

The PluriLum assay: A novel stem cell-based assay for testing of chemicals' embryotoxic effects

Rie Vinggaard

Cell Toxicology team, National Food Institute, Technical University of Denmark

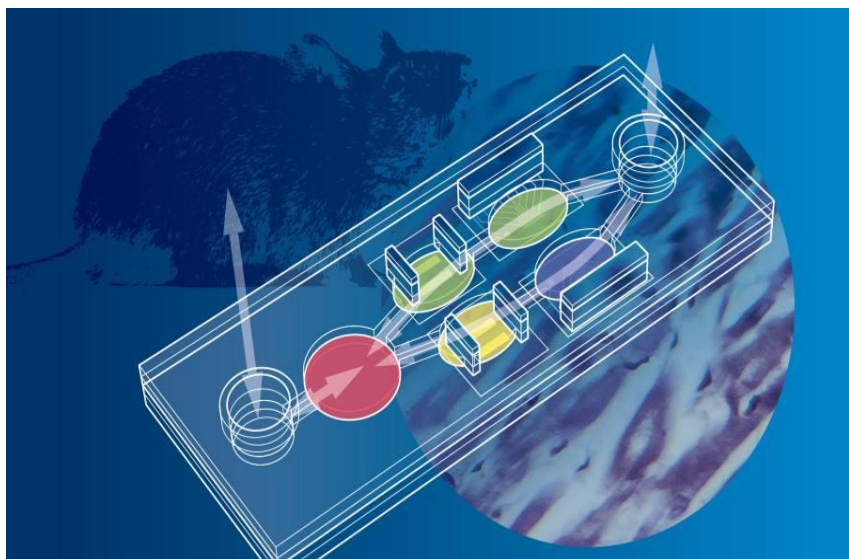
The Danish 3R Center

Annual meeting 8-9 Nov, 2022

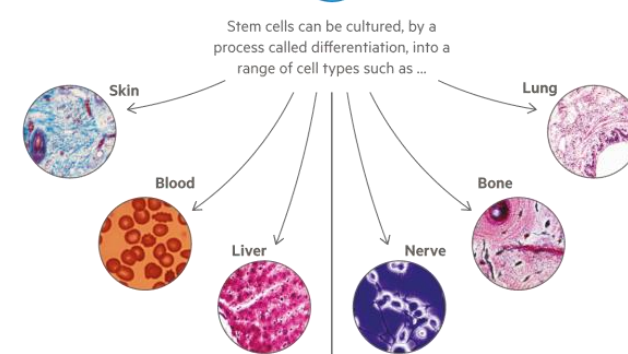
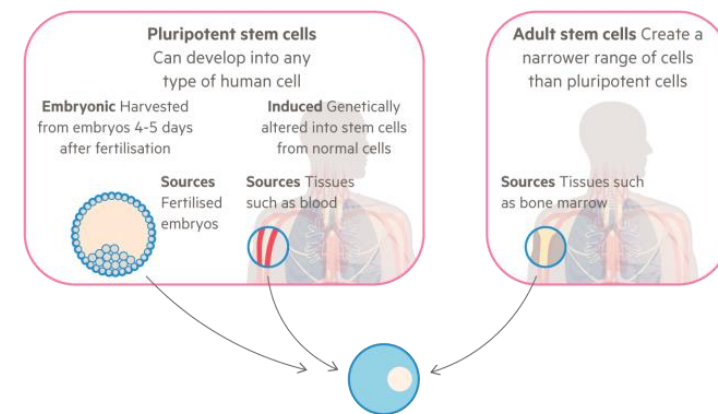
Financial Times Aug 14, 2022

How science is getting closer to a world without animal testing

By Clive Cookson, Hannah Kuchler and Joe Miller

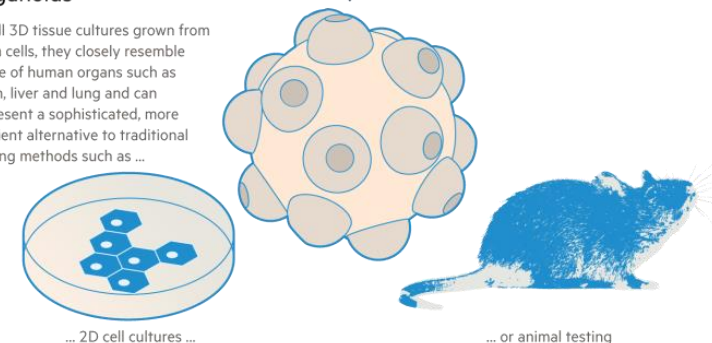


Stem cell types



Organoids

Small 3D tissue cultures grown from stem cells, they closely resemble those of human organs such as brain, liver and lung and can represent a sophisticated, more efficient alternative to traditional testing methods such as ...

