DEUTSCHES ZENTRUM
ZUM SCHUTZ VON
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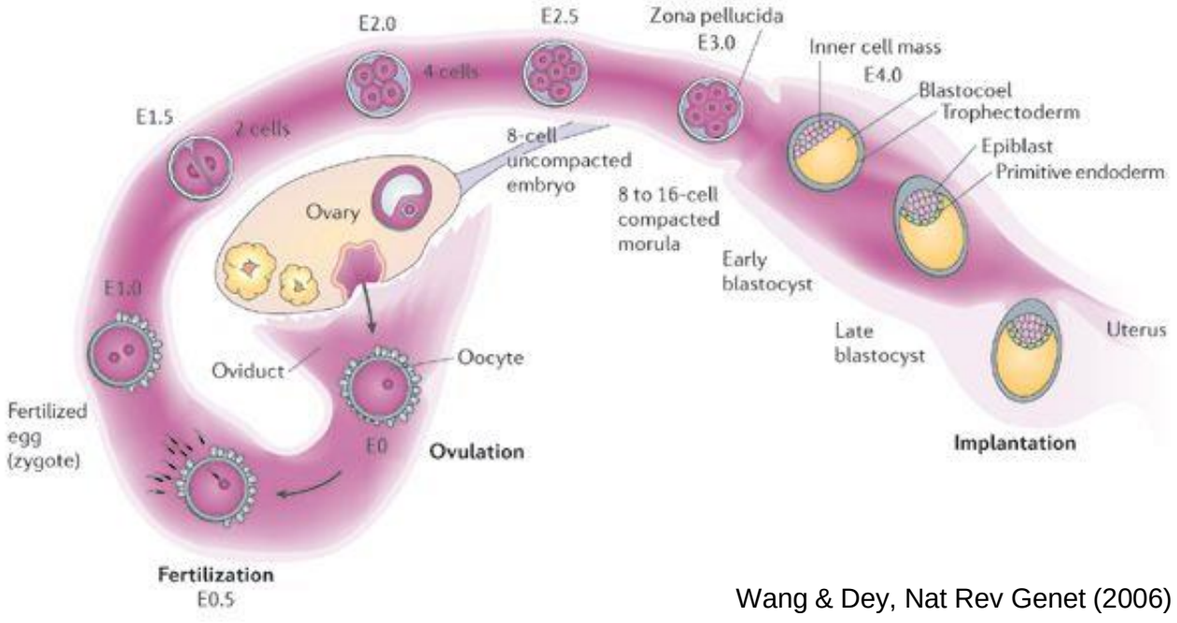
BfR
Bundesinstitut für Risikobewertung

GERMAN FEDERAL INSTITUTE
FOR RISK ASSESSMENT

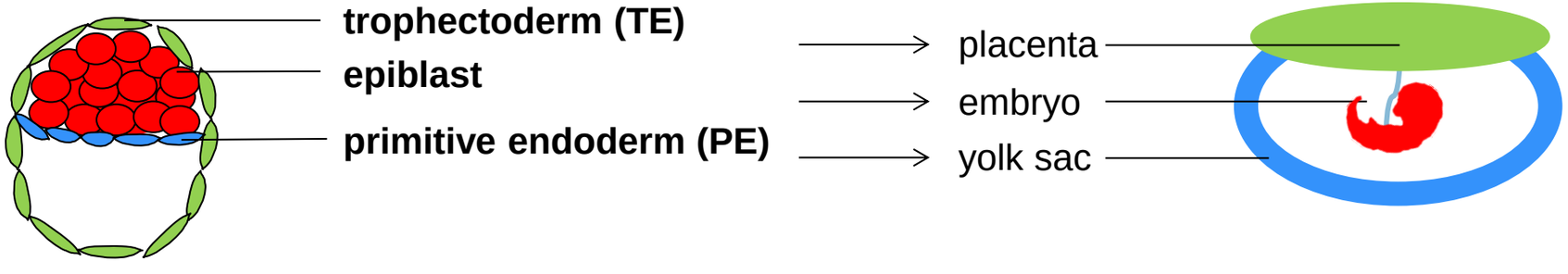
Improved embryoid bodies for studying development, embryotoxicity, and placental function *in vitro*

