Einführung in die Stammzell- und Embryonenforschung II (ESF-II/9) WS2022/23

Zur Herstellen von Lebewesen aus einer Stammzelle

Biologische Grundlagen – Stand der Forschung – Gesellschaftliche Auswirkungen

4. Doppelstunde

Teil 2 Herstellung von Lebewesen - Stand der Forschung (3. bis 6. Doppelstunde)

2.1. Ex vivo Embryonen aus einer pluripotenten Stammzellen

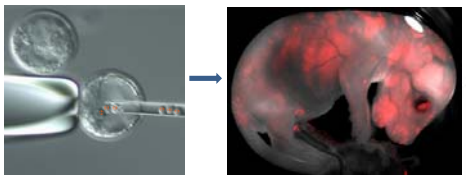
- 2.1.1. Blastoide - Herstellung von Blastozysten aus Stammzellen
- 2.1.2. Gastruloide – Gastrulation in Stammzellaggregaten
- 2.1.3. Embryoids - Synthetische Embryonen

2.2. Ex vivo Keimzellen aus pluripotenten Stammzellen

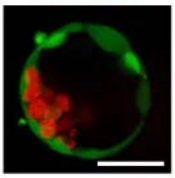
- 2.2.1. Der weibliche und männliche Reproduktionszyklus ex vivo
- 2.2.2. Herstellung von Zygoten - In vitro Fertilisation und Klonen
- 2.2.3. Herstellung von künstlichen Plazenten aus Stammzellen
- 2.2.4. Herstellung von Mäusen aus Stammzellen in Leihmüttern

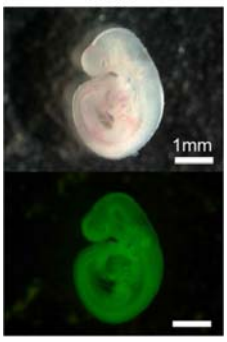
Ad 2.1.3. Vorgeschichte:

1. Chimera formation Injektion von ESCs in das Blastocoel von Blastozysten (1984)



TS cells = 4N
ES cells = 2N





2. Tetraploidaggregation (1990)

Zygoten → 2Blastomeren Stadium → Elektrofusion: 4n Zygote → Morula

Sandwich aus 4N-Molula - zu testende ESCs oder iPSCs - 4N-Molula

→ Blastocyst; besteht nur aus 4N Trophektoderm und 2N ICM

Einpflanzen in pseudo-schwangere Maus → 100%-ige ESC-abstammende Maus in F0

DOI:10.1038/s41598-018-33690-7

Teil 2 Herstellung von Lebewesen - Stand der Forschung (3. bis 6. Doppelstunde)

2.1. Ex vivo Embryonen aus einer pluripotenten Stammzellen

2.1.3. Embryoids - Synthetische Embryonen

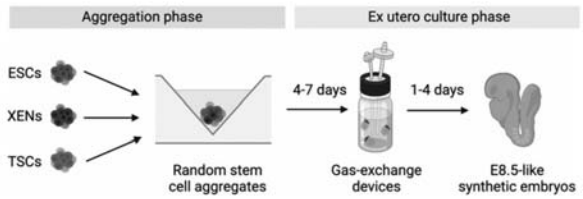


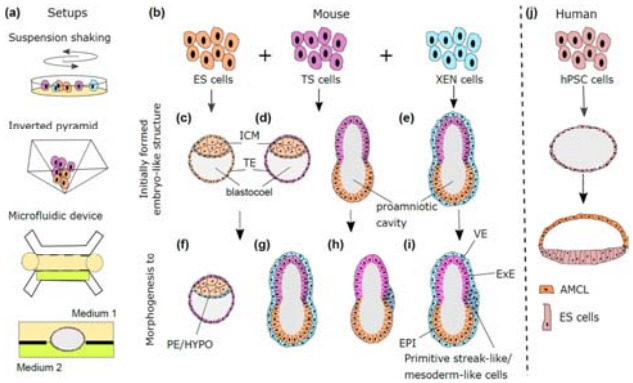
FIG. 1. Synthetic embryos cultured until an E8.5-like stage. In the first phase, embryonic and extraembryonic stem cells are combined into aggregates, which are cultured to promote the formation of self-organized synthetic embryos resembling E5.5–6.5 stages. In the second phase, successfully aggregated structures are selectively picked and transferred into gas-exchanging bioreactors, where they fully develop through gastrulation and into early neurulation and organogenesis.