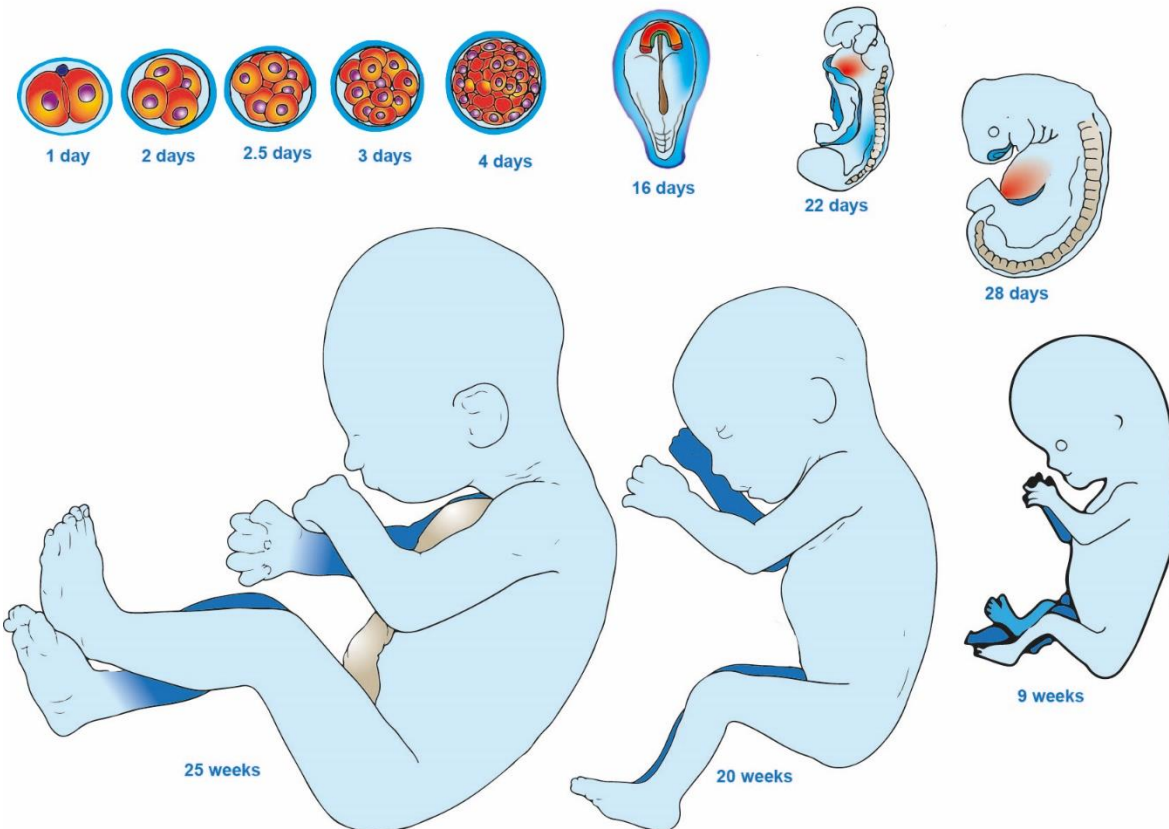


Graphic: Ian Bott
Sources: HSCI; Nature; Mayo Clinic; NCBI; FT research
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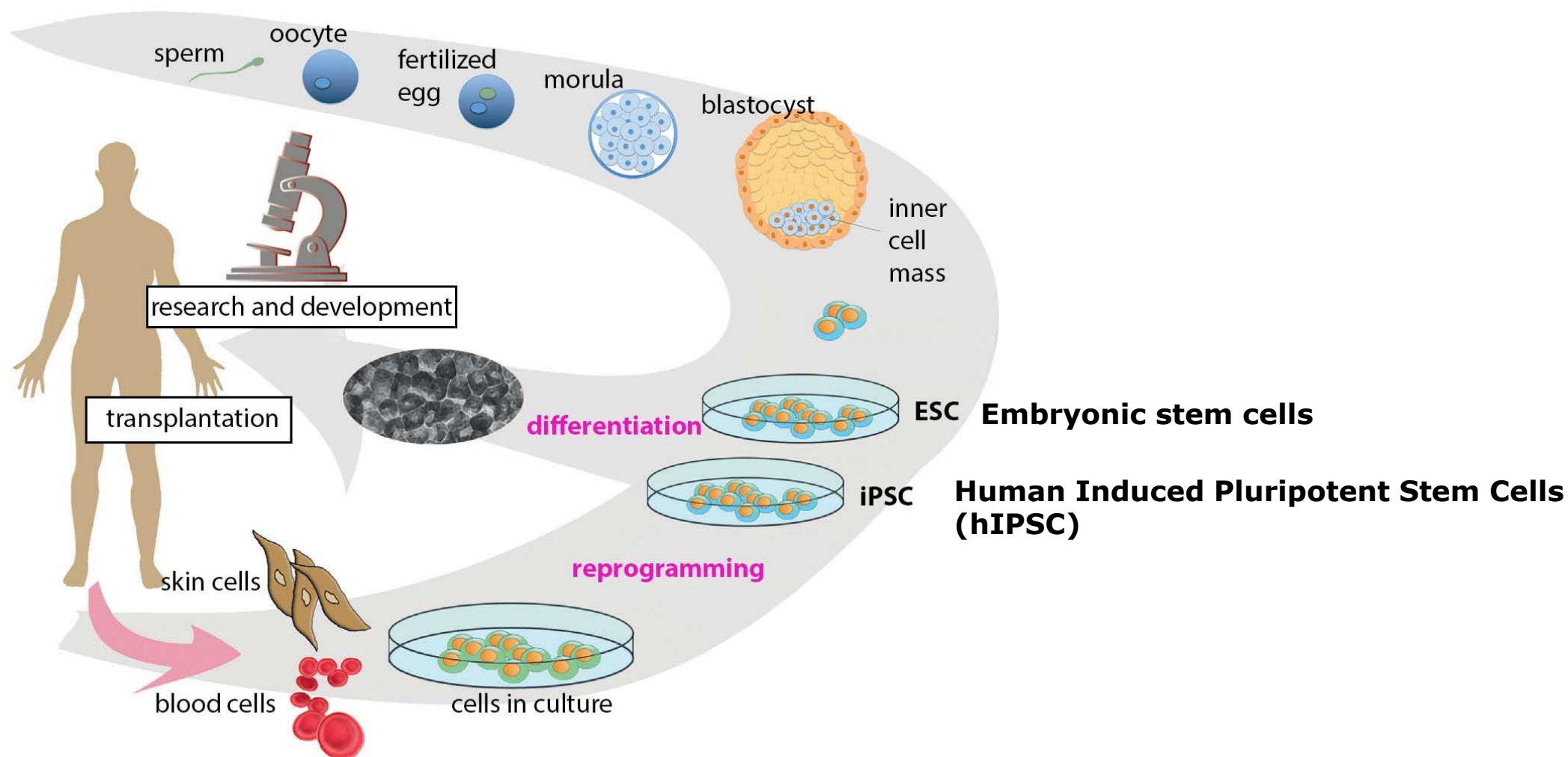
Prenatal developmental toxicity

Human prenatal development:

