EXTENDED EMBRYO CULTURE -- GOING BEYOND DAY 6

Tiencheng Arthur Chang, PhD, HCLD, ELD, CC
University of Texas Health Science Center
San Antonio, TX

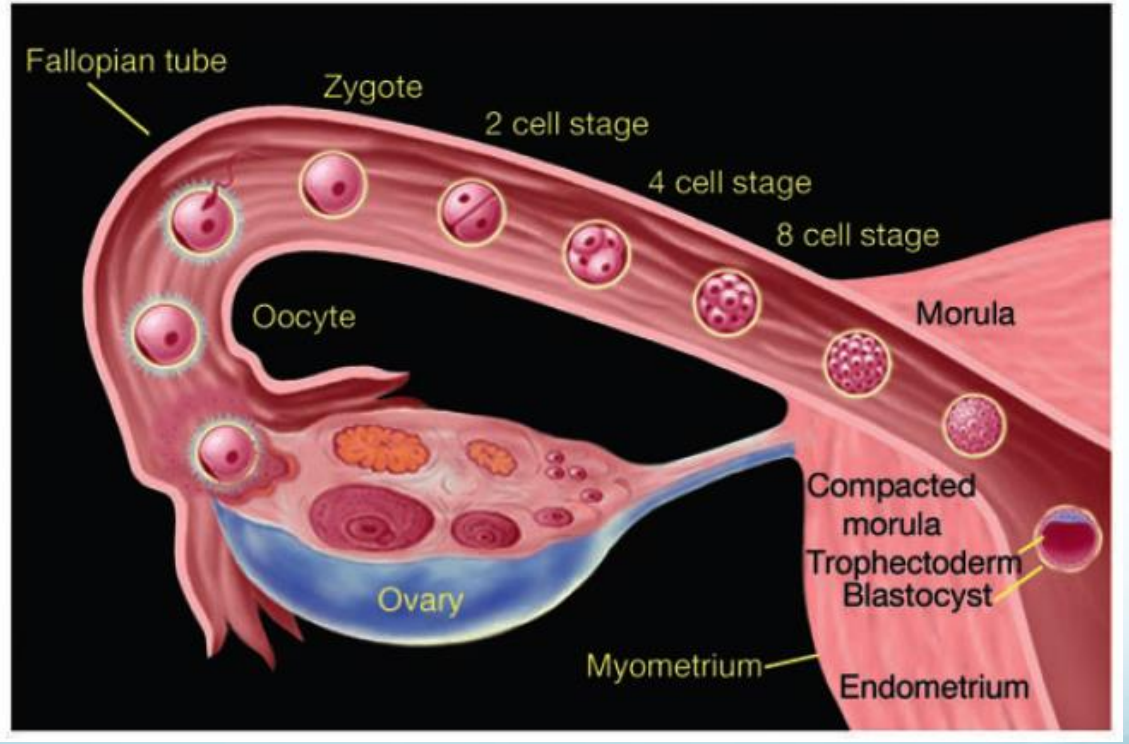
LEARNING OBJECTIVES

1. Overall concept of extended embryo culture
2. Current trends of pre-implantation embryo culture
3. Extended embryo culture at peri- and post-implantation stages: current progress, potentials and limitations
4. Ethical and technological challenges of post-implantation embryo culture

DISCLOSURE

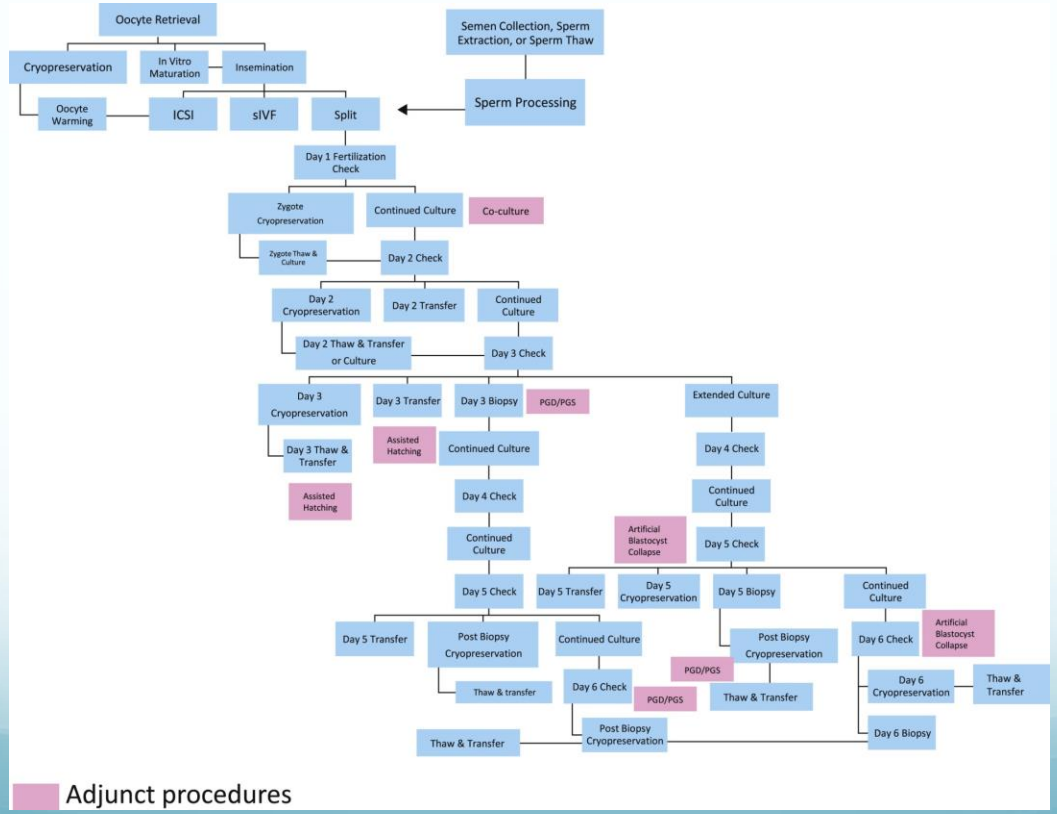
- Associate Professor, University of Texas Health Science Center
- Lab Director, UT Health San Antonio - Women's Health Center, and DFW Center for Fertility and IVF
- Consultant, Irvine Scientific

EARLY STAGES OF HUMAN EMBRYO DEVELOPMENT



Red-Horse et al, J Clin Invest 2004

LABORATORY WORKFLOW – IVF AND PRE-IMPLANTATION EMBRYO CULTURE



Alikani et al., Fert Steril 2014

CONCEPT OF IN VITRO EMBRYO CULTURE

- Embryo culture
  - In vivo
  - Ex vivo / in vitro, pre-implantation (D0-D7)
    - Intravaginal (e.g., INVOCell)
    - In the ART laboratory: most commonly practiced methods today
    - In the ART laboratory: on-the-chip, microfluidics, other novel methods
  - Ex vivo / in vitro, peri- and post-implantation (beyond D7)
    - In the ART/research laboratory: embryo development beyond the 7^{th} day post fertilization
    - In other biotechnology laboratory: artificial utero environment

IN VITRO EMBRYO CULTURE

- Animal models *(McLaren and Biggers, Nature 1958)*
- Early days of clinical attempts *(Edwards, Bavister, and Steptoe, Nature 1969; Edwards, Steptoe, and Purdy, Nature 1970)*
- Methodology revisions and improvements throughout the past few decades
- Current methods commonly practiced by REI clinics
- Other novel methods and progress in the animal models

CHECKPOINTS DURING EMBRYO CULTURE

- Embryo culture is one of the most critical parts of ART success
- Checkpoints:
  - Oocyte maturity and quality
  - Sperm quality and decision on insemination methods
  - Insemination (conventional or ICSI) / micromanipulation
  - D0 fertilization check
  - D1-D3 pre-compaction embryo culture and evaluation
  - D3-D5/6/7 extended culture into blastocyst and evaluation
  - Biopsy/PGT, embryo transfer, cryopreservation
  - (Peri-implantation and post-implantation)

PREIMPLANTATION EMBRYO DAY 3-7 EXTENDED CULTURE

EXTENDED EMBRYO CULTURE

- Rationale:
  - Better self-selection; easier/higher accuracy to select good quality embryo(s) by embryologist
  - Improved embryonic-uterine synchrony, reduced uterine contractility/embryo expulsion, favorable uterine environment
  - Higher IR; lower multiple BR; also higher % euploid embryos *(Harton et al. Fertil Steril 2013; Demko et al. Fertil Steril 2016)*
  - Pre-requisite for blastocyst biopsy for PGT.

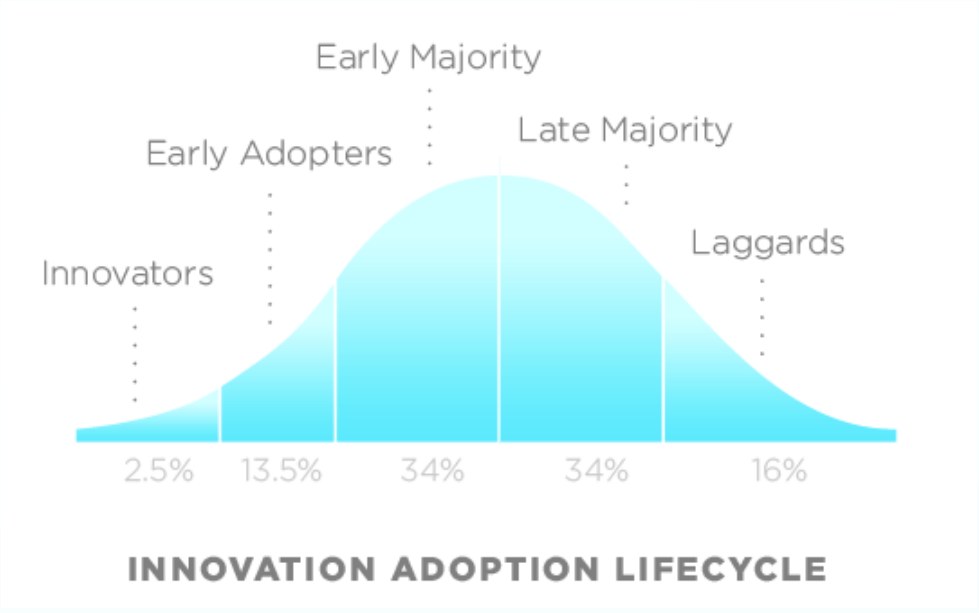
- Risks:
  - Embryos in sub-optimal condition longer (*in vitro* environment is sub-optimal compared with in vivo environment)
  - More liability to the lab; requires rigorous quality management
  - Lower # available of embryos; higher % dropout/no ET rate and consequent trauma to patient

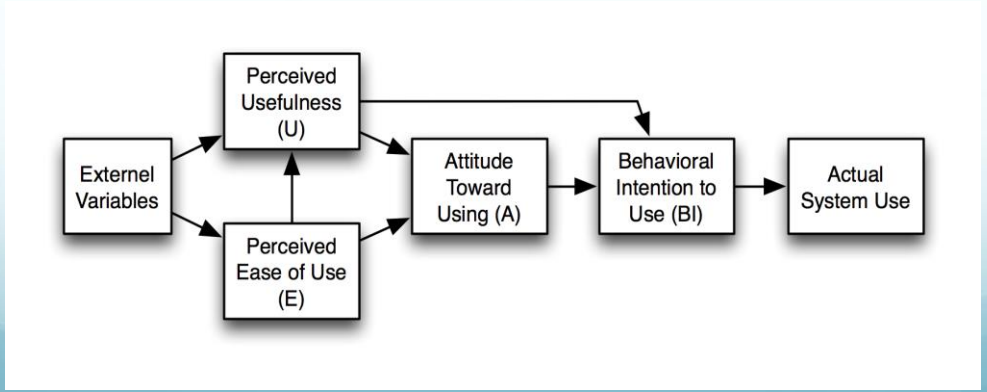
FACTORS ON PREIMPLANTATION STAGE EXTENDED EMBRYO CULTURE

- Culture medium
- pH
- CO_2, O_2, and gas mixture
- Temperature
- Air and water quality; humidity
- Co-culture (cumulus cells, uterine cells, or non-reproductive tract cells)
- Other factors

TECHNOLOGY ADOPTION IN ART

- Formal medical research *vs.* innovative treatment
- Technology adoption in IVF: 6.5 million babies have been born through IVF since the birth of Louise Brown in 1978 *(ESHRE Focus on Reproduction, July 2016)*
- Most ART technologies spread into clinics through innovative treatment:
  - Example: ICSI, no RCT, no sufficient animal studies prior to clinical application
  - Business competition-driven and patient-driven technology adoption