Marlon Schneider

Developmental biology – pre-implantational stages

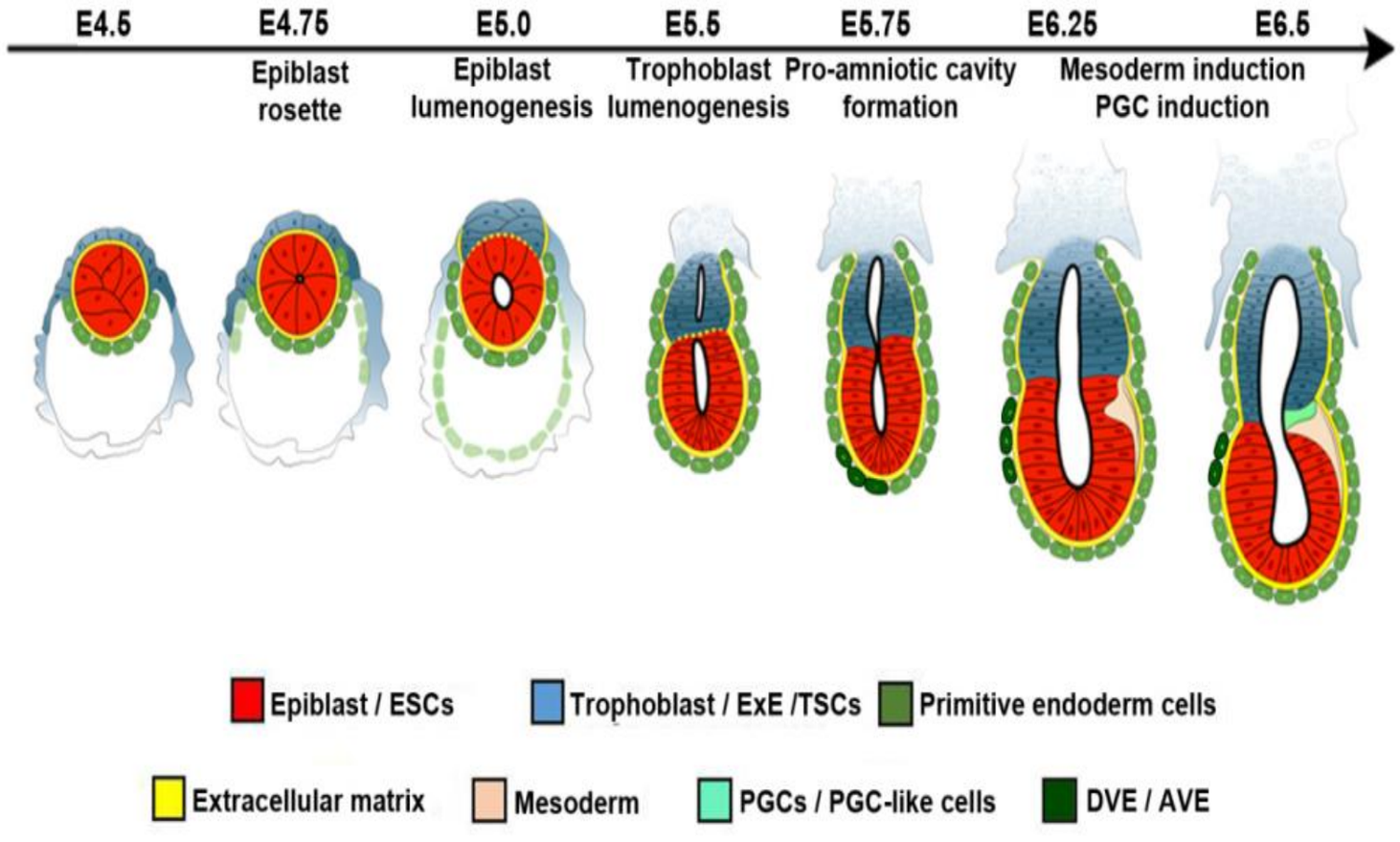


Wang & Dey, Nat Rev Genet (2006)



Late blastocyst
>100 cells
E4.5

Developmental biology – post-implantational development



Harrison et al., Science (2017)