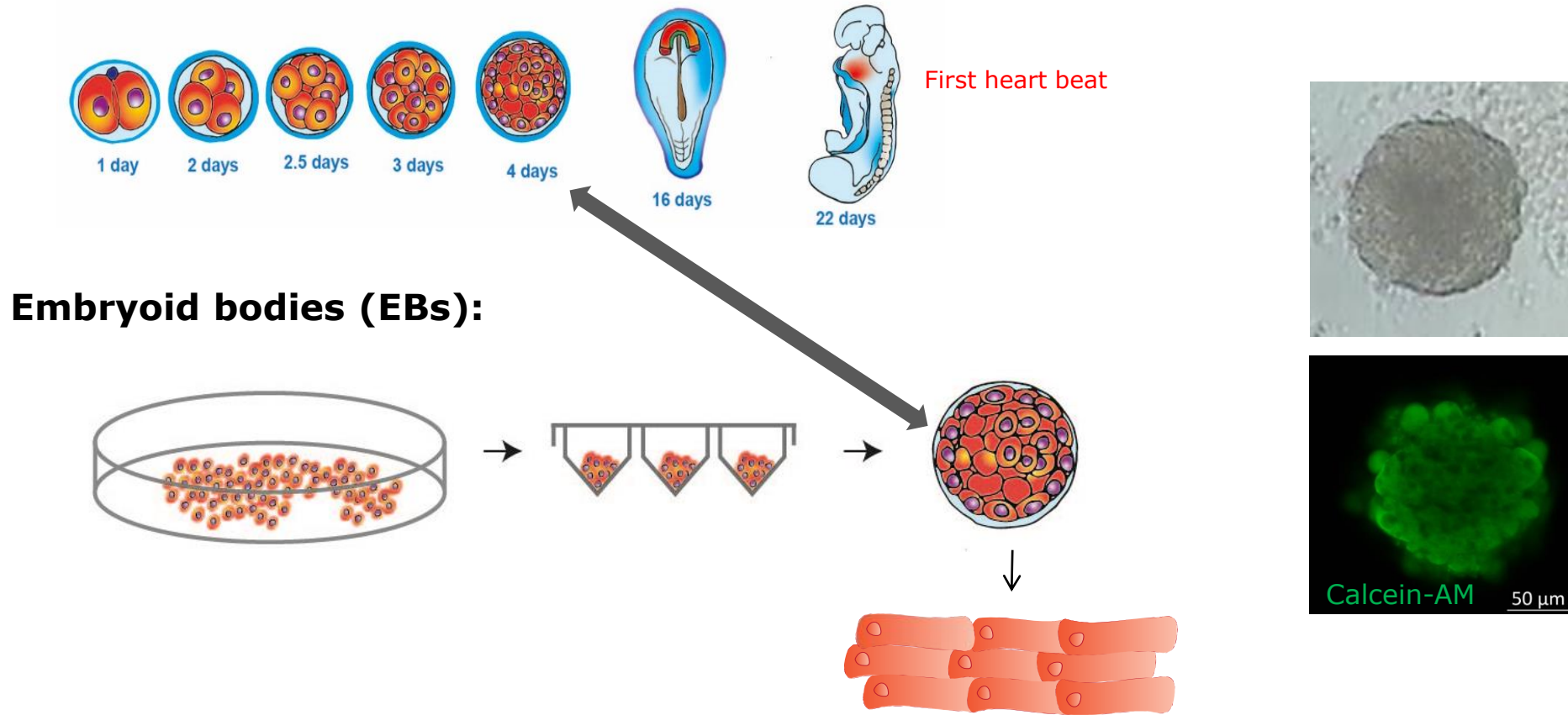
Example of developmental toxicity:
Thalidomide (1960s)