Early Majority
Early Adopters
Late Majority
Laggards
Innovators
2.5%
13.5%
34%
34%
16%
INNOVATION ADOPTION LIFECYCLE


Perceived Usefulness (U)
Externel Variables
Attitude Toward Using (A)
Behavioral Intention to Use (BI)
Actual System Use
Perceived Ease of Use (E)

ADD-ONS DURING EMBRYO CULTURE

- Types of add-ons/adjuvant treatments:
  - Micromanipulation/mechanical approaches
  - Preimplantation genetic testing (PGT)
  - Proteins/chemical approaches
  - Other additional components and culture environment/system
- Pros and cons of add-ons/adjuvant treatments:
  - Utilization of gametes and embryos
  - Effect on IR, LBR
  - Cost effectiveness
  - Psychological effects on patient

EXAMPLES OF DEVELOPMENT IN PREIMPLANTATION EXTENDED EMBRYO CULTURE

- Single-step / continuous culture medium composition
- Oxygen concentration
- Lactate concentration
- Humidity

FACTORS ON EMBRYO CULTURE: MEDIUM COMPOSITION

- Fertilization medium composition example: Sodium Chloride, Potassium Chloride, Calcium Chloride, Potassium Phosphate, Magnesium Sulfate, Sodium Bicarbonate, Glucose, Lactate Na Salt, Sodium Pyruvate, Glycine, L-Alanine, L-Arginine HCl, L-Asparagine, L-Aspartic Acid, L-Cystine, L-Glutamic Acid, Glycyl-Glutamine, L-Histidine, L-Isoleucine, L-Leucine, L-Lysine HCl, L-Methionine, L-Phenylalanine, L-Proline, L-Serine, L-Threonine, L-Tryptophan, L-Tyrosine, L-Valine, EDTA, Phenol Red, Gentamicin Sulfate

- Embryo culture medium composition example: Sodium Chloride, Sodium Pyruvate, L-Arginine, L-Threonine, Potassium Chloride, L-Alanine, L-Cystine, L-Tryptophan, Calcium Chloride, L-Asparagine, L-Histidine, L-Tyrosine, Potassium Phosphate, L-Aspartic Acid, L-Isoleucine, L-Valine, Magnesium Sulfate, L-Glutamic Acid, L-Leucine, Glycyl-L-Glutamine, Sodium Bicarbonate, Glycine, L-Lysine, EDTA, Glucose, L-Proline, L-Methionine, Phenol Red, Sodium Lactate, L-Serine, L-Phenylalanine, Protein Supplement, Human α- and β-globulins, Gentamicin Sulfate

FACTORS ON EMBRYO CULTURE: MEDIUM COMPOSITION

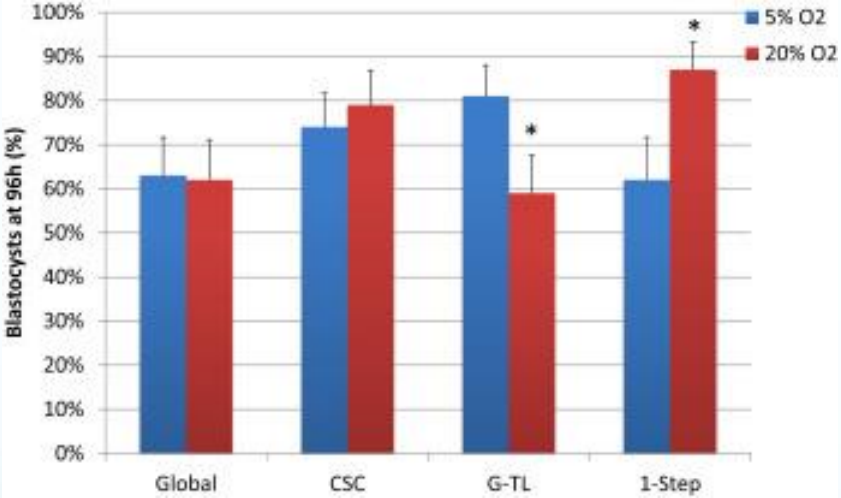
- In vitro culture is suboptimal than in vivo environment
- Mimicking tubal/uterine secretion components or adding novel components
- Glucose, pyruvate, lactate to help the embryo passing developmental blocks; ratios of energy substrates
- Amino acids
  - Short term deprivation of a.a. causes damage *(Gardner and Lane, Hum Reprod 1996)*
  - Concerns: ammonium buildup during medium storage and culture *(Kleijkers et al Hum Reprod 2016);* dipeptite form glutamine reduces ammonium buildup; dipeptite form of other a.a.
- Organic and inorganic salts

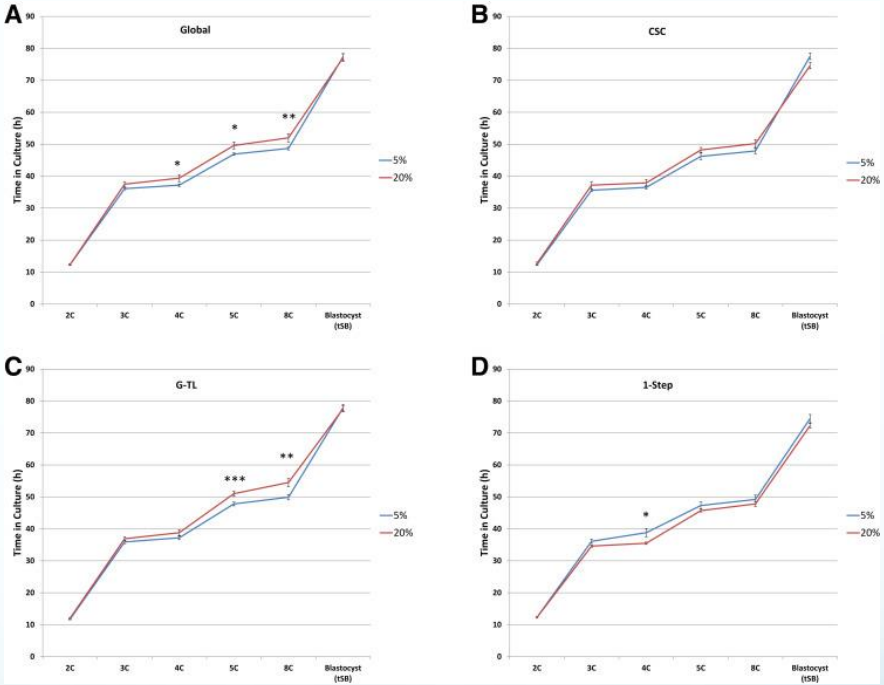
FACTORS ON EMBRYO CULTURE: MEDIUM COMPOSITION

- Embryo culture media: sequential (D0 insemination/fertilization, D1-D3 cleavage stage, D3-D7 blastocyst stage) and single-step (D0/D1-D7)
- Composition of commonly used sequential and single step media varies widely in glucose, pyruvate, lactate, amino acids *(Morbeck et al, Fert Steril 2014 & 2017)*
- Single-step / continuous culture medium has become popular in recent years:
  - Utilization in time-lapse imaging
  - Less handling of embryo: lower risk of error and contamination
  - Reduces time, workload, and cost (especially for small volume clinics)

FACTORS ON EMBRYO CULTURE: MEDIUM COMPOSITION

- Human oviduct: low glucose (G), high pyruvate (P) and lactate (L); uterus: high glucose, low pyruvate
- Amino acids to overcome effect of glucose during D0-D3 *(Gardner and Lane, Hum Reprod 1996)*
- Commercial single-step media: L/P ratio = 18-21
- Interaction of medium x oxygen concentration, and presence of protein, have impact on blastocyst development *(Morbeck et al, Fert Steril 2014 & 2017)*
- DNA methylation kinetics and hydroxymethylation modification depend on types of medium *(Salvaing et al, Hum Reprod 2016)*





Development of individually cultured 1-cell mouse embryos to the expanded blastocyst stage in four commercially available culture media containing protein *(Morbeck et al, Fert Steril 2017)*

FACTORS ON EMBRYO CULTURE: MEDIUM COMPOSITION

- More info needed from manufacturers
- US FDA does not require clinical trials on new culture medium based on components present in existing commercial medium approved by FDA
- Studies, most with sequential media, showed conflicting data of clinical outcomes in birth weight *(Eaton et al, Hum Reprod 2012; Nelissen et al, Hum Reprod 2012; Vergouw et al, Hum Reprod 2012; Lin et al, Hum Reprod 2013; Dumoulin et al, Hum Reprod 2015)*
- No sufficient data on effect of single-step medium
- Difference between single-step and sequential media from the same manufacturer

FACTORS ON EMBRYO CULTURE: OXYGEN CONCENTRATION

- Atmospheric oxygen is ~21%
- Physiological concentrations of oxygen in the female tract are around 5% (2-8%, hypoxia) (Fischer and Gardner, *J Reprod Fertil 1993*, Gardner, *Reprod BioMed Online 2016*)
- Atmospheric oxygen is stressful to mammalian embryos (e.g., ability to regulate ammonium) *(Wale and Gardner, Biol Reprod 2013; Awonuga et al, Biol Reprod 2013)*
- Adjusting CO_2, O_2 and N_2 concentration (e.g., 6%, 5%, 89%) in gas supply to control oxygen level in the incubator

FACTORS ON EMBRYO CULTURE: OXYGEN CONCENTRATION

- Ultra-low oxygen level, 2% O_2, exists in several mammalian species and human:
  - Uterine environment / implantation site *(Fischer and Gardner, J Reprod Fertil 1993)*
  - First few weeks of fetal-placental blood supply *(Yedwab, Fertil Steril 1976; Jauniaux et al, Am J Obstet Gynecol 2001)*
- Ultra-low 2% O_2 has been demonstrated beneficial for stem cell physiology *(Mohyeldin et al, Cell Stem Cell 2010)*

FACTORS ON EMBRYO CULTURE: OXYGEN CENCENTRATION

- Stem cell lineages:
  - 20-30% of blastocyst = ICM → embryonic stem cell (ESC)
  - 70-80% of blastocyst = trophectoderm → trophoblast stem cell (TSC) *(Thouas et al, Reprod BioMed Online 2001)*
- Research have shown 2% hypoxia condition promotes TSC growth and progressive invasion *(Zhou, Placenta 2011)*

FACTORS ON EMBRYO CULTURE: LACTATE CONCENTRATION

- Glucose in the culture medium → converted to pyruvate → lactate, by lactate dehydrogenase (LDH), with the concomitant production NADH → NAD+
- Glucose consumption increases in extended culture → lactate increases/accumulates → reduced pyruvate conversion by LDH and oxidation. Negative influence on embryo metabolism
- Low lactate may keep a more efficient metabolic rate and reduce pH fluctuations, and consequently, less lab workload on medium change.
- Removal of lactate is also important in ultra-low oxygen culture environment (2%, as previously discussed) *(Bolnick et al, JARG 2017)*

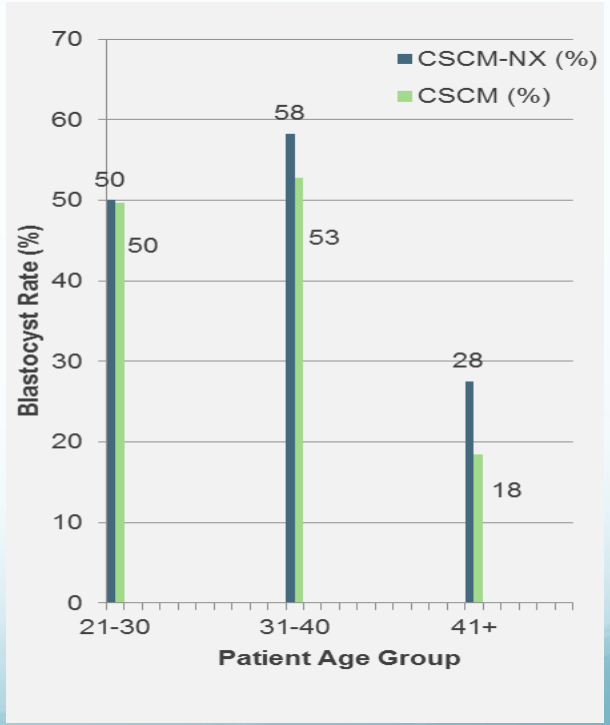
FACTORS ON EMBRYO CULTURE: LACTATE CONCENTRATION

- Recent study showed correlation of low lactate level single-step continuous medium with higher embryo developmental capacity in blastocyst rate and ongoing pregnancy *(White et al, abstract, ESHRE 2017 abstract).*
- Promising direction of culture medium development.