Background

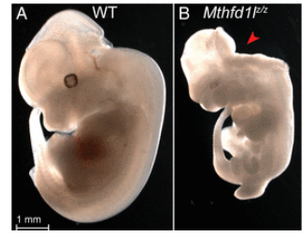
Basic and applied research

- 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



Momb et al. , PNAS (2013)

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 foetuses 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

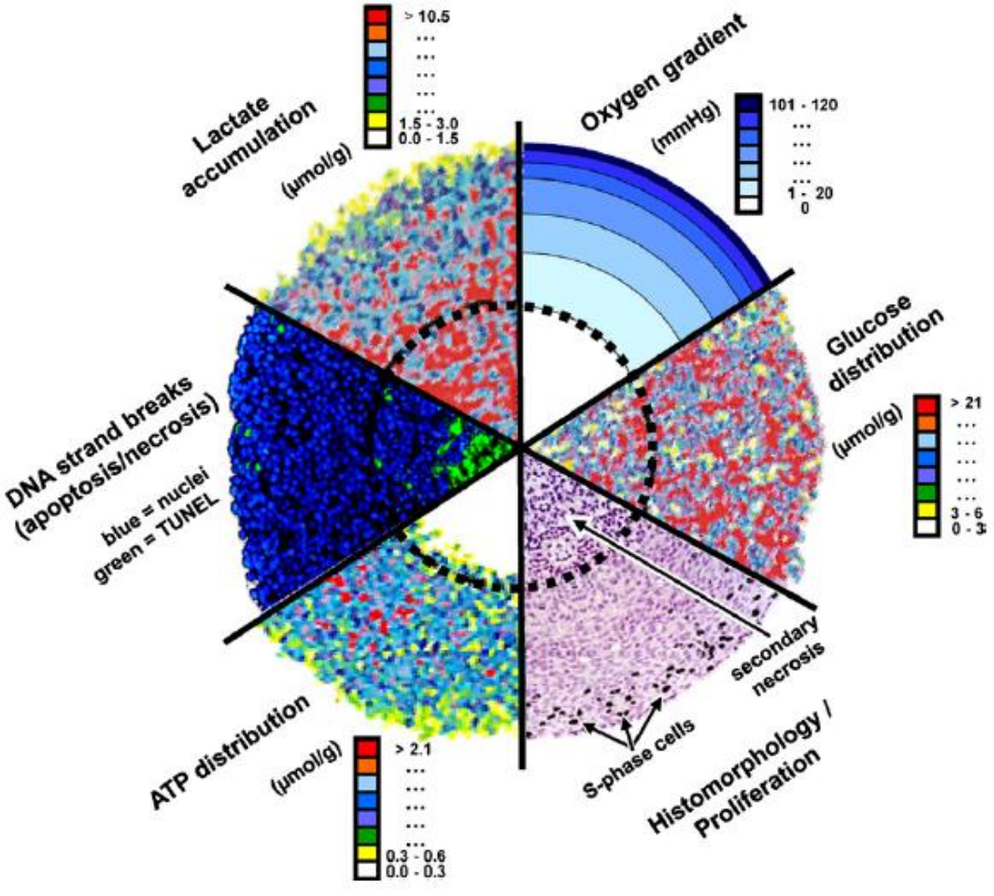
2. Limb bud micromass (MM) assay

- *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)

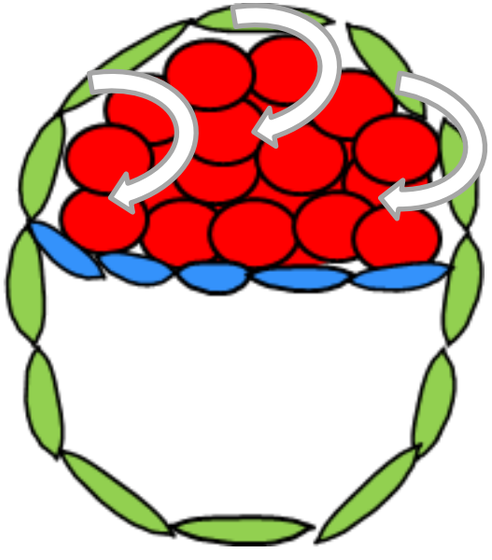
- employs embryonic stem cells and 3T3 cells
- composed of two procedures: cytotoxicity (3T3 and D3) and differentiation (D3) assays
- less expensive, no animals needed
- biological relevance?