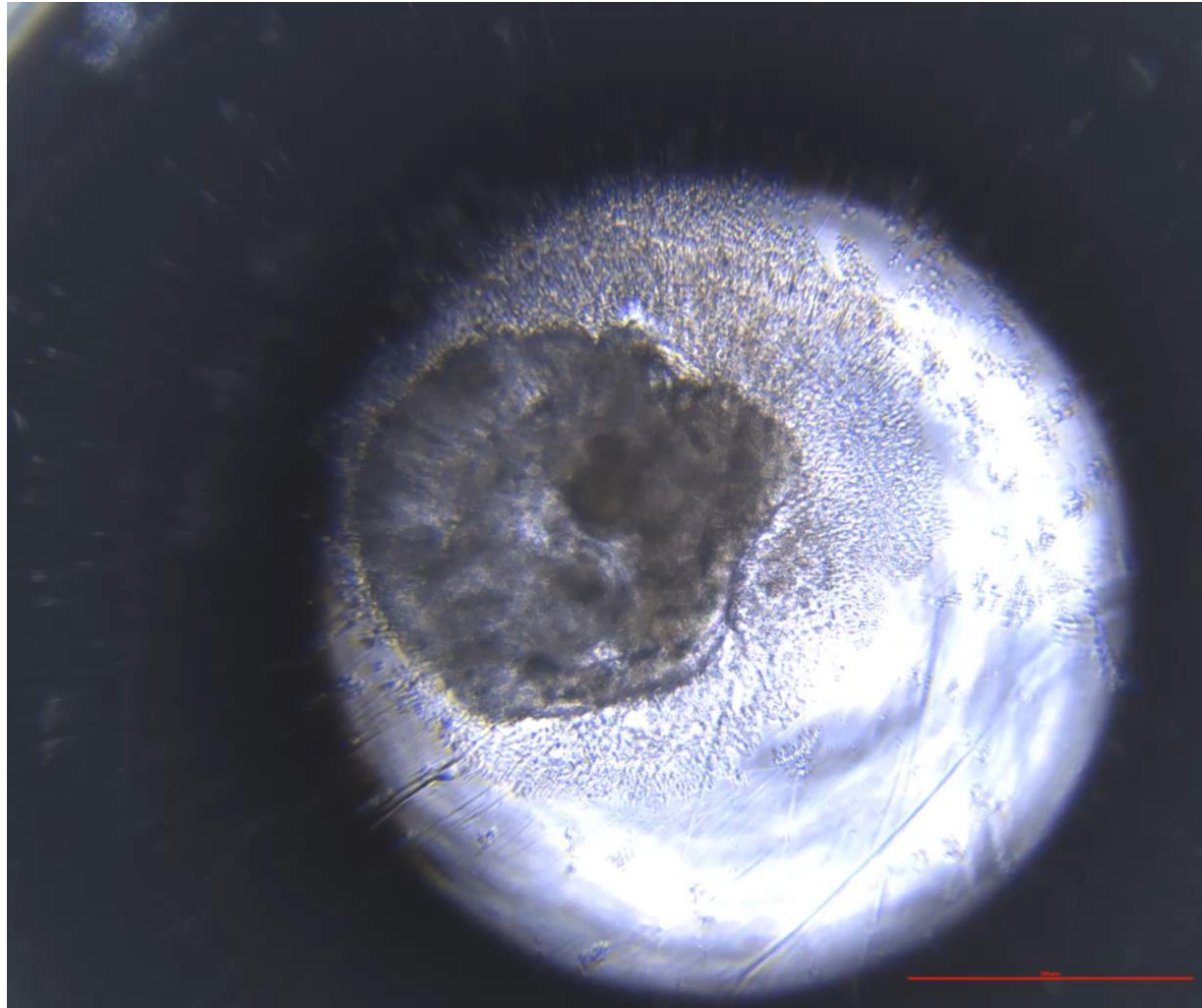
Human-induced pluripotent stem cells have no ethical issues