FACTORS ON EMBRYO CULTURE: LACTATE CONCENTRATION

- Clinical study vs. regular (high) lactate medium: increased blastocyst rates in advanced maternal age patients (9%-56% improvement), overall higher ongoing pregnancy (55% vs. 45%) *(Ni and White, ASPIRE 2018 Conference)*

- Need PGT-A data to provide more insights on effect of low lactate culture



FACTORS ON EMBRYO CULTURE: HUMIDITY

- Dry vs. humid incubators
- More significant osmolarity shift (increase) and lower embryo developmental capacity (blastocyst formation and utilization rate) in dry incubators, even with oil overlay, than humidified incubators (same model dry incubator with water wells placed inside) *(Fawzy et al, Fertil Steril 2017)*
- More studies, with larger sample size and different incubator models, will be needed to further investigate on this topic.

EMBRYO CULTURE INTO DAY 7

- Currently, most IVF labs select embryos at Day 5-6 and often use Day 6 as cut-off for embryo culture
- Embryos not suitable for transfer or cryopreservation after Day 6 are routinely discarded
- Proportion of cryopreserved blastocysts: ~65% for Day 5, 30% for Day 6, and 5% for Day 7 *(Kovalevsky et al, Fertil Steril, 2013)*
- PGT-A with Day 7 embryo = ~2-8%.

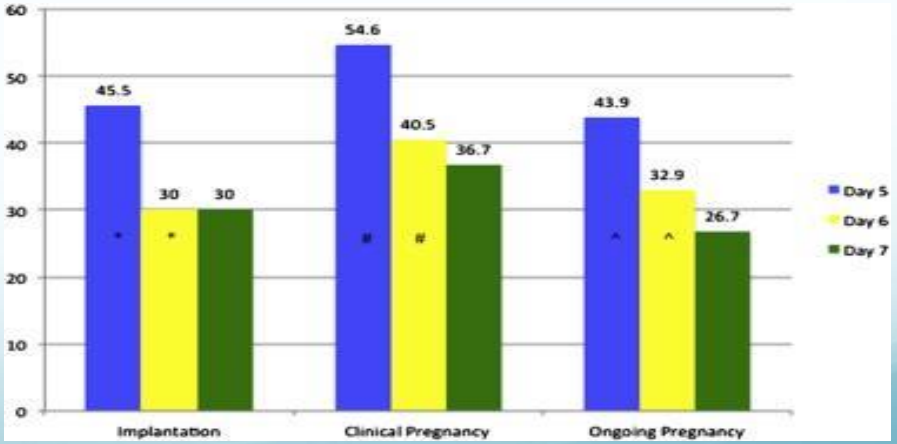
EMBRYO CULTURE INTO DAY 7

- Culture into Day 7 still increases the number of usable embryos per IVF cycle (review) *(Hammond et al, Hum Reprod 2018)*
- Transferring Day 7 vitrified blastocysts yielded lower but still acceptable life birth rates (25.1-25.6% vs 46.5% Day 5 and 41.4% Day 6) and seems safe for the offspring (single center retrospective study) *(Du et al, Hum Reprod 2018)*

EMBRYO CULTURE INTO DAY 7

- Day 7 blastocysts outcomes (all FET data): 17-56% implantation rates, 20-56% clinical pregnancy rates, and 11-42% live birth rates. *(Hammond et al, Hum Reprod 2018)*

- Thaw survival, ongoing PR, IR from Day 7 cryopreserved embryos were lower than Day 5/6 cryopreserved embryos *(Kovalevsky et al., Fertil Steril 2013)*

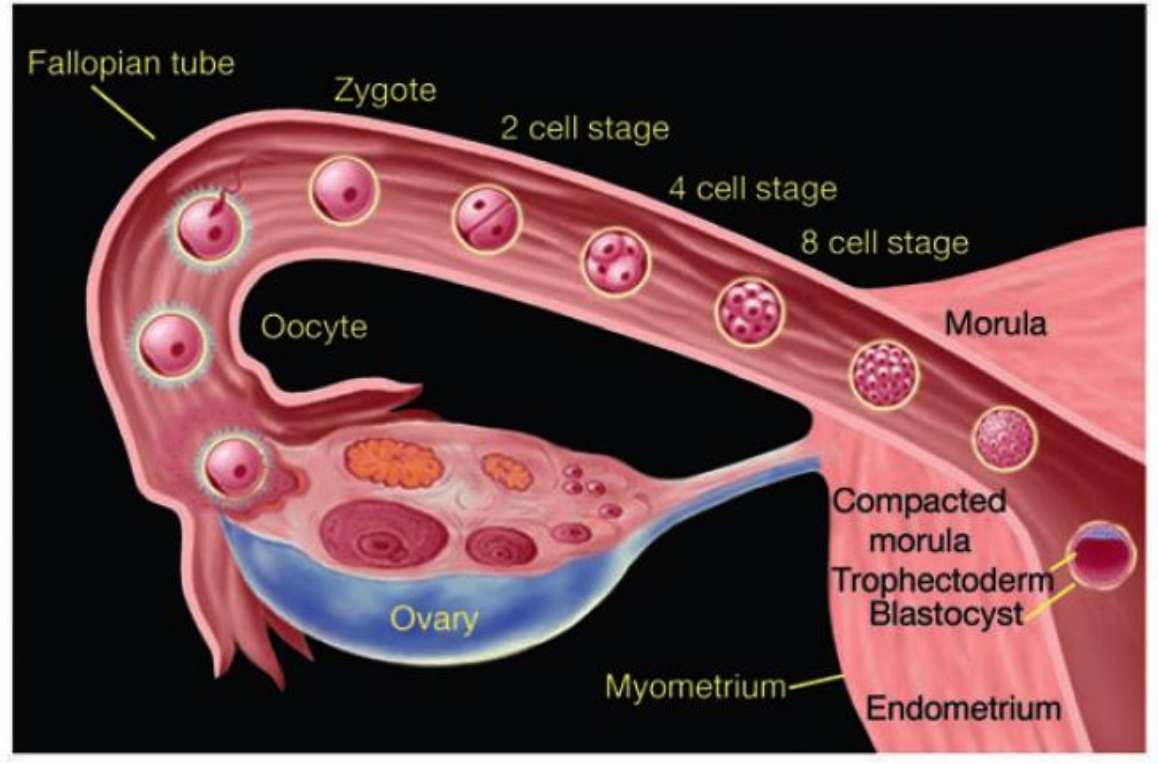


PGT-A with Day 7 Blastocysts

- PGT-A study: with high aneuploidy, D7 blastocysts could still yield live birth *(Su et al, RB&E 2016)*
- Euploidy in expanded blastocysts at D7 = 31.3%, vs. D5 65.8% vs. D6 49.7% *(Tiegs et al, Fertil Steril 2018)*
- Day 7 blastocysts have a lower, but clinically important potential → biopsy on Day 7 is less ideal, but still feasible
- Data from Day 7 blastocysts were small size studies. More research is needed.

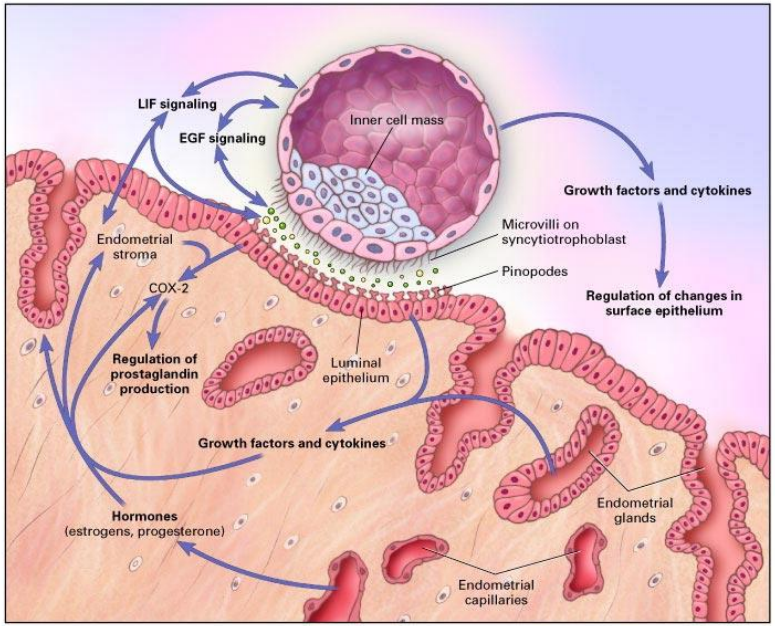
EXTENDED EMBRYO CULTURE BEYOND THE BLASTOCYST STAGE

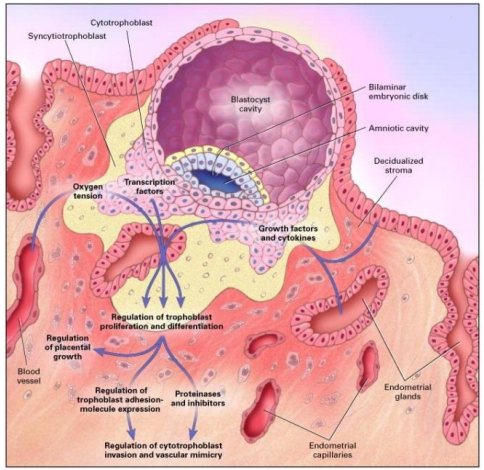
EARLY STAGES OF HUMAN EMBRYO DEVELOPMENT

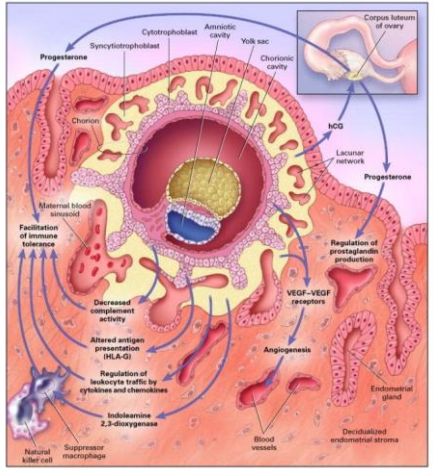


Red-Horse et al, J Clin Invest 2004

BLASTOCYST IMPLANTATION







Norwitz et al., NEJM 2001

TABLE 1. FACTORS ASSOCIATED WITH IMPLANTATION AND THE MAINTENANCE OF EARLY PREGNANCY.*

FACTOR	EXAMPLES	SUGGESTED ROLE
Hormones	Estradiol-17β; progesterone	Promote proliferation and differentiation of endometrial stromal and epithelial cells
	Human chorionic gonadotropin	Maintains progesterone release from corpus luteum
Changes in endometrial luminal epithelium	Pinopodes; alterations in adhesion-molecule and mucin expression	Facilitate blastocyst capture and attachment; promote trophoblast differentiation and invasion
Cytokines and growth factors	Leukemia inhibiting factor; heparin-binding epidermal growth factor; hepatocyte growth factor; interleukin; vascular endothelial growth factor	Facilitate signaling between blastocyst and uterus; regulate endometrial prostaglandin production; promote endometrial invasion, proliferation, and differentiation; regulate endometrial vascular permeability and remodeling
Immunologic factors	Interleukin-10; Crry (complement regulator)	Immunosuppression
	HLA-G	Prevents immune recognition and rejection of fetal semi-allograft
	Indoleamine 2,3-dioxygenase	Degrades tryptophan, which is essential for macrophage action
Trophoblast proteinases, inhibitors, and adhesion molecules	Matrix metalloproteinases–tissue inhibitor of metalloproteinases; cathepsin B and L; cadherins; integrins	Regulate trophoblast invasion; facilitate trophoblast vascular mimicry
Other factors	Cyclooxygenase-2	Regulates prostaglandin production
	Oxygen tension	Regulates the balance between trophoblast proliferation and differentiation

*This table highlights some of the more important factors and is not intended to be all-inclusive.

SCIENTIFIC NEEDS AND FACTORS ON PERI- AND POST-IMPLANTATION STAGE EMBRYO CULTURE

- Embryo culture beyond the 7th day: remains one of the least studied areas compared with studies focusing on the pre-implantation embryo (D0-D7) and fetal/placental (1st trimester)
- Limited available materials and ethical concerns
- Critical stage of embryo development and maternal-embryo interaction
- In vitro models as a testing platform to study pregnancy loss, drug development, and toxicity
- Methodology:
  - Extended culture of preimplantation embryo (animal/human)
  - “Synthetic entities”: construction with stem cell (ESC, iPSC, etc.)