DOI: 10.1089/cell.2022.0111

2018

Figure 5. The use of pluripotent stem cells to generate embryo-like structures. Mouse embryo-like structures are shown ...

Mit TSCs und XEN
Bis zum egg cylinder gebracht.



Embryo implantation in the laboratory: an update on current techniques
Samuel Ojosnegros, Anna Seriola, Amélie L Godeau, Anna Veiga

Hum Reprod Update, Volume 27, Issue 3, May-June 2021, Pages 501–530, https://doi.org/10.1093/humupd/dmaa054

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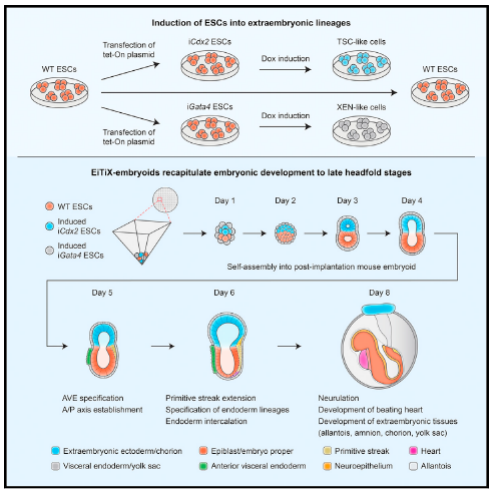
2.1.3. Embryoids - Synthetische Embryonen

10 / 2022

Mouse embryo model derived exclusively from embryonic stem cells undergoes neurulation and heart development

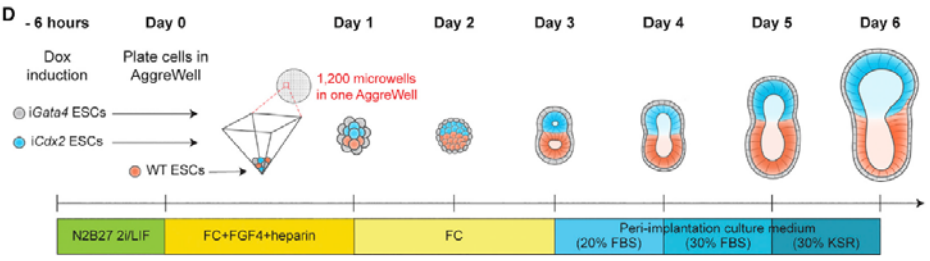
Cell Stem Cell

Kasey Y.C. Lau,[1,3] Heman Rubinstein,[2,3] Carlos W. Gantner,[1] Ron Hadas,[1,2] Gianluca Amadei,[1,4,5] Yonatan Stelzer,[1,6,*] and Magdalena Zernicka-Goetz[1,4,5,7,*]



