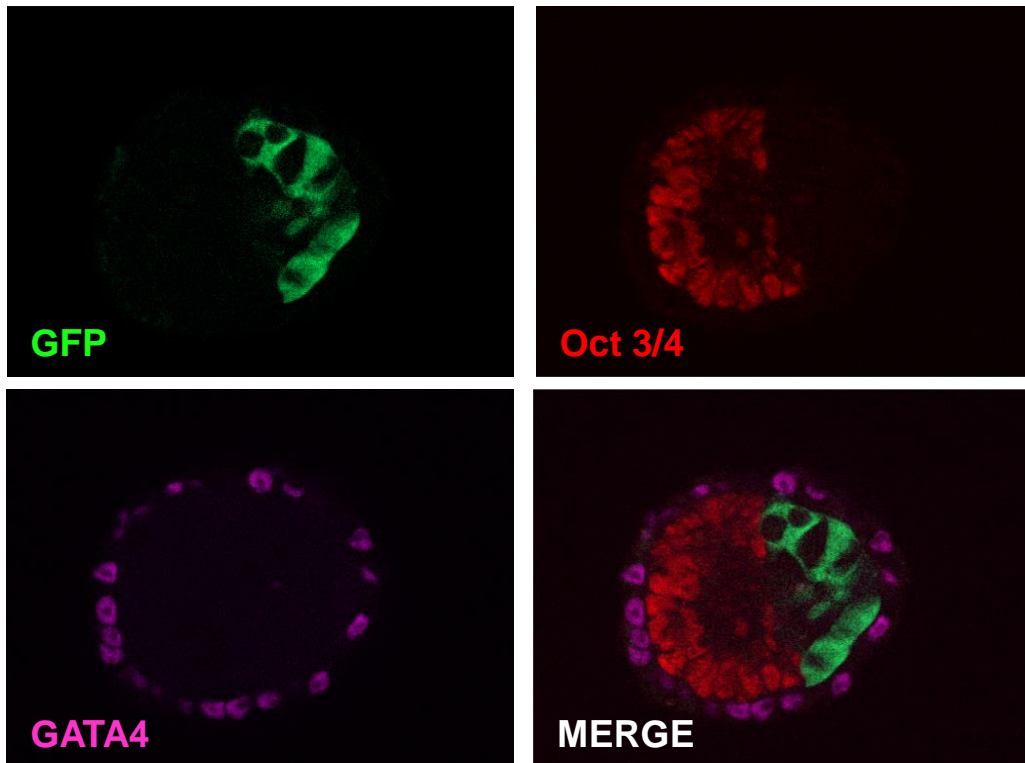
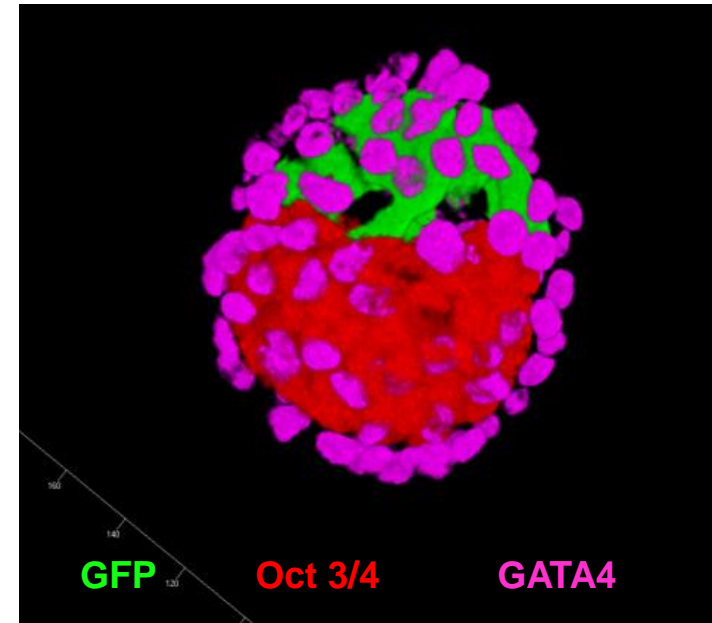
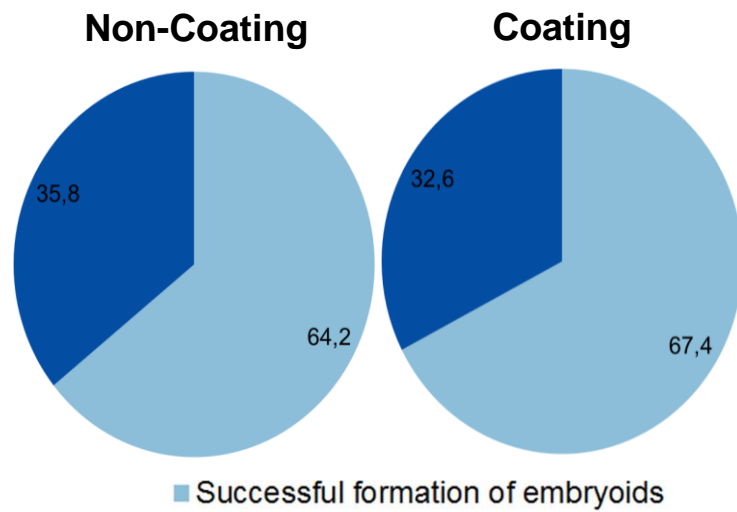
Cell culture format