NEEDS AND FACTORS ON PERI- AND POST-IMPLANTATION STAGE EMBRYO CULTURE

- Factors affecting peri- and post-implantation stage in vitro embryo development
  - Co-culture/feeder cell
  - Extracellular matrix (ECM)
  - Medium (embryo culture-based, conditioned, stem cell-based)
  - Oxygen level (5%, 2%, etc.)
  - Micro-environment for embryonic and stem-cell niche
  - Two-dimensional (2D) *vs.* three-dimensional (3D) culture
  - Other factors

EFFECT OF 2D VERSUS 3D CULTURE

- Preimplantation two-dimensional (2D) culture environment for post-implantation extended culture
- Concept of three-dimensional (3D) culture, *e.g.*, using Matrigel, Hydrogel, artificial scaffolds, to mimic *in vivo* environment
- Progress in 2D and 3D embryo culture:
  - Animal models: mouse, domestic animals, nonhuman primates
  - Experiments using human embryos

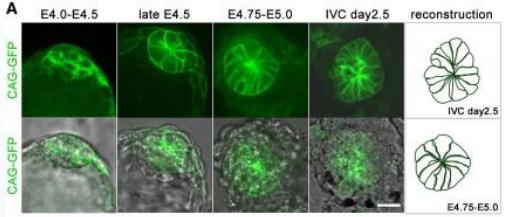
ANIMAL MODEL APPROACHES TO CULTURE EMBRYOS BEYOND THE ROUTINE IVF PROCEDURE

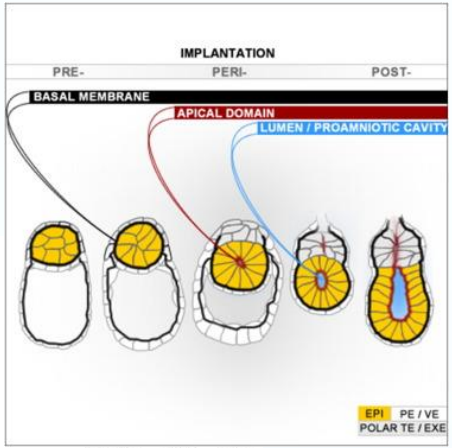
- Rodent embryos:
  - Experimental methodology
  - Application to human embryology
- Domestic animal embryos:
  - Experimental methodology
  - Agriculture application
  - Application to human embryology
- Nonhuman primate (NHP) embryos:
  - Experimental methodology
  - Similarity between NHP and human embryos

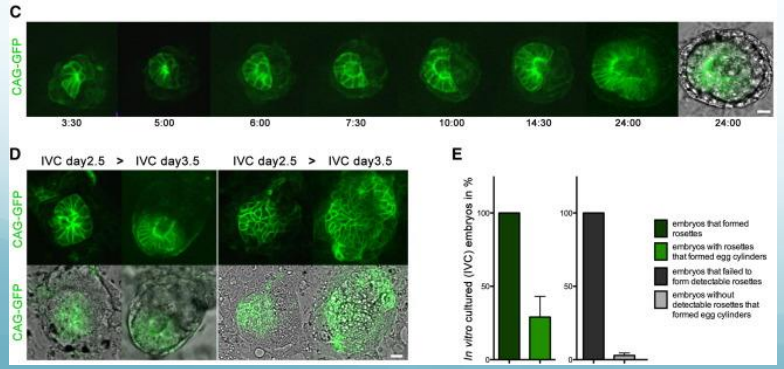
PERI-IMPLANTATION MOUSE EMBRYO CULTURE AND SELF-ORGANIZATION

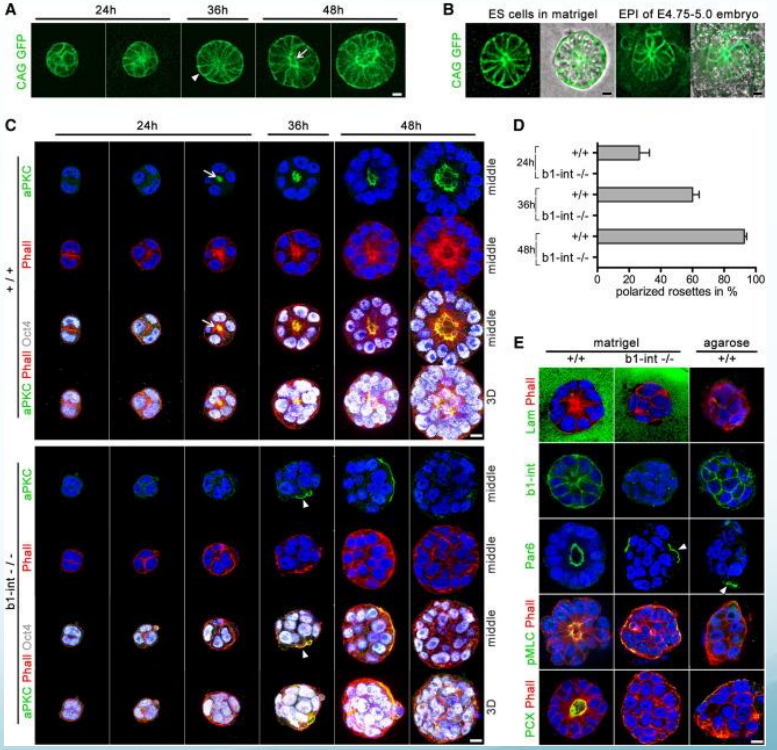
- Mouse embryo implantation model *(Bedzhov and Zernicka-Goetz, Cell 2014)*
- Embryos attached to culture dish in a 2D system showed progressive growth
- Cultured embryo showed markers of embryonic (OCT4, NANOG), trophoblastic lineages (Cytokeratin 7, and later on, HCGB)
- Basal membrane function can be substituted in vitro by extracellular matrix (ECM) proteins: ES cells can be induced to form similar polarized rosettes and initiate lumenogenesis.

PERI-IMPLANTATION MOUSE EMBRYO CULTURE AND SELF-ORGANIZATION







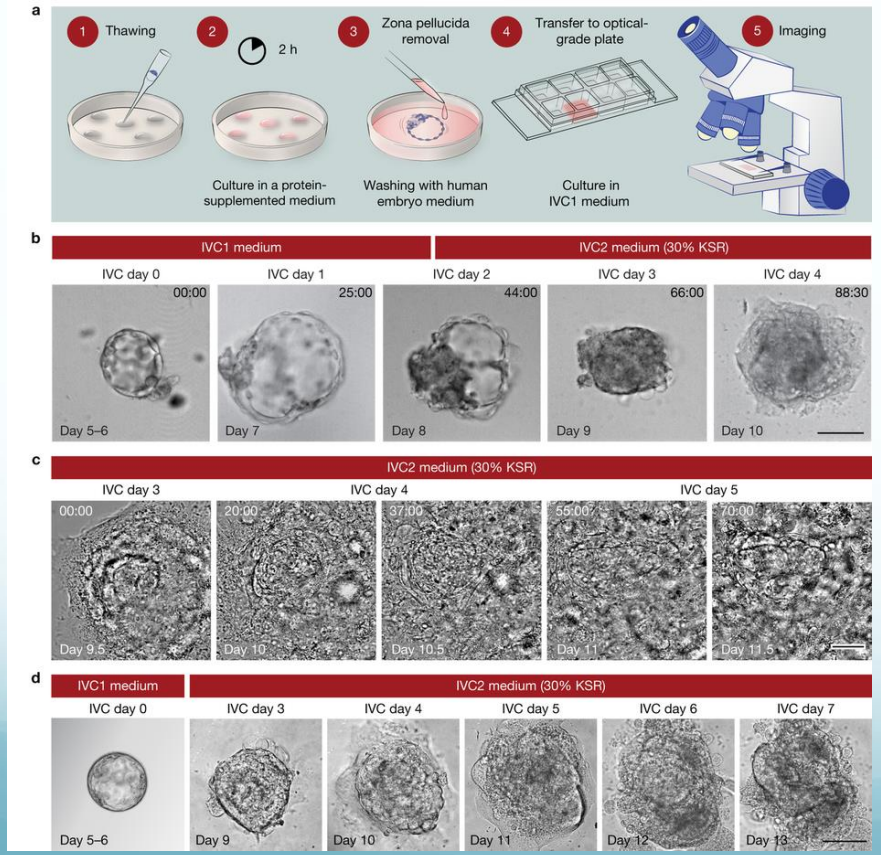


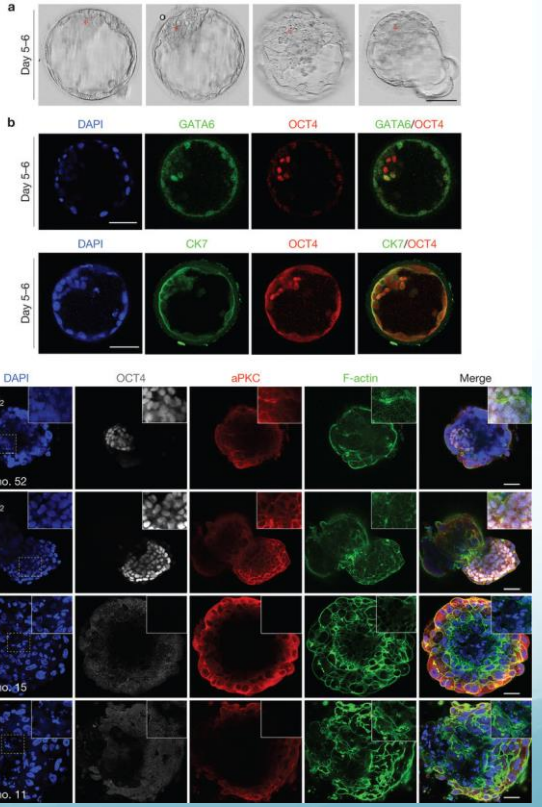
Bedzhov and Zernicka-Goetz, Cell 2014

PERI- / POST-IMPLANTATION HUMAN EMBRYO 2D CULTURE AND SELF-ORGANIZATION

- Human embryos donated for research
- Embryos attached to culture dish in a 2D system showed self-organization and key landmarks without maternal tissues: epiblast expansion, lineage segregation, bi-laminar disc formation, amniotic sac cavitation, and trophoblast diversification *(Deglincerti et al, Nature 2016; Shahbazi, Nat Cell Biol 2016)*
- Markers: OCT4, NANOG (ICM/epiblast);CDX2, GATA3 (TE); cytokeratin 7 (TE/trophoblast); HCGB after day 12 (syncytiotrophoblast)
- Developmental capacity of such embryos remains unclear: genetic and epigenetic modifications