Drawbacks of embryoid bodies as an experimental tool



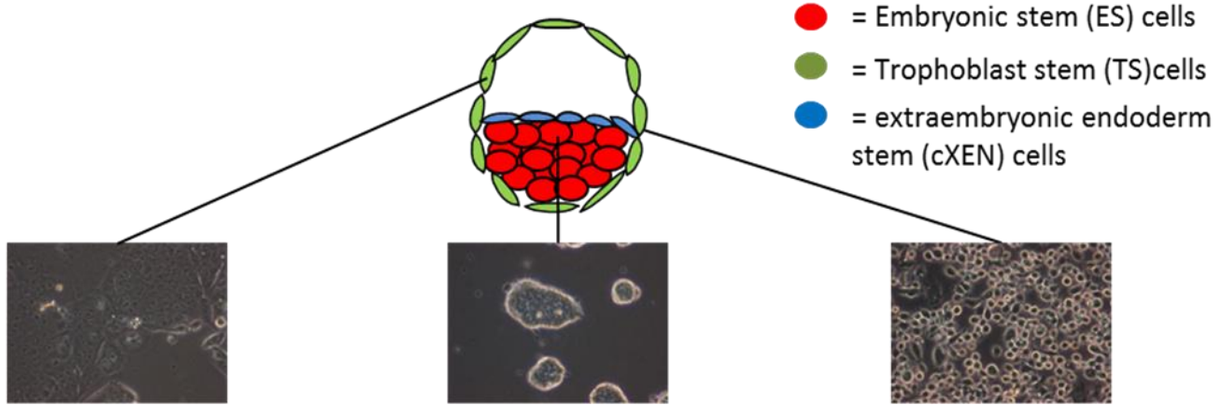
Hirschhäuser et al., J Biotechnol (2010)