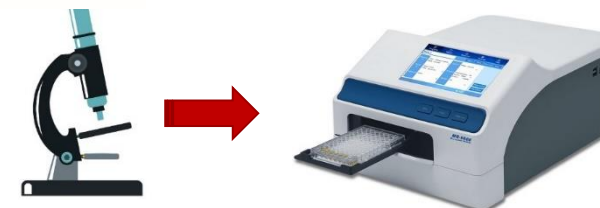
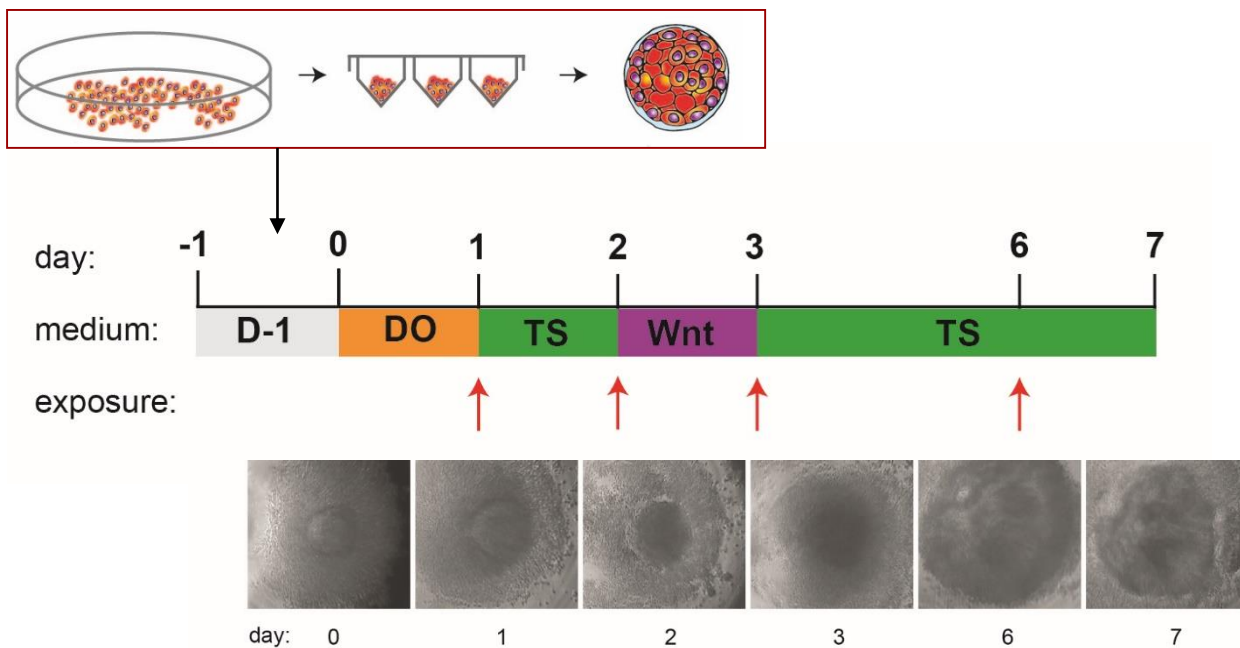
hIPSC can form cell aggregates called embryoid bodies that mimic the blastocyst



EBs mimic the developing embryo and can