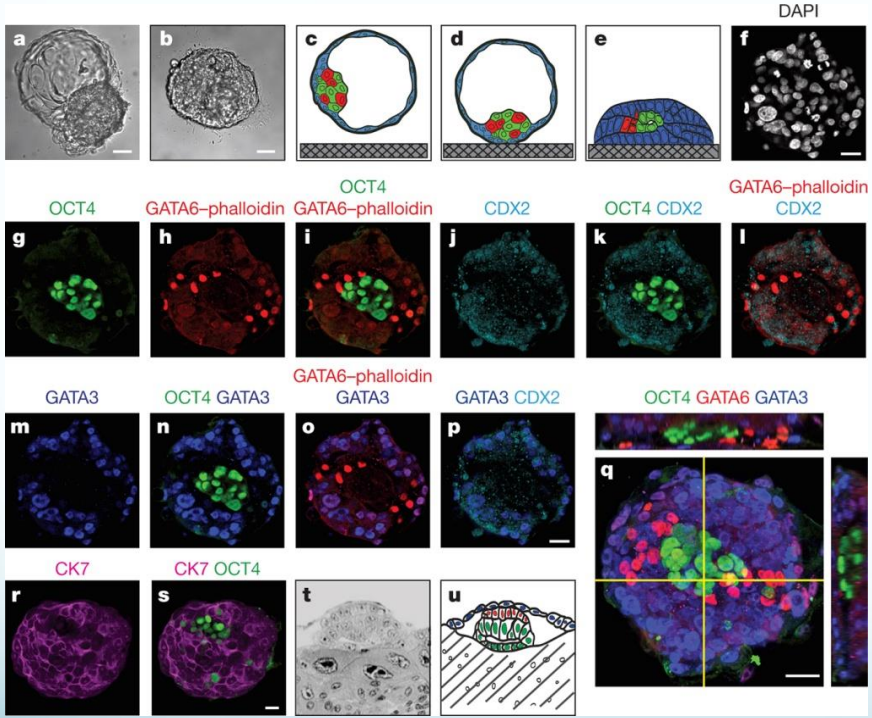
PERI- / POST-IMPLANTATION HUMAN EMBRYO 2D CULTURE

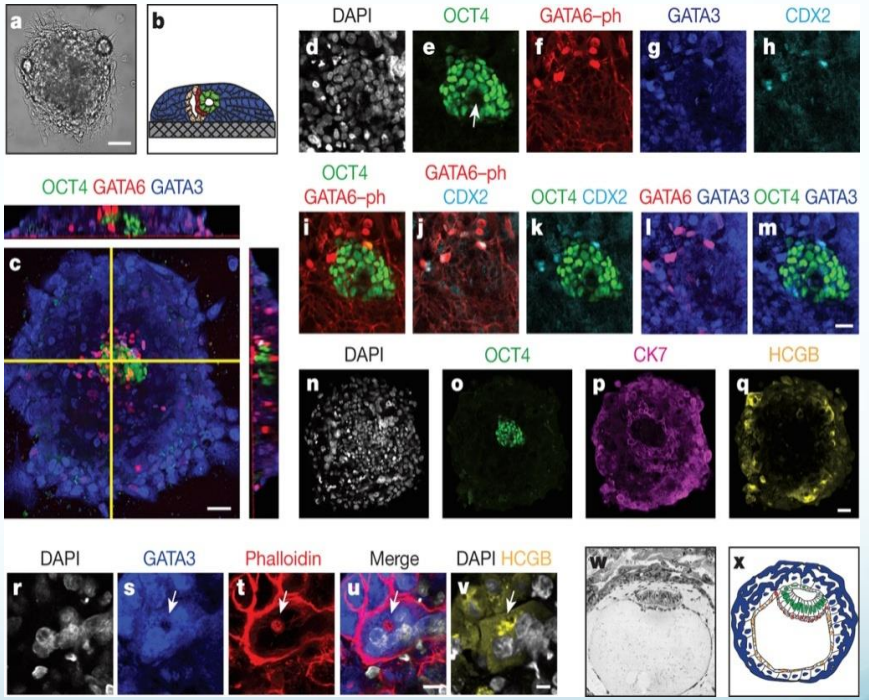




Shahbazi, Nat Cell Biol 2016

PERI- / POST-IMPLANTATION EMBRYO CULTURE – HUMAN EMBRYO 2D CULTURE





Day 8 (left) human embryos display transcriptional and morphological self-organization; day 12 (right) exhibit TE cellular phenotypes (*Deglincerti et al, Nature 2016)*

IN VITRO STEM CELL-DERIVED EMBRYONIC DEVELOPMENT

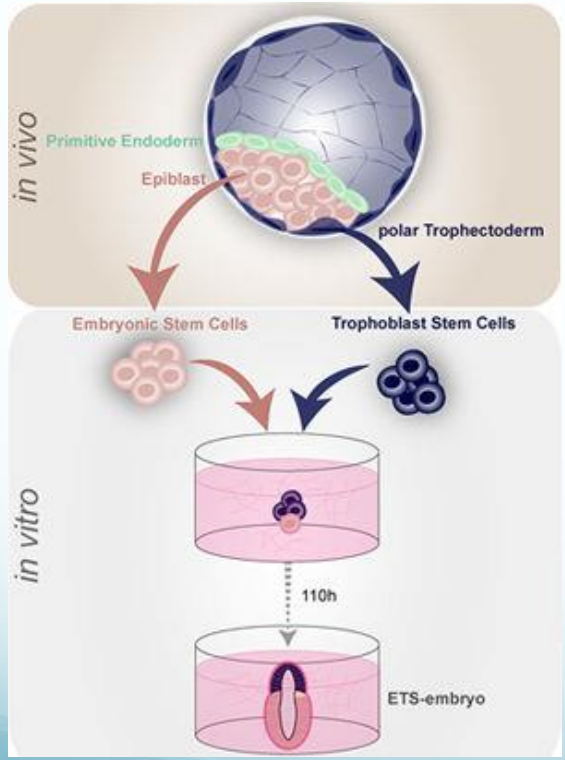
- Combined mouse embryonic stem cells (ESC) and trophoblast stem cells (TSC) in a 3D scaffold to mimic embryogenesis (*Harrison et al., Science 2017*); ESC and TSC aggregated and formed blastocyst-like structure, and triggered decidualization in utero after implantation (*Rivron et al., Nature 2018*)

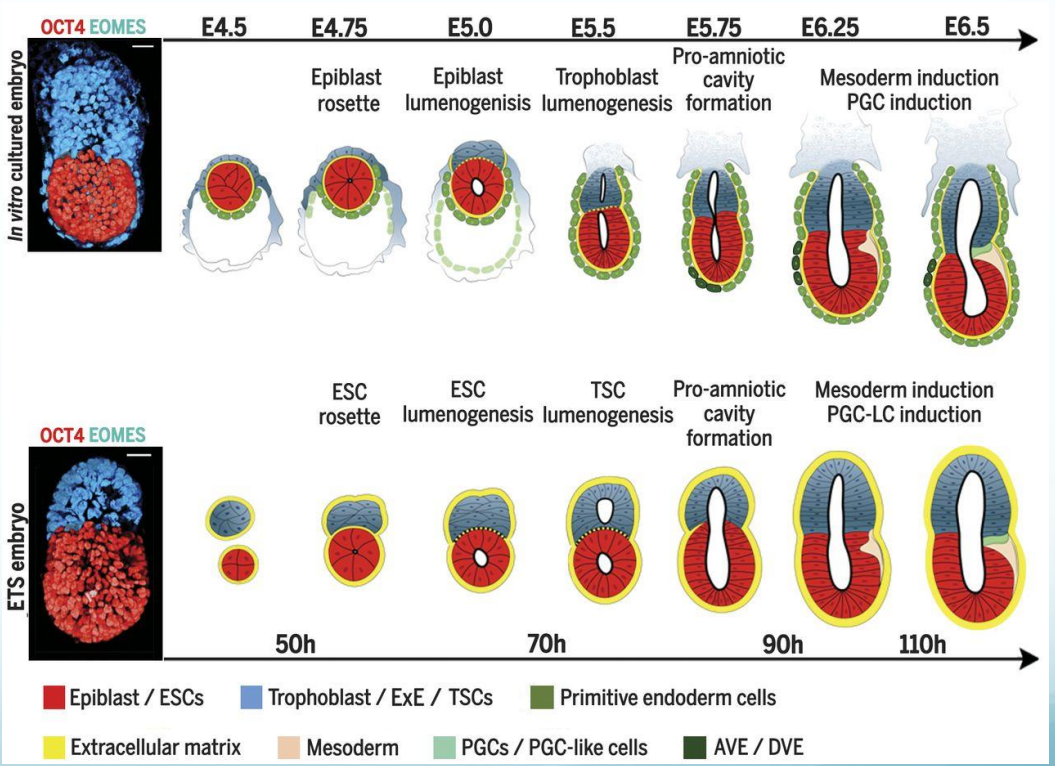
- Self-assembly *in vitro* embryogenesis showed critical steps of embryonic development including germ layer specification, embryonic and extraembryonic compartment development, pro-amniotic cavity formation

- Human pluripotent stem cell (hPSC)-based post-implantation amniotic sac development without maternal or extraembryonic tissues *(Shao et al, Nat Comm 2017)*