https://doi.org/10.1016/j.stem.2022.08.013

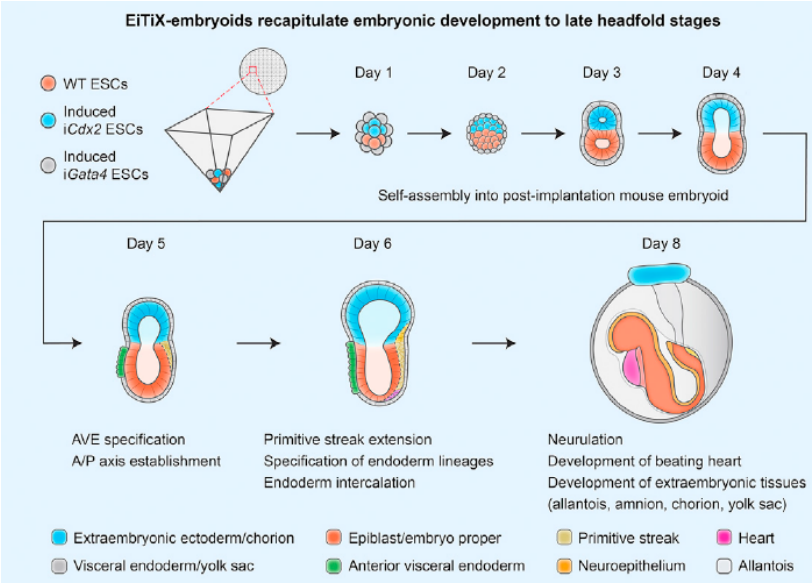
2.1.3. Embryoids - Synthetische Embryonen



(D) Schematic of EiTiX-embryoid generation. FBS, fetal bovine serum; KSR, knockout serum replacement.

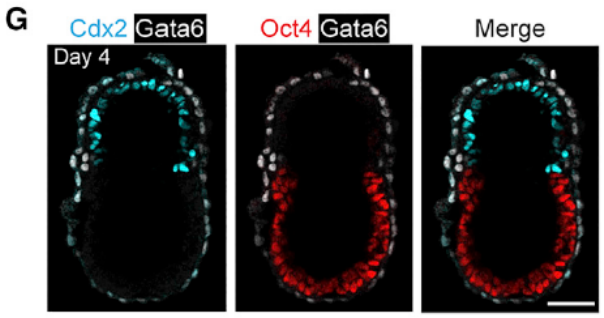
https://doi.org/10.1016/j.stem.2022.08.013

2.1.3. Embryoids - Synthetische Embryonen



https://doi.org/10.1016/j.stem.2022.08.013

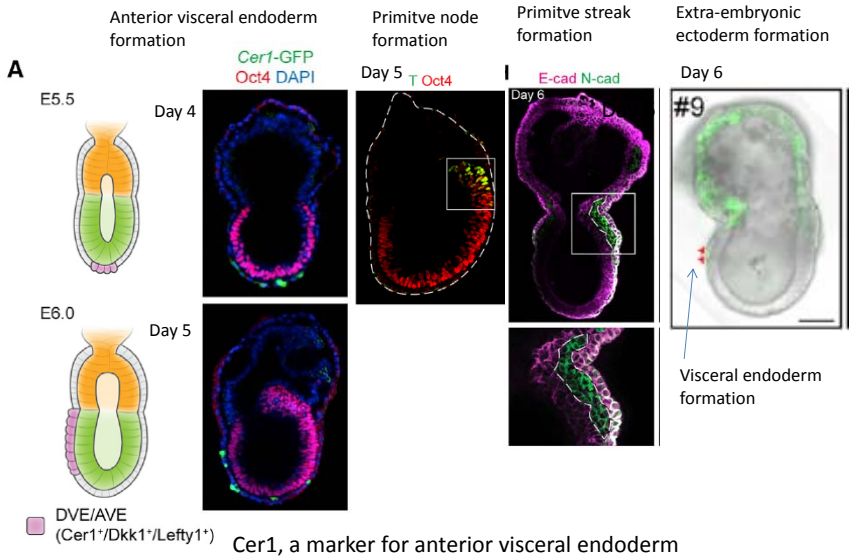
2.1.3. Embryoids - Synthetische Embryonen