Unphysiological gradients of cell proliferation, viability and metabolism



Lack of inductive signals

Approach - *Self-assembly of all three cell types*



Cell number

650 cells/ embryoid

↓

↓

↓

36 cells/ embryoid

Cell ratio

50% ES
50% TS

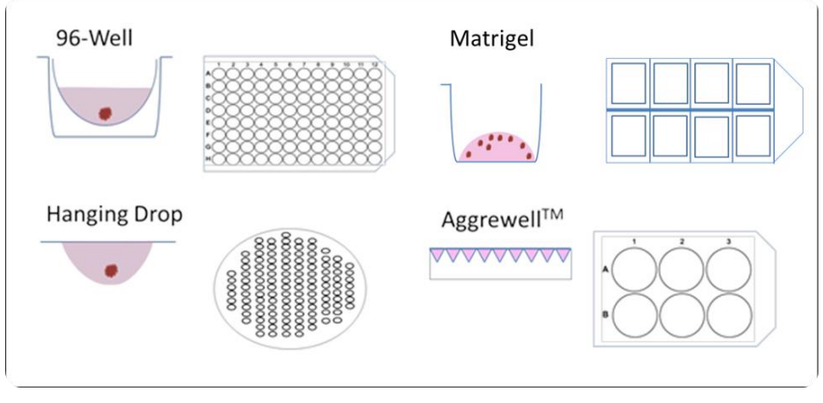
or

30% ES
70% TS

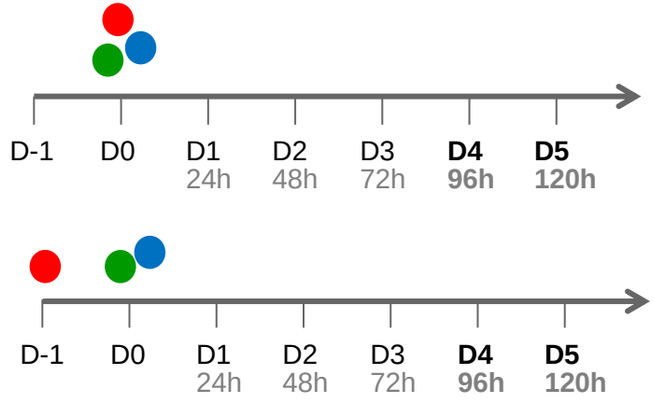
or

8% ES
8% XEN
83% TS

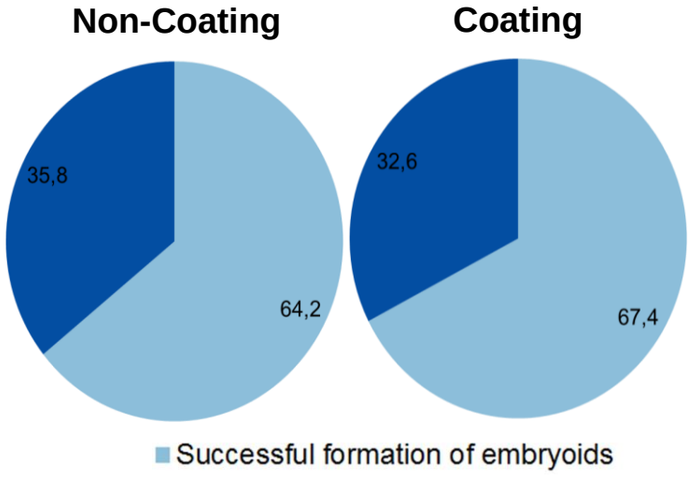
Cell culture format

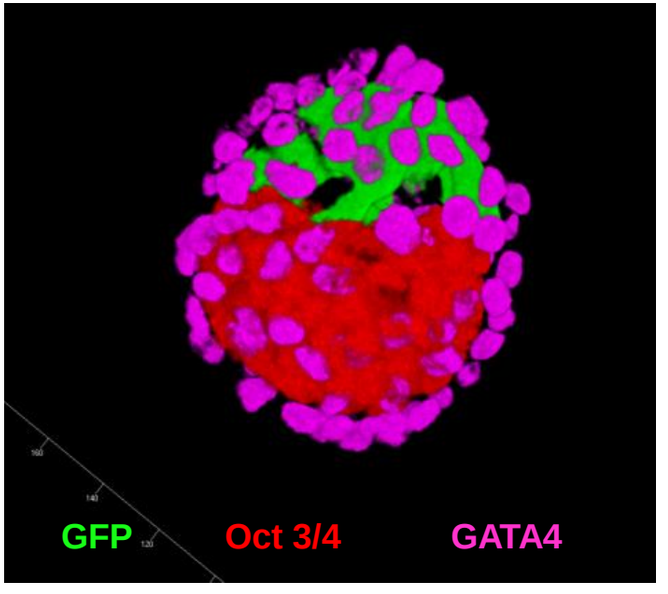


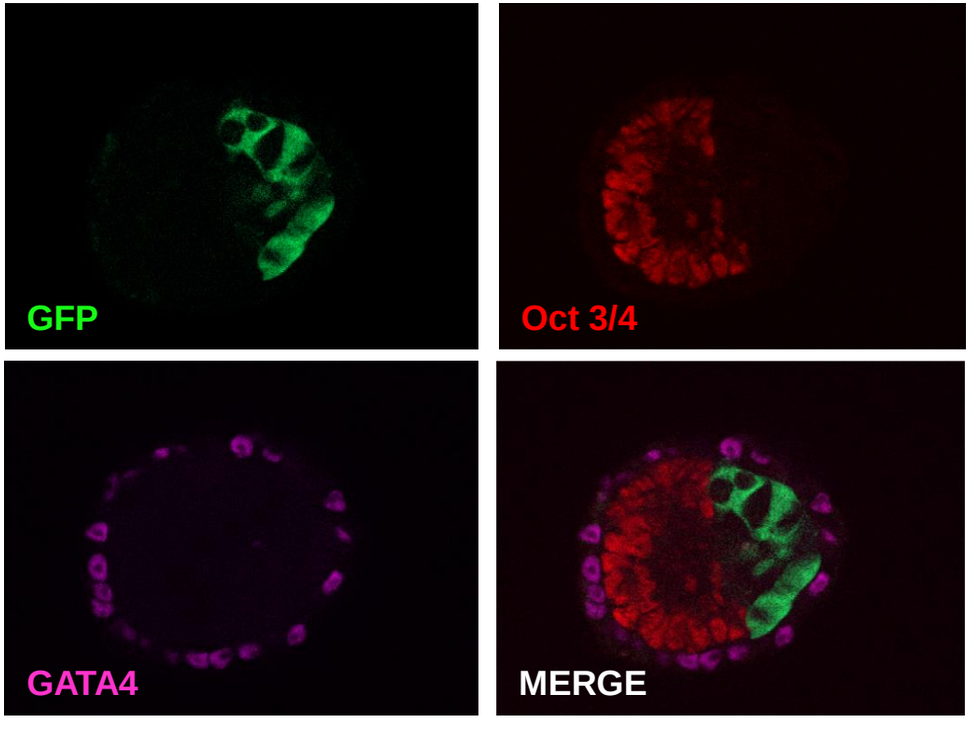
Coating vs. non-coating