- Self-organization and differentiation after placed on gel bed and ECM overlay

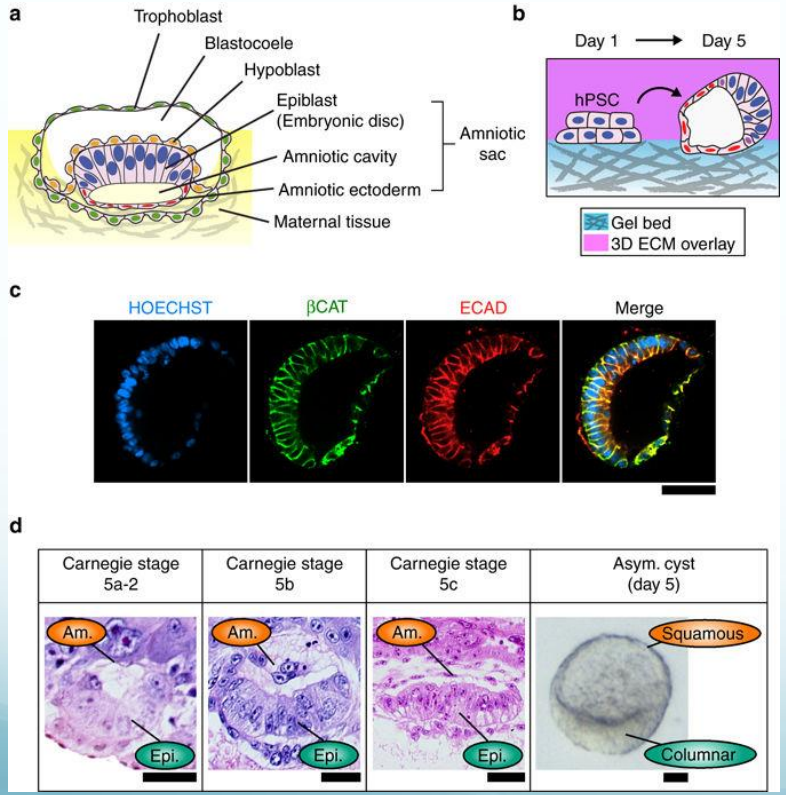
IN VITRO STEM CELL-DERIVED EMBRYONIC DEVELOPMENT

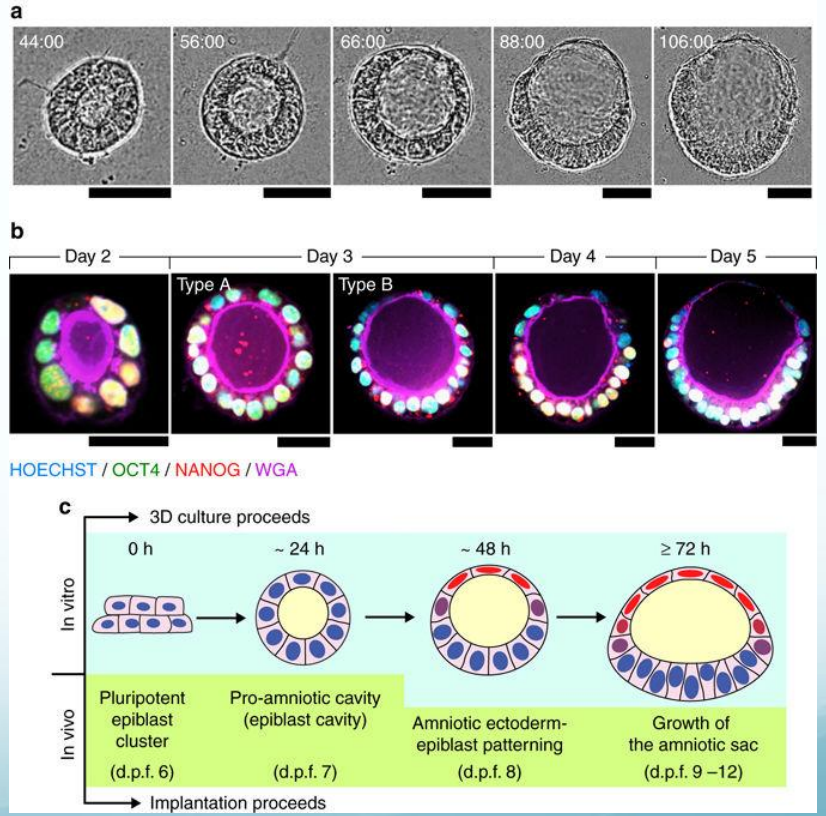




Kubaczka and Daley, Cell Res 2017; Harrison et al., Science 2017

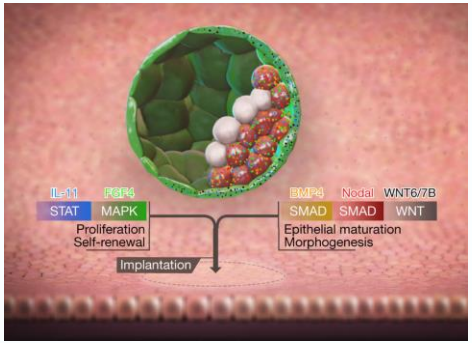
IN VITRO STEM CELL-DERIVED EMBRYONIC DEVELOPMENT

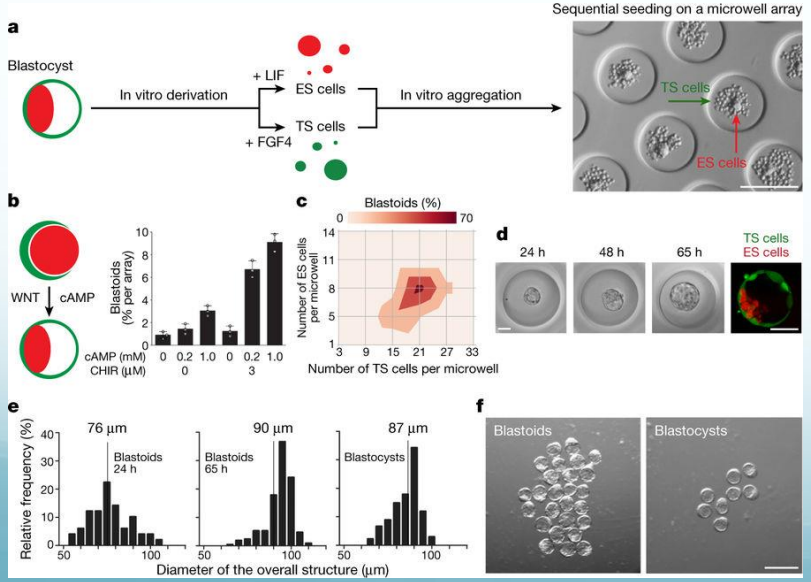


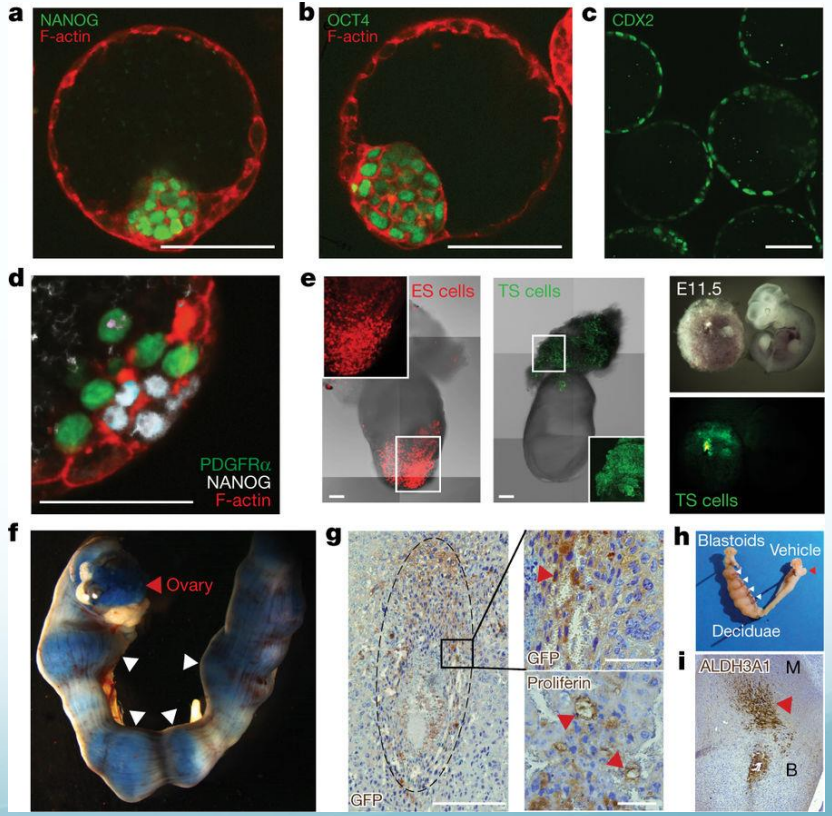


Shao et al, Nat Comm 2017

IN VITRO STEM CELL-DERIVED MOUSE BLASTOCYST-LIKE STRUCTURES







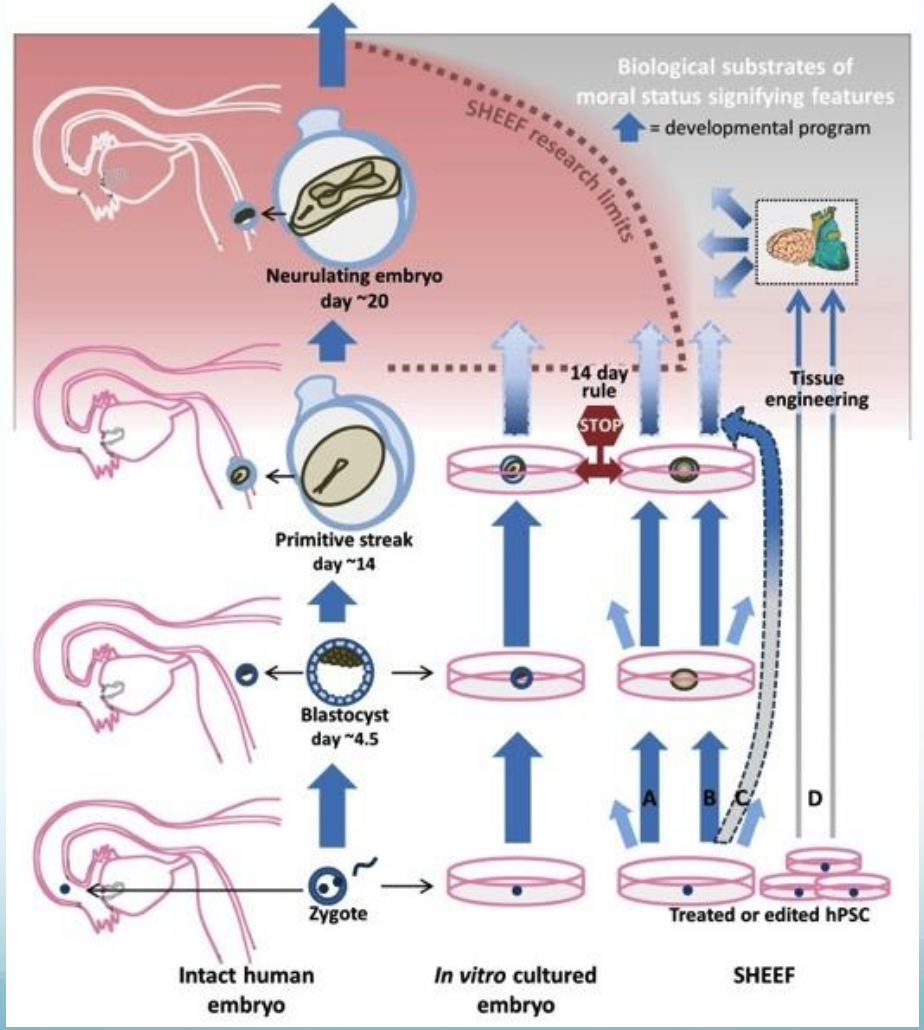
Rivron et al, Nature 2018

HUMAN EMBRYO EXTENDED CULTURE -- ETHICAL CONCERNS

- The “14-day rule” on embryo culture recommended by several commissions: *Ethics Advisory Bard 1979 (USA), Warnock 1984 (UK), NIH 1994 (USA)*
- Concept of 14-day rule on experiments: intact human embryos not allowed to develop beyond the earlier of day 14 or appearance of the primitive streak (PS) *(Hyun et al, Nature 2016; Aach et al, eLife 2017)*
- Moral status of the embryo
  - At the moment of conception, or
  - Increasing in moral status as the embryos develop
- Synthetic embryo/entity and embryo-like structure with potential human organism

HUMAN EMBRYO EXTENDED CULTURE – ETHICAL CONCERNS

- Principle of International Society of Stem Cell Research (ISSCR) guidelines *(Kimmelman, et al, Lancet 2016)*
  - Integrity of the research enterprise
  - Primary of patient welfare
  - Respect for research participants
  - Transparency
  - Social justice



Aach et al, eLife 2017

NONHUMAN PRIMATE EMBRYOS IN EXTENDED CULTURE BEYOND DAY 14

POST-IMPLANTATION 3D EMBRYO CULTURE - NONHUMAN PRIMATES

- Rhesus monkey embryos
- IVF derived preimplantation embryo culture
  - Matrigel embedding, with combination feeder cell co-culture
    (Douglas et al, Endo Rev 2009; Chang et al, RB&E 2018)
- Growth up to D53 post fertilization:
  - ICM → embryonic structure
  - TE → invasive outgrowth of trophoblastic structures
  - Elevated hCG secretion
  - Embryonic, trophoblast and proliferation markers

EMBRYO DEVELOPMENT IN THE HUMAN AND NONHUMAN PRIMATES

Table 1.
Comparison of in vitro embryonic development rates in humans and nonhuman primate species.

	Human [39]	**Cynomolgus macaque [33]**	**Rhesus macaque [27, 34–36]**	**African Green Monkey [37]**	**Squirrel Monkey [38]**	**Marmoset [14, 15]**
D1	2 pronuclei		2 cells	2 cells	2 cells	2 cells
D2	2–4 cells		4–8 cells	2–4 cells	4 cells	2–4 cells
D3	8–16 cells	>8 cells	8 cells	4 cells	8 cells	4–8 cells
D4	Compacted morula		16 cells	4–8 cells	Blastocyst	8–16 cells
D5	Blastocyst	Morula	Morula	8–16 cells	Blastocyst	16 cells Morula
D6	Hatching blastocyst	Morula blastocyst	Early blastocyst	Morula		Morula
D7	Implantation	Blastocyst	Blastocyst-expanded blastocyst	Early blastocyst-blastocyst		Morula blastocyst
D8		Blastocyst	Expanded blastocyst	Blastocyst		Blastocyst
D9		Blastocyst	Hatching blastocyst	Blastocyst-expanded blastocyst		Blastocyst
D10				Expanded blastocyst		Blastocyst
D11						Expanded blastocyst

*Bracketed numbers denote reference citations.

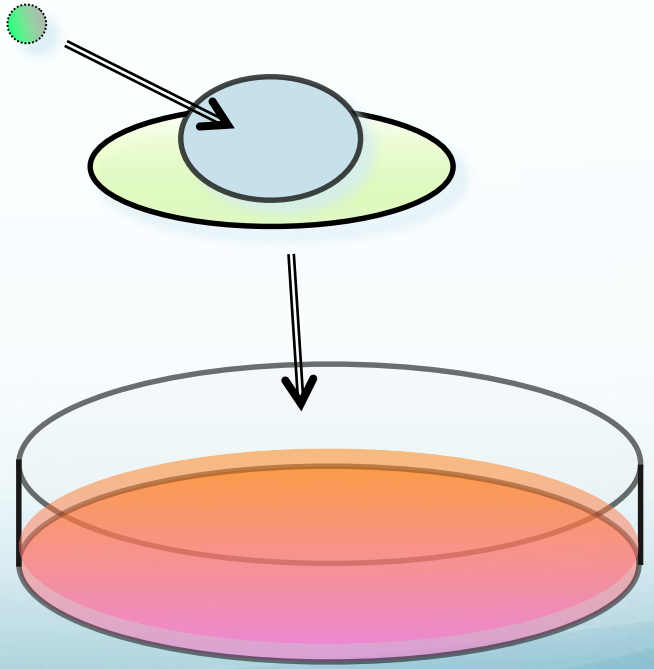
Kropp, Di Marzo, and Golos, Biol Reprod 2017

POST-IMPLANTATION 3D EMBRYO CULTURE - NONHUMAN PRIMATES

Blastocyst

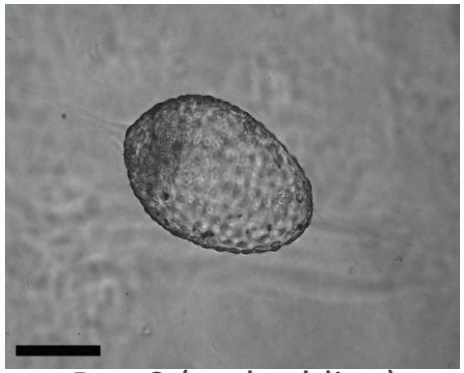
Matrigel Raft:
Coverslip coated in Matrigel