https://doi.org/10.1016/j.stem.2022.08.013

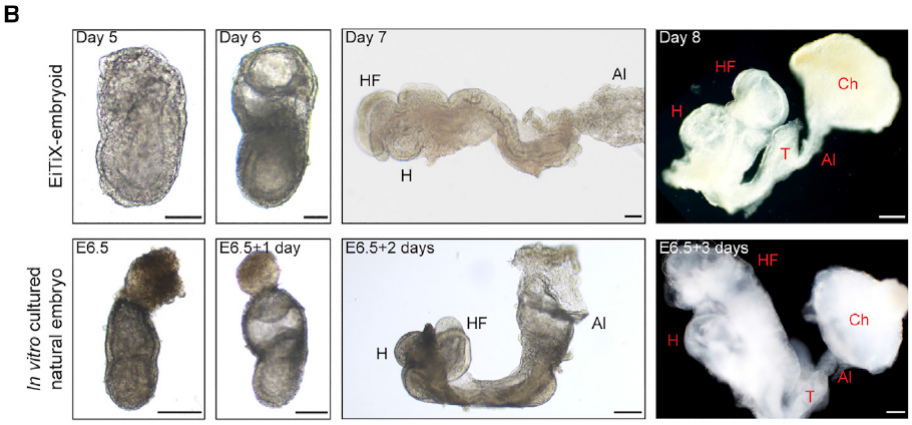
(G) Day 4 EiTiX-embryoids stained to reveal Cdx2 (cyan), Oct4 (red), and Gata6 (white).

2.1.3. Embryoids - Synthetische Embryonen

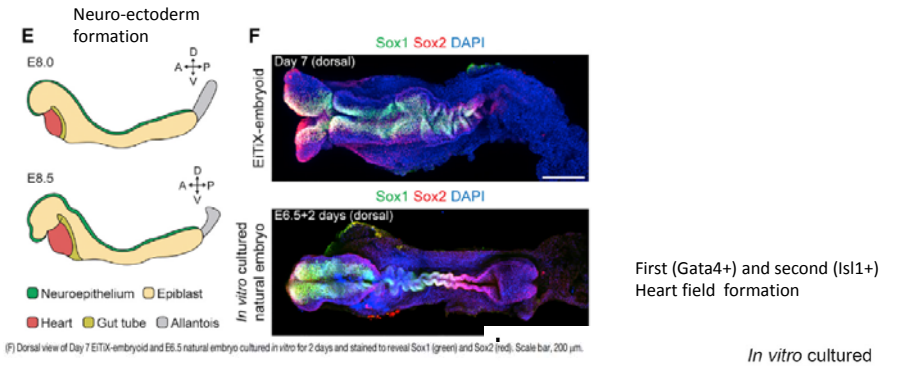


Cer1, a marker for anterior visceral endoderm
T = Brachyury, a marker for mesoderm