Coating vs. non-coating



Results – Successful self-assembly



Sozen et al., Nat Cell Biol (2018)

Approach: Coating, 50/50 Medium, 3 ES/ 3 XEN /30 TS

„Synthetic embryos“ are a hot topic

Science

RESEARCH ARTICLES

Cite as: S. E. Harrison *et al.*,
Science 10.1126/science.aal1810
(2017).

Assembly of embryonic and extra-embryonic stem cells to mimic embryogenesis in vitro

Sarah Ellys Harrison,^{1*} Berna Sozen,^{1,2*} Neophytos Christodoulou,¹ Christos Kyprianou,¹ Magdalena Zernicka-Goetz^{1†}

ETS (ES + TS) embryos

- Combination of ES and TS cells (50%/50%)
- Similar to mouse embryos at 5-6 days after fertilization
- Formation rate: 22%

Blastoids

- Combination of ES and TS cells (29%/71%)
- Delayed addition of TS cells to the ES cell aggregate
- Similar to mouse embryos at 3.5 days after fertilization
- Formation rate: 70%

ETX (ES + TS + XEN) embryos

- Combination of ES, TS and XEN cells (23%, 60%, 17%)
- Similar to mouse embryos at 5.5 days after fertilization
- Formation rate: 70% → 29.8% specific morphology

LETTER

<https://doi.org/10.1038/s41586-018-0051-0>

Blastocyst-like structures generated solely from stem cells

Nicolas C. Rivron^{1,2*}, Javier Frias-Aldeguer^{1,2}, Erik J. Vrij¹, Jean-Charles Boisset², Jeroen Korving², Judith Vivie^{2,3}, Roman K. Truckenmüller¹, Alexander van Oudenaarden², Clemens A. van Blitterswijk^{1,5} & Niels Geijsen^{2,4,5}