Results – Sucessful 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%

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. Vrij[1], Jean-Charles Boisset[2], Jeroen Korving[2], Judith Vivié[2,3], Roman K. Truckenmüller[1], Alexander van Oudenaarden[2], Clemens A. van Blitterswijk[1,5] & Niels Geijsen[2,4,5]

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%

nature cell biology

TECHNICAL REPORT

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

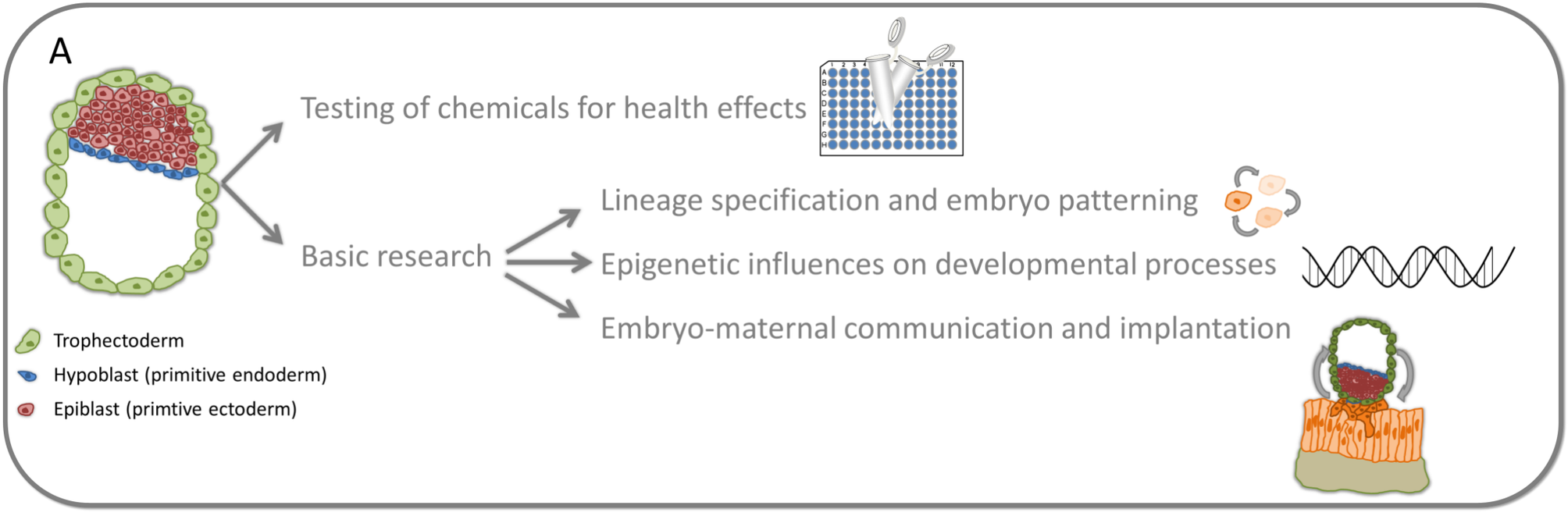
Self-assembly of embryonic and two extra-embryonic stem cell types into gastrulating embryo-like structures