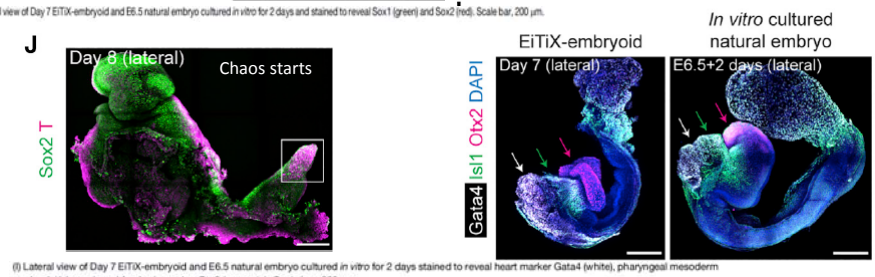
Vergleich mit natürlichen Embryonen



2.1.3. Embryoids - Synthetische Embryonen



First (Gata4+) and second (Isl1+) Heart field formation



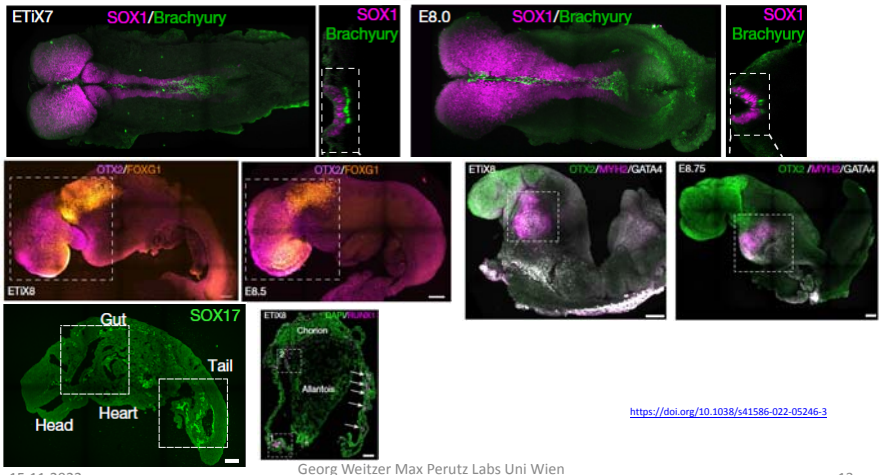
Embryo model completes gastrulation to neurulation and organogenesis

8 / 2022

https://doi.org/10.1038/s41586-022-05246-3
Received: 9 November 2021
Accepted: 17 August 2022
Published online: 25 August 2022

Gianluca Amadei, Charlotte E. Handford, Chengxiang Qiu, Joachim De Jonghe, Hannah Greenfeld, Martin Tran, Beth K. Martin, Dong-Yuan Chen, Alejandro Aguilera-Castrejon, Jacob H. Hanna, Michael B. Elowitz, Florian Hollfelder, Jay Shendure, David M. Glover & Magdalena Zernicka-Goetz

Primitve streak- Fore, mid & hindbrain – heart - fore & hind gut - allantois and chorion formation.

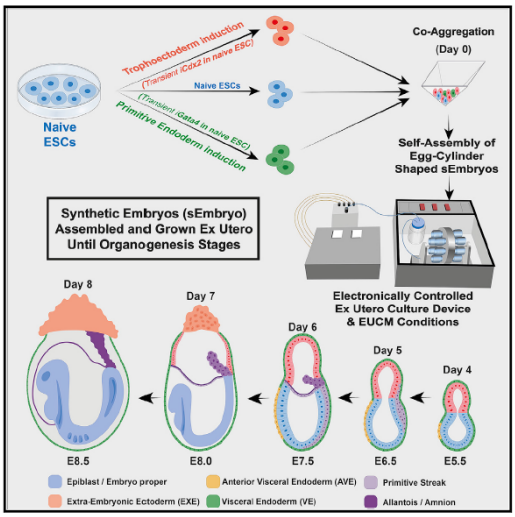


https://doi.org/10.1038/s41586-022-05246-3

Post-gastrulation synthetic embryos generated *ex utero* from mouse naive ESCs

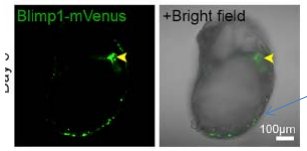
9/2022

Shadi Tarazi, Alejandro Aguilera-Castrejon, Carine Joubran, Nadir Ghanem, Shahd Ashouokhi, Francesco Roncato, Emilie Wildschutz, Montaser Haddad, Bernardo Oldak, Elidet Gomez-Cesar, Nir Livnat, Sergey Viukov, Dmitry Lokshtanov, Segev Naveh-Tassa, Max Rose, Suhair Hanna, Calanit Raanan, Ori Brenner, Merav Kedmi, Hadas Keren-Shaul, Tsvee Lapidot, Itay Maza, Noa Novershtern, and Jacob H. Hanna
Department of Molecular Genetics, Weizmann Institute of Science, Rehovot 76100, Israel