differentiate into most cell types of the body, e.g cardiomyocytes



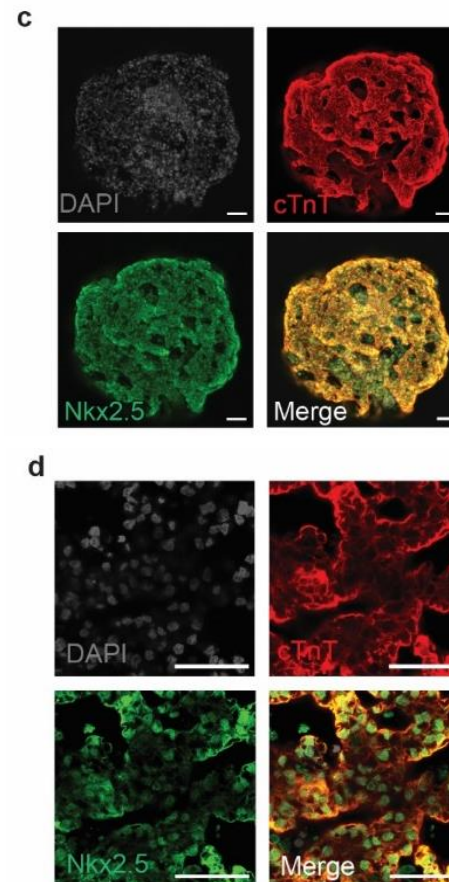
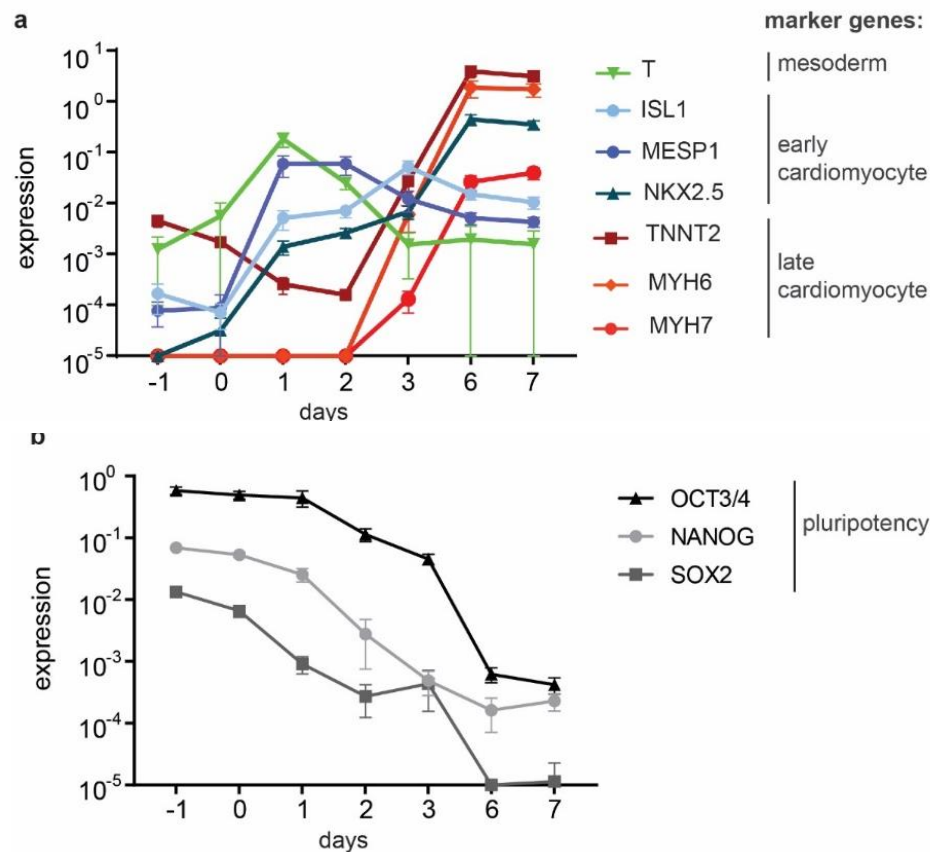
Development of the reporter gene assay PluriLum