Feeder cell plated
35 mm 6-well dish

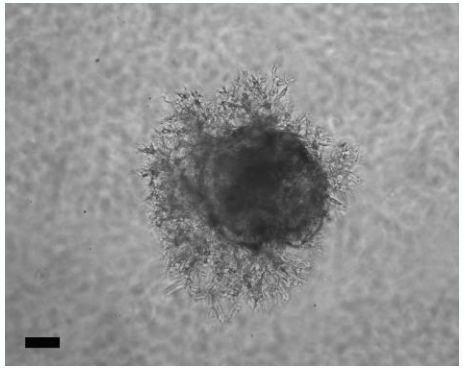


Chang et al, RB&E 2018

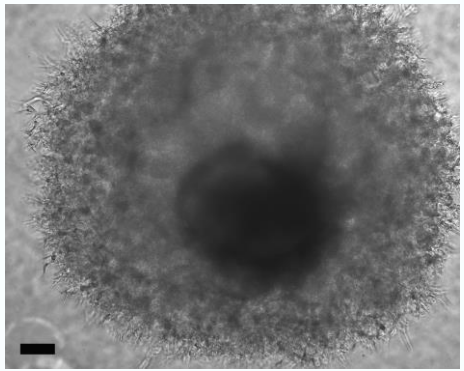
POST-IMPLANTATION 3D EMBRYO CULTURE – NONHUMAN PRIMATES



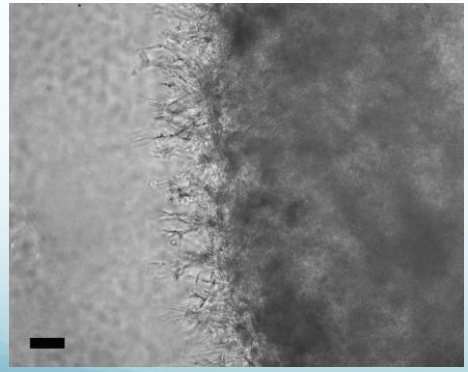
Day 0 (embedding)



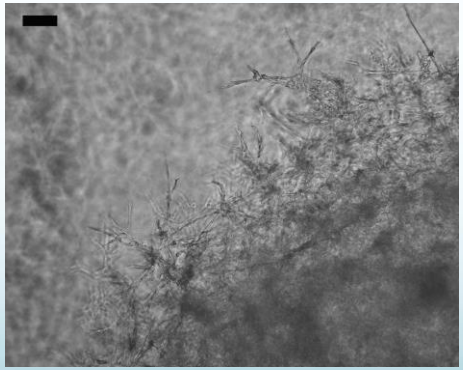
Day 6 post embedding



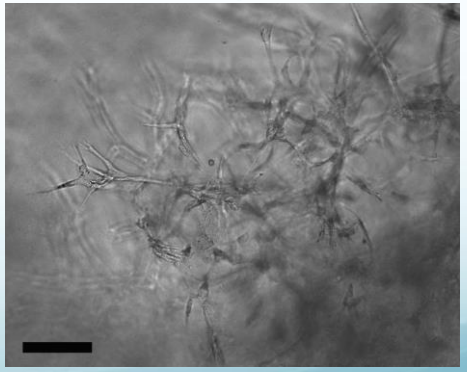
Day 9



Day 19



Day 26

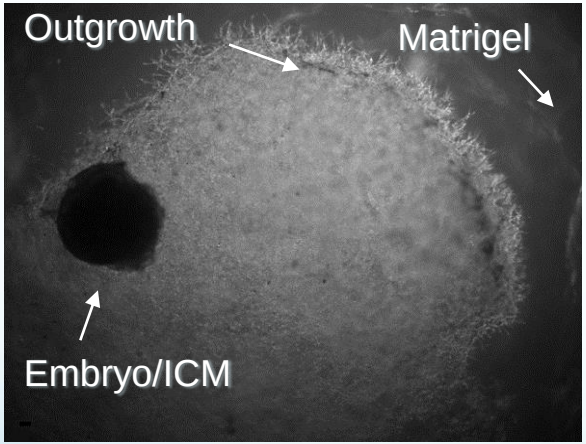


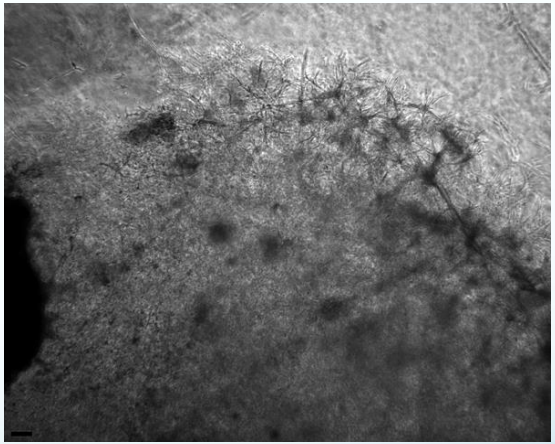
Day 38

Bar = 100 μm

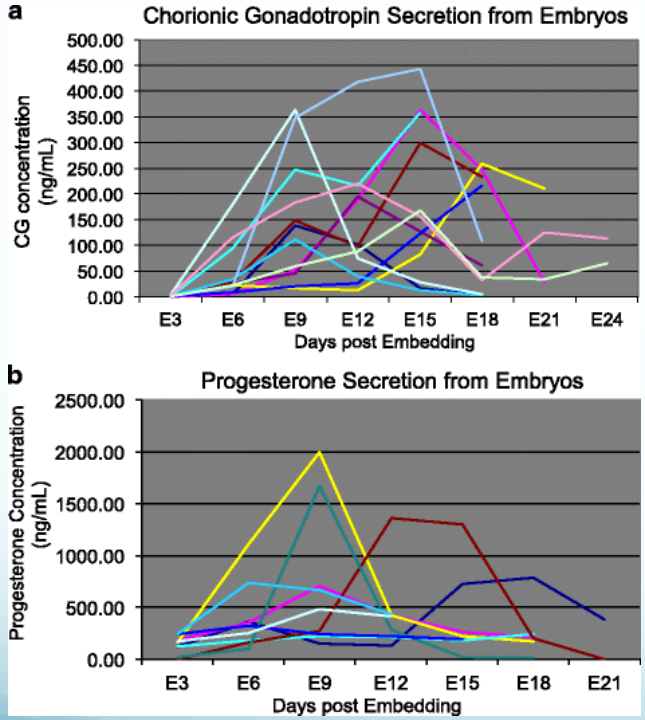
Chang et al, RB&E 2018

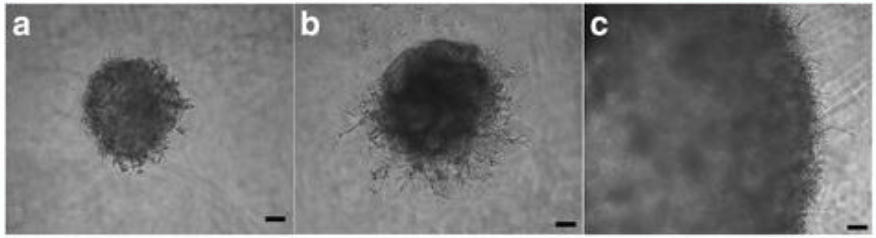
DEVELOPMENT OF RHESUS MONKEY IVF-DERIVED EMBRYO IN MATRIGEL (53 DAYS POST-FERTILIZATION)