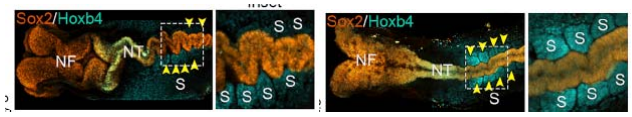
https://doi.org/10.1016/j.cell.2022.07.028

Primordial germ cells

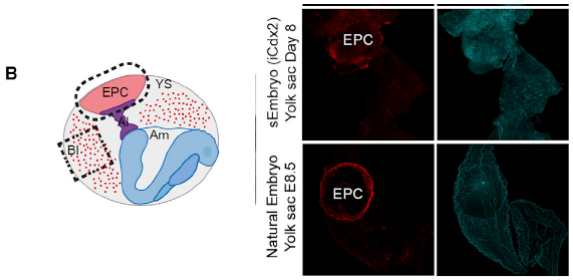


Induziert vom posterioren Visceralen Endoderm (PVE) und inhibiert durch sezernierte Faktoren vom AEV.

Somite formation



Ectoplacental cone formation



43 printed pages
24 co-autors

https://doi.org/10.1016/j.cell.2022.07.028

2.1.3. Embryoids - Synthetische Embryonen

2021

Möglicher Ersatz für die Verwendung echter menschlicher Embryonen

Article

Single-cell transcriptomic characterization of a gastrulating human embryo

Richard C. V. Tyser[1,6], Elmir Mahammadov[2,3,4,6], Shota Nakanoh[5], Ludovic Vallier[5], Antonio Scialdone[2,3,4,7]✉ & Shankar Srinivas[1,7]✉

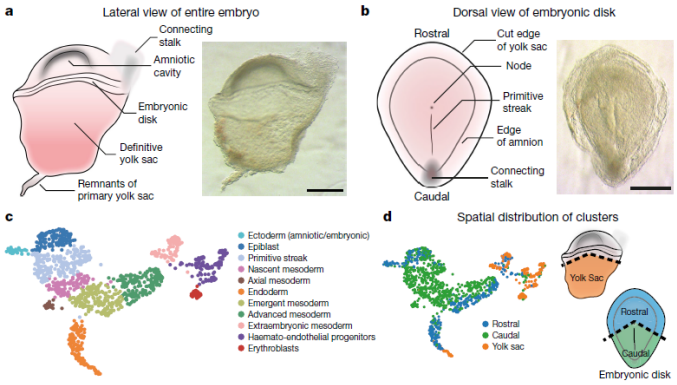


Fig. 1 | Morphological and transcriptional characterization of a CS7 human gastrula. a, Lateral view of the intact CS7 human embryo. Scale bar, 500 µm. **b**, Dorsal view of the dissected embryonic disk showing the primitive streak and node. Scale bar, 500 µm. **c**, Uniform manifold approximation and projection (UMAP) of all the cells computed from genes with highly variable expression. **d**, UMAP and schematics highlighting the anatomical region that cells were collected from (see also Extended Data Fig. 1b).

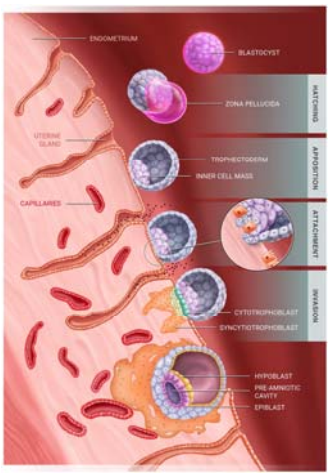
https://doi.org/10.1038/s41586-021-04158-y

Expression profiles in human dpf 14-17 embryos is very similar to murine E7.0 – 7.5 embryos except for the hematopetic cells which corresponds best to E8.5 murine embryos. → Comparison of these data with human sEmbryo are pending.

Improvements of the sEmbryo model:
Increase the fidelity and timing of trophopblast and extra-embyonic endoderm differentiation. Adapting oxygen and nutrition supply.