LAUSCHKE K ET AL. *A novel human pluripotent stem cell based assay to predict developmental toxicity.*
Arch.Toxicol 94(11), 3831, 2020

LAUSCHKE K, TRESCHOW AF ET AL. *Creating a human NKX2.5 reporter stem cell line for developmental toxicity testing.*
Arch Toxicol 95, 1659, 2021

Pluripotency and cardiomyocyte markers behave as expected



LAUSCHKE K ET AL. A novel human pluripotent stem cell based assay to predict developmental toxicity. Arch.Toxicol 94(11), 3831, 2020

LAUSCHKE K, DALGAARD MD, EMNÉUS J, VINGGAARD AM. Transcriptomic changes upon epoxiconazole exposure in a human stem cell-based model of developmental toxicity. CHEMOSPHERE, 284, 131225, 2021.

DAVIDSEN N, ROSENMAI AK, LAUSCHKE K, SVINGEN T, AND VINGGAARD AM. Developmental effects of PFOS, PFOA and GenX in a human induced pluripotent stem cell differentiation model. CHEMOSPHERE 279, 130624, 2021.

How many animals are saved?

- The required animal numbers and associated costs for *in vivo* tests to accomplish **REACH legislation requirements** for chemicals were evaluated
- The most conservative estimate of **68,000 chemicals** was carried through current testing requirements using the most optimistic assumptions
- Reproductive toxicity testing requires **90% of all animal use and 70% of the required costs** for registration
- The **prenatal developmental toxicity study (OECD TG 414)** may be the *in vivo* assay that the **PluriLum** may replace
- TG414 requires at least **20 pregnant female rats or rabbits** per dose (3 doses/chemical plus control)
- Average price of **63,100€/rat study** and **92,500€/rabbit study** was estimated
- Total no. of animals required was **4,351,591 rats**, and if the 2nd study is included **2,434,790 rabbits**
- Thus, the potential for reducing the use of animals for this endpoint is significant

Rovida & Hartung. Re-evaluation of animal numbers and costs for *in vivo* tests to accomplish REACH legislation requirements for chemicals - a report by the transatlantic think tank for toxicology (t(4)). ALTEX 26, 187-208, 2009.

Acknowledgements



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Department of Biotechnology and Biomedicine

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