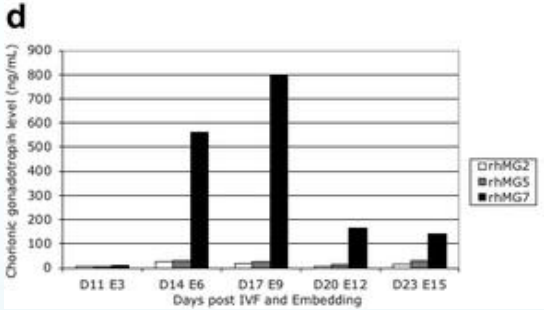


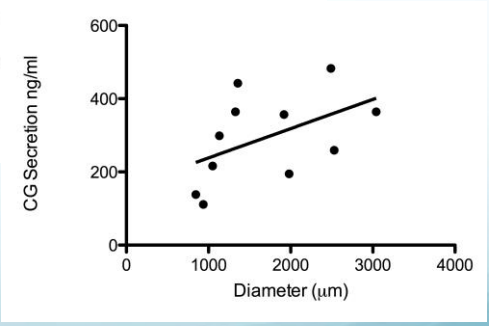


HORMONAL SECRETION PROFILE AMONG RHESUS EMBRYOS CULTURE IN MATRIGEL



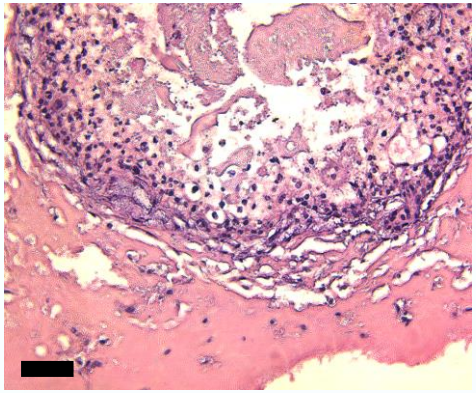




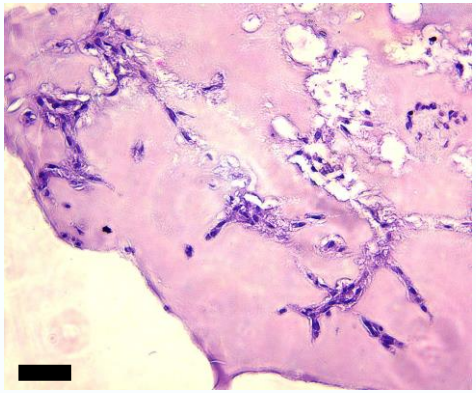


Chang *et al, RB&E 2018*

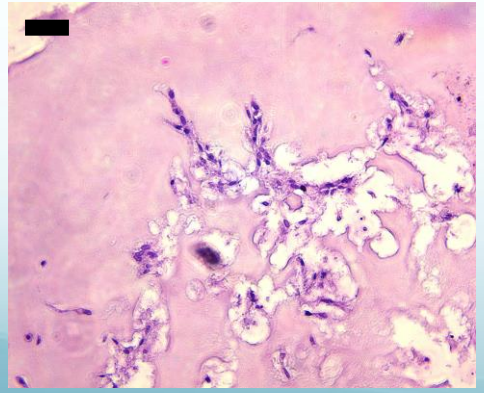
EMBRYONIC AND EXTRAEMBRYONIC OUTGROWTH: HEMATOXYLIN AND EOSIN (H&E) STAINING



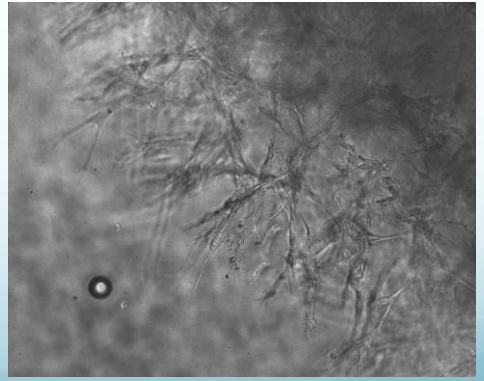
H&E



H&E

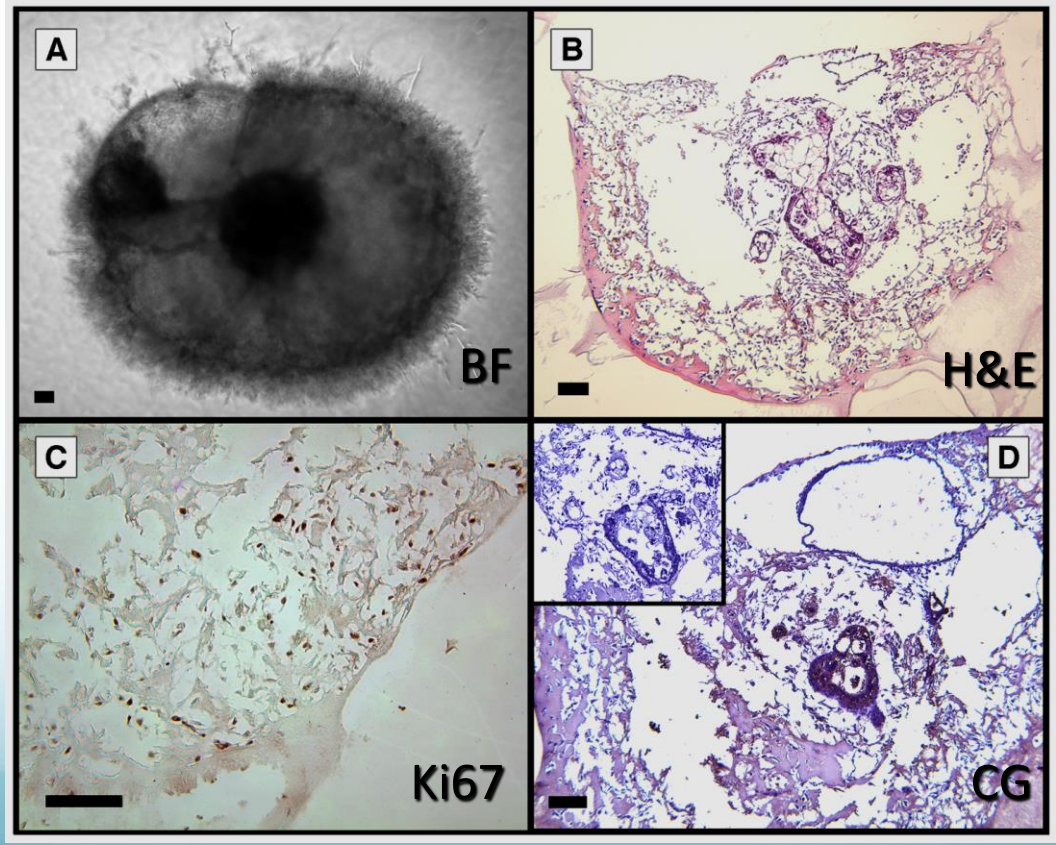


H&E

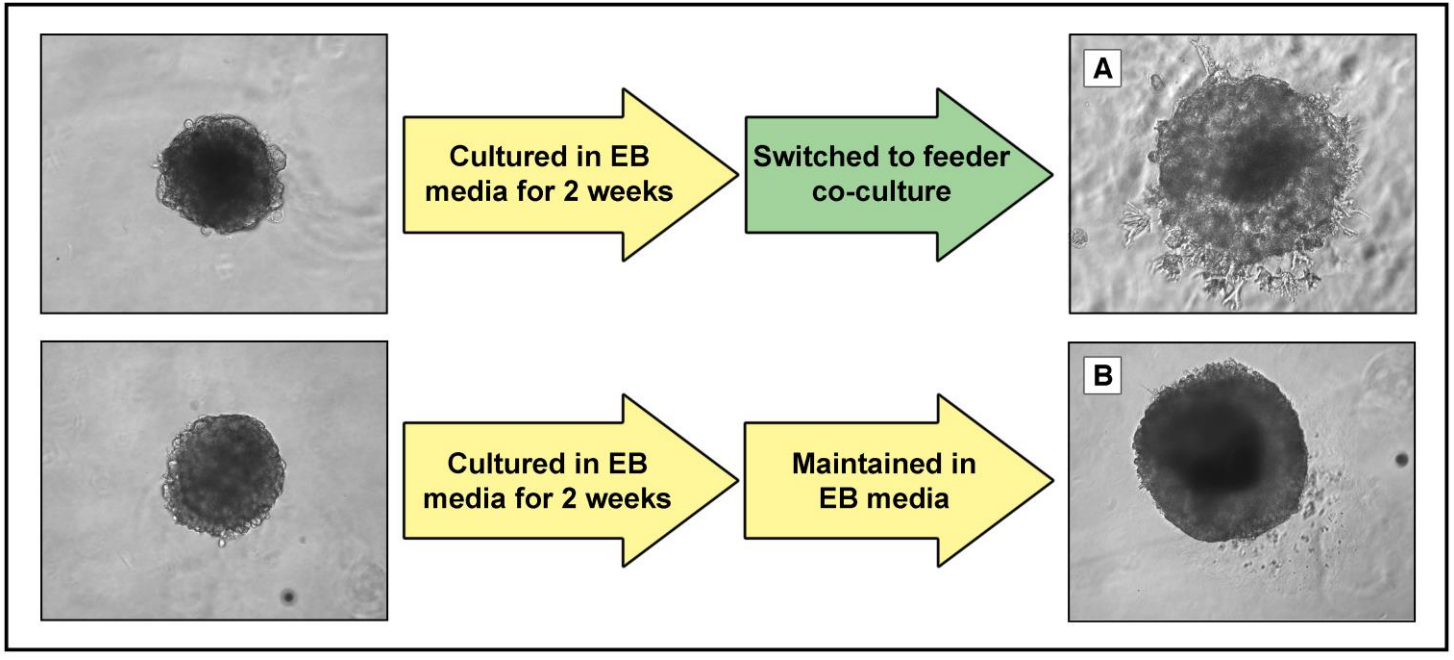


Brightfield

EMBRYO-DERIVED OUTGROWTH, PROLIFERATION, AND CG SECRETION

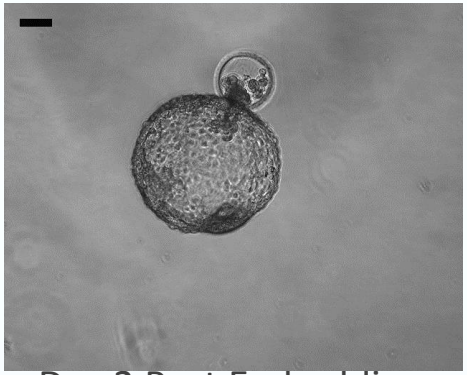


EFFECT OF CO-CULTURE ON TROPHOBLAST INVASION

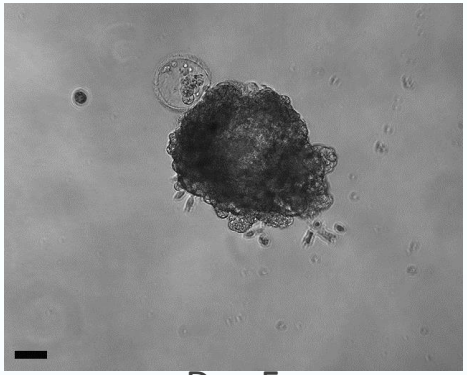


Chang et al, manuscript in preparation

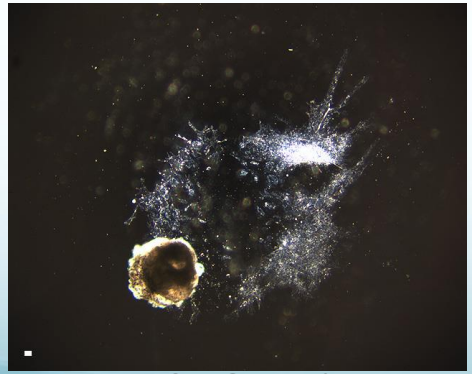
COMPARATIVE 2D CULTURE: POORER DEVELOPMENT



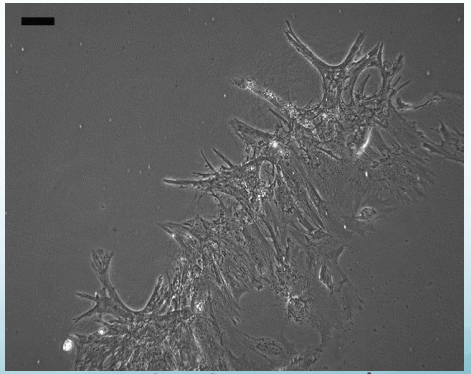
Day 2 Post Embedding



Day 5



Day 25 Overview



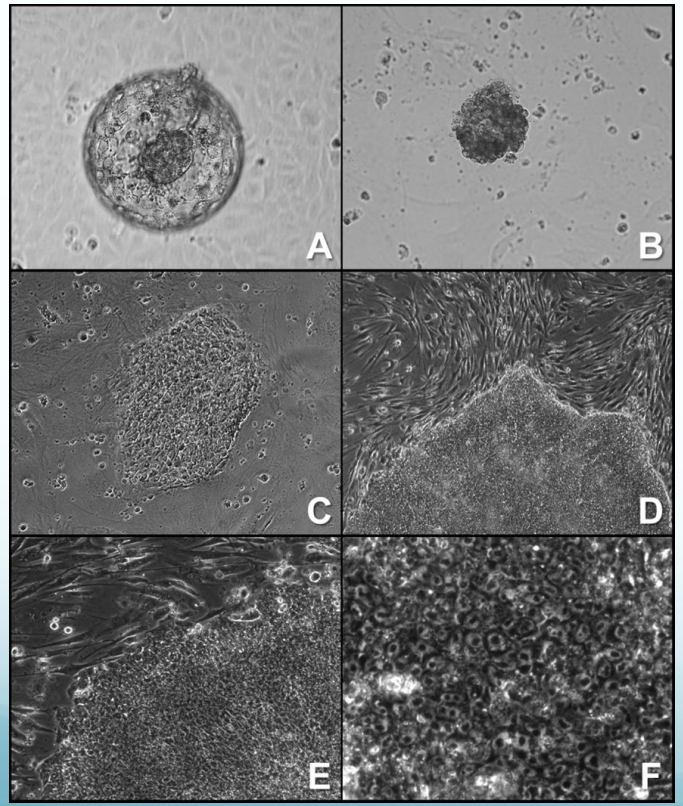
Day 25 Outgrowths

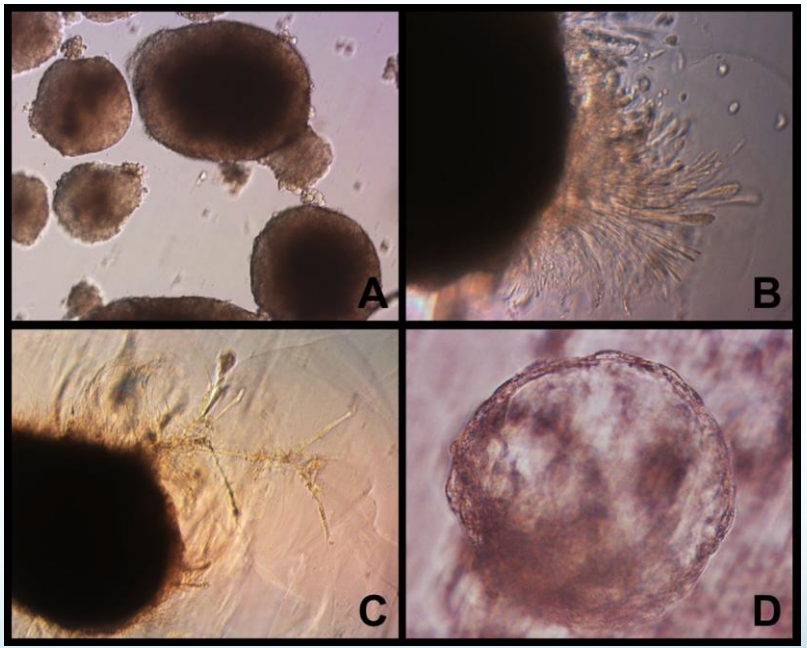
Chang et al, manuscript in preparation

POST-IMPLANTATION 3D EMBRYO CULTURE - NONHUMAN PRIMATES

- IVF-ICSI derived preimplantation embryo culture
- Baboon embryonic stem cell (ESC) derivation
- Baboon ESC-derived embryoid body
- ESC aggregates → embryoid body → embryo-like formation after Matrigel embedding *(Chang et al, Stem Cell & Dev 2011 and unpublished data)*

BABOON ESC-DERIVED EMBRYOID BODY SELF-ORGANIZATION





Chang et al, Stem Cell & Dev 2011

FUTURE DIRECTIONS

- Optimizing culture environment, e.g., dynamic O_2
- 3D printing: embryonic/extraembryonic structure, extracellular matrix (ECM), uterine/scaffold structure
- Biofabrication with multiple types of biopolymers (Matrigel, hydrogel/alginate, hyaluronic acid, etc.)
- Dynamically reconfigurable architectures to support further downstream development and embryo-maternal interaction
- Gene editing to repair *in vitro* developing embryos, and replacement of embryo into uterus for continuing pregnancy

TAKE-HOME MESSAGE

- Interaction of critical factors on preimplantation embryo extended culture: *it's the system, not one single factor*
- Embryo culture into Day 7 should be considered for routine practice
- Embryo culture beyond Day 7: currently at early stage research; future therapeutic applications and testing platform
- Ethical concerns and current technical limitations on extended embryo culture
- Need more research in *in vitro* extended embryo culture and artificially constructed extraembryonic support system

ACKNOWLEDGEMENTS

- University of Texas Health Science Center, San Antonio, TX
  - Department of Obstetrics and Gynecology, School of Medicine
- NIH-NCRR Southwest National Primate Research Center, San Antonio, TX
- University of Wisconsin, Madison, WI
  - Dept. of Obstetrics and Gynecology, School of Medicine and Public Health
  - Department of Comparative Bioscience, School of Veterinary Medicine
- NIH-NCRR Wisconsin National Primate Research Center, Madison, WI