nature
cell biology

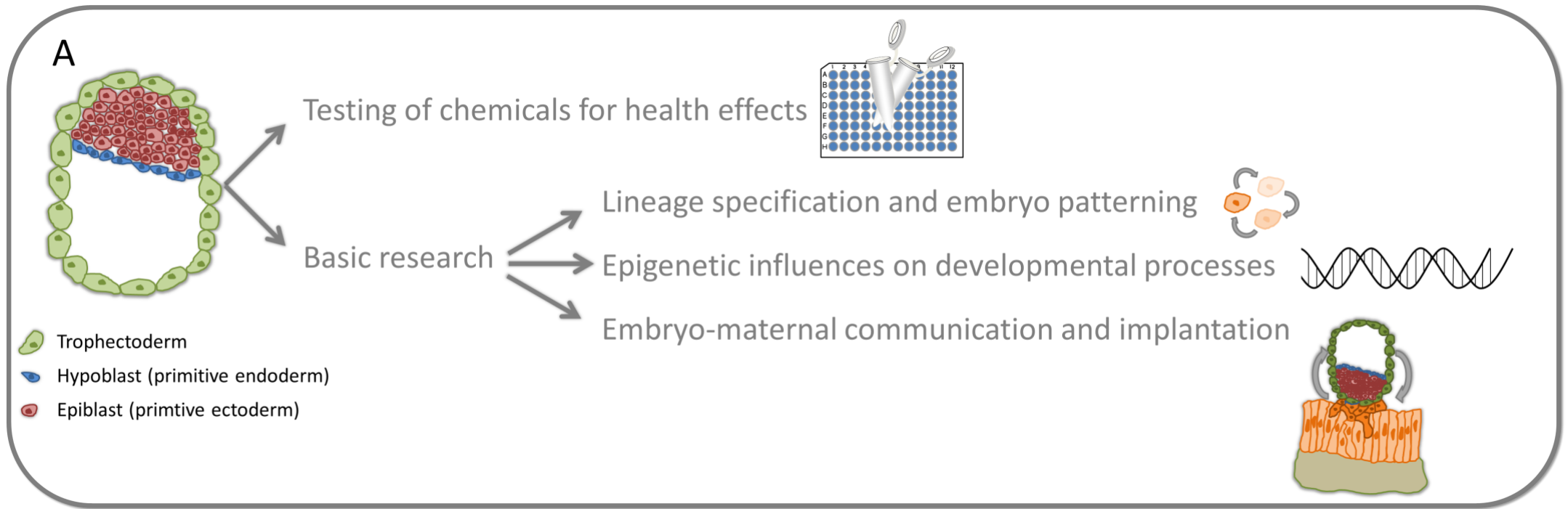
TECHNICAL REPORT

<https://doi.org/10.1038/s41556-018-0147-7>

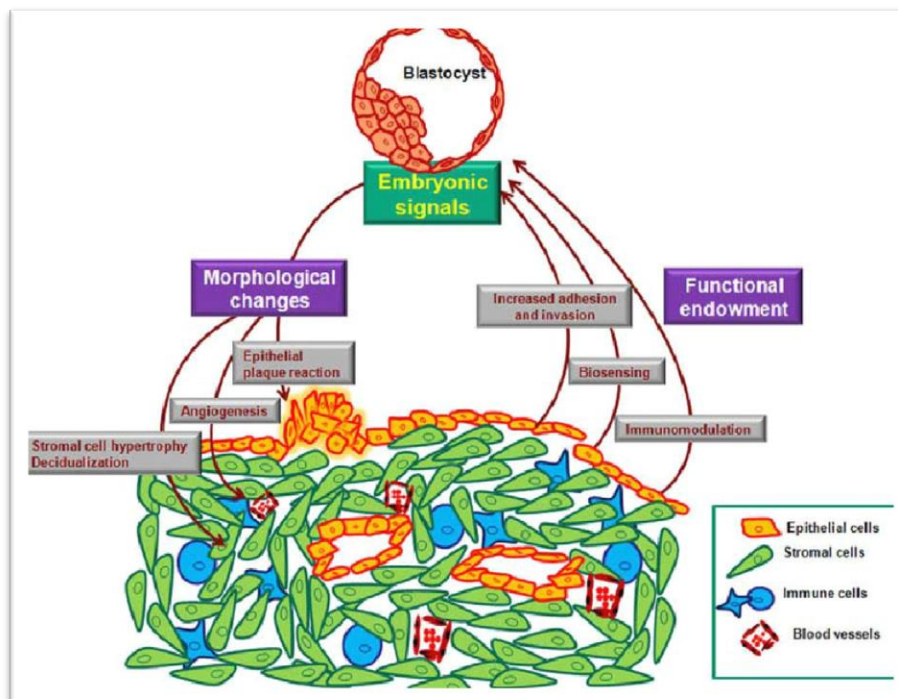
Self-assembly of embryonic and two extra-embryonic stem cell types into gastrulating embryo-like structures

Berna Sozen^{1,2,10}, Gianluca Amadei^{1,10}, Andy Cox¹, Ran Wang³, Ellen Na⁴, Sylwia Czukiewska¹, Lia Chappell⁵, Thierry Voet^{5,6}, Geert Michel⁷, Naihe Jing^{3,8}, David M. Glover⁹ and Magdalena Zernicka-Goetz^{1*}

Potential applications



Outlook



Knöspel et al, Biol Rep (2018)

Modi et al., Front Biosci (2012)

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TS cells

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Thank you for your attention

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