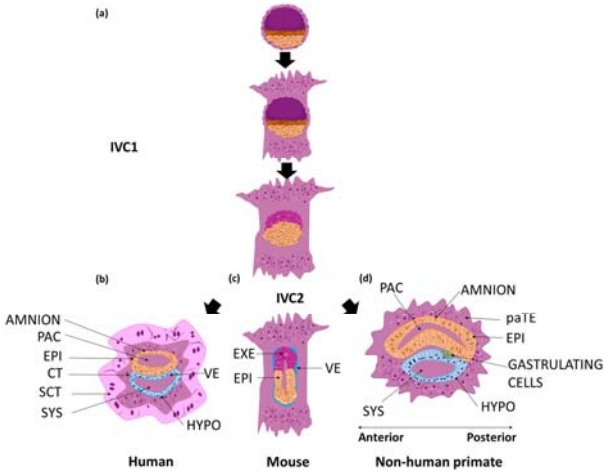
Improvement of artificial placentas and uteri →

In vitro Nidation / Einnistung von Embryonen ex vivo / Plazentaimitate



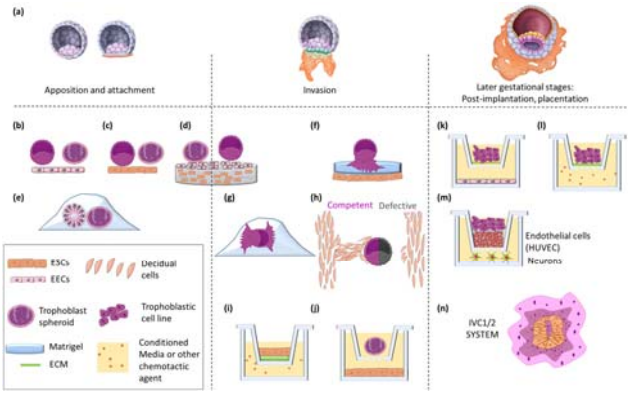
Hum Reprod Update, Volume 27, Issue 3, May-June 2021, Pages 501–530, https://doi.org/10.1093/humupd/dmaa054

Figure 4. The sequential culture media strategy applied to different species. A sequential culture media strategy uses ...



Hum Reprod Update, Volume 27, Issue 3, May-June 2021, Pages 501–530, https://doi.org/10.1093/humupd/dmaa054

Figure 3. Schematic representation of the geometries used to study embryo implantation in vitro. (a) The different ...



Hum Reprod Update, Volume 27, Issue 3, May-June 2021, Pages 501–530, https://doi.org/10.1093/humupd/dmaa054

Embryo Implantation: War in Times of Love

Nancy Ashary , Abhishek Tiwari , Deepak Modi

•DOI: 10.1210/en.2017-03082 Review 2018 Feb 1;159(2):1188-1198. doi: 10.1210/en.2017-03082.

Abstract

Contrary to widespread belief, the implantation of an embryo for the initiation of pregnancy is like a battle, in that the embryo uses a variety of coercive tactics to force its acceptance by the endometrium.

At 25 years chance to become pregant ist 25% per month! (over 38 years: 7 → 1%)

We propose that embryo implantation involves a three-step process:

(1) Identification of a receptive endometrium;

(2) Superimposition of a blastocyst-derived signature onto the receptive endometrium …

(3) Breaching by the embryo and trophoblast invasion, culminating in decidualization and placentation.

…

2.1.3. Embryoids - Synthetische Embryonen

Resumée

Synthetische Embryonen entwickeln sich in wenigen Prozent der Fälle bis zum Beginn der Organogenes.

Die Plazentaentwicklung kann noch nicht nachgestellt werden.

Notwendige Güterabwägung: → siehe letzte Vorlesungseinheit

Ethische Dimension dieser Experimente:	Potenciality	Potenzialität
	Autonomy	Autonomie
	Contingency	Bedingtheit