Berna Sozen[1,2,10], Gianluca Amadei[1,10], Andy Cox[1], Ran Wang[3], Ellen Na[4], Sylwia Czukiewska[1], Lia Chappell[5], Thierry Voet[5,6], Geert Michel[7], Naihe Jing[3,8], David M. Glover[9] and Magdalena Zernicka-Goetz[1*]

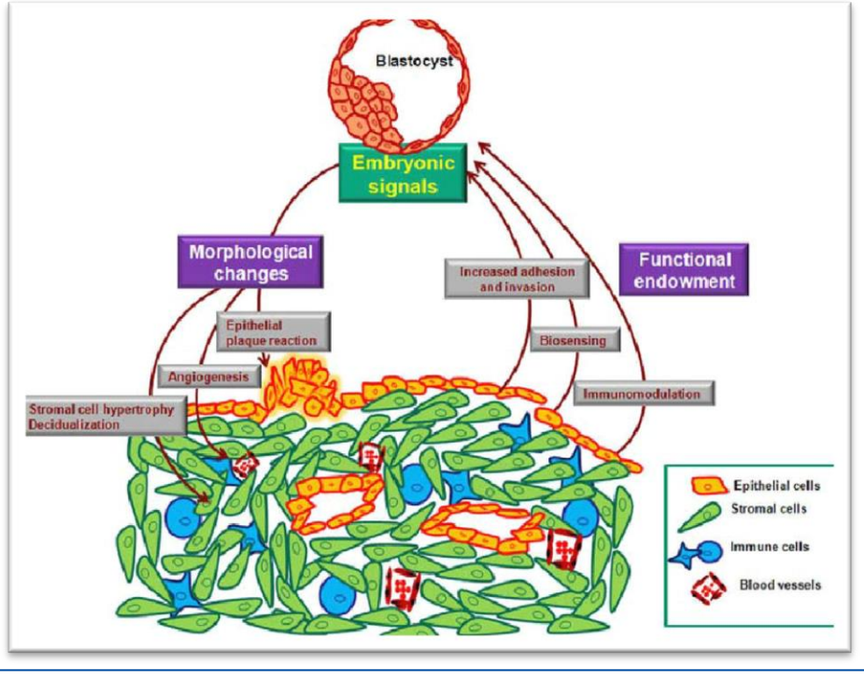
ETX (ES + TS + XEN) embryos

- 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

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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References

Wang H, Dey SK. Roadmap to embryo implantation: clues from mouse models. Nat Rev Genet. 2006 Mar;7(3):185-99. Review.

Harrison SE, Sozen B, Christodoulou N, Kyprianou C, Zernicka-Goetz M. Assembly of embryonic and extraembryonic stem cells to mimic embryogenesis in vitro. Science. 2017 Apr 14;356(6334). pii: eaal1810. doi: 10.1126/science.aal1810.

Matsuo I, Hiramatsu R. Mechanical perspectives on the anterior-posterior axis polarization of mouse implanted embryos. Mech Dev. 2017 Apr;144(Pt A):62-70. doi: 10.1016/j.mod.2016.09.002. Epub 2016 Sep 30. Review.

Hirschhaeuser F, Menne H, Dittfeld C, West J, Mueller-Klieser W, Kunz-Schughart LA. Multicellular tumor spheroids: an underestimated tool is catching up again. J Biotechnol. 2010 Jul 1;148(1):3-15. doi: 10.1016/j.jbiotec.2010.01.012.

Modi DN, Godbole G, Suman P, Gupta SK. Endometrial biology during trophoblast invasion. Front Biosci (Schol Ed). 2012 Jan 1;4:1151-71. Review.

Saitou M, Yamaji M. Germ cell specification in mice: signaling, transcription regulation, and epigenetic consequences. Reproduction. 2010 Jun;139(6):931-42. doi: 10.1530/REP-10-0043. Epub 2010 Apr 6. Review.

Momb J, Lewandowski JP, Bryant JD, Fitch R, Surman DR, Vokes SA, Appling DR. Deletion of Mthfd1l causes embryonic lethality and neural tube and craniofacial defects in mice. Proc Natl Acad Sci U S A. 2013 Jan 8;110(2):549-54. doi: 10.1073/pnas.1211199110. Epub 2012 Dec 24.