

# Embryology III

## PERIMPLANTATION DEVELOPMENT

autumn 2024

# Placentation and establishment of pregnancy

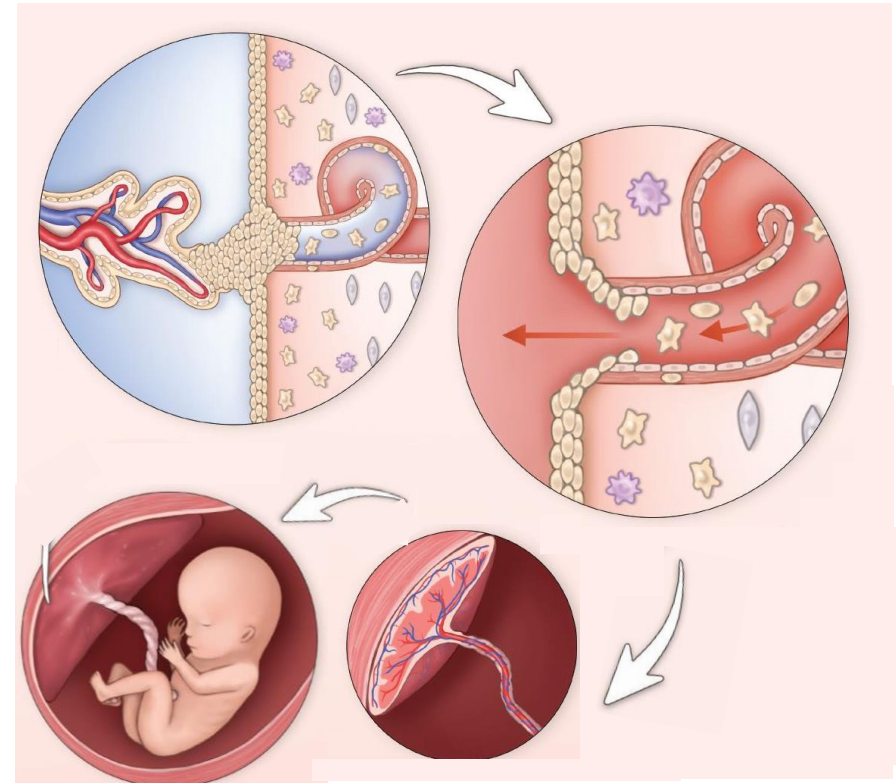
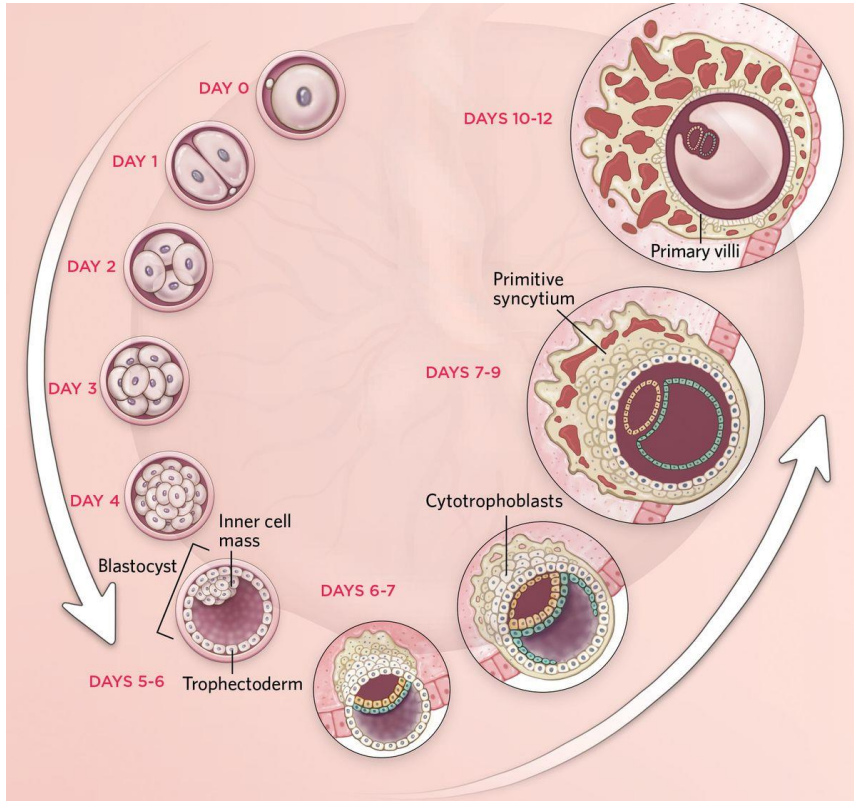


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# Embryo nutrition



## 1) Cytotrophic nutrition

- from conception (D0) to D10 pc
- use of maternal cellular sources
- quite metabolism

## 2) Histiotropic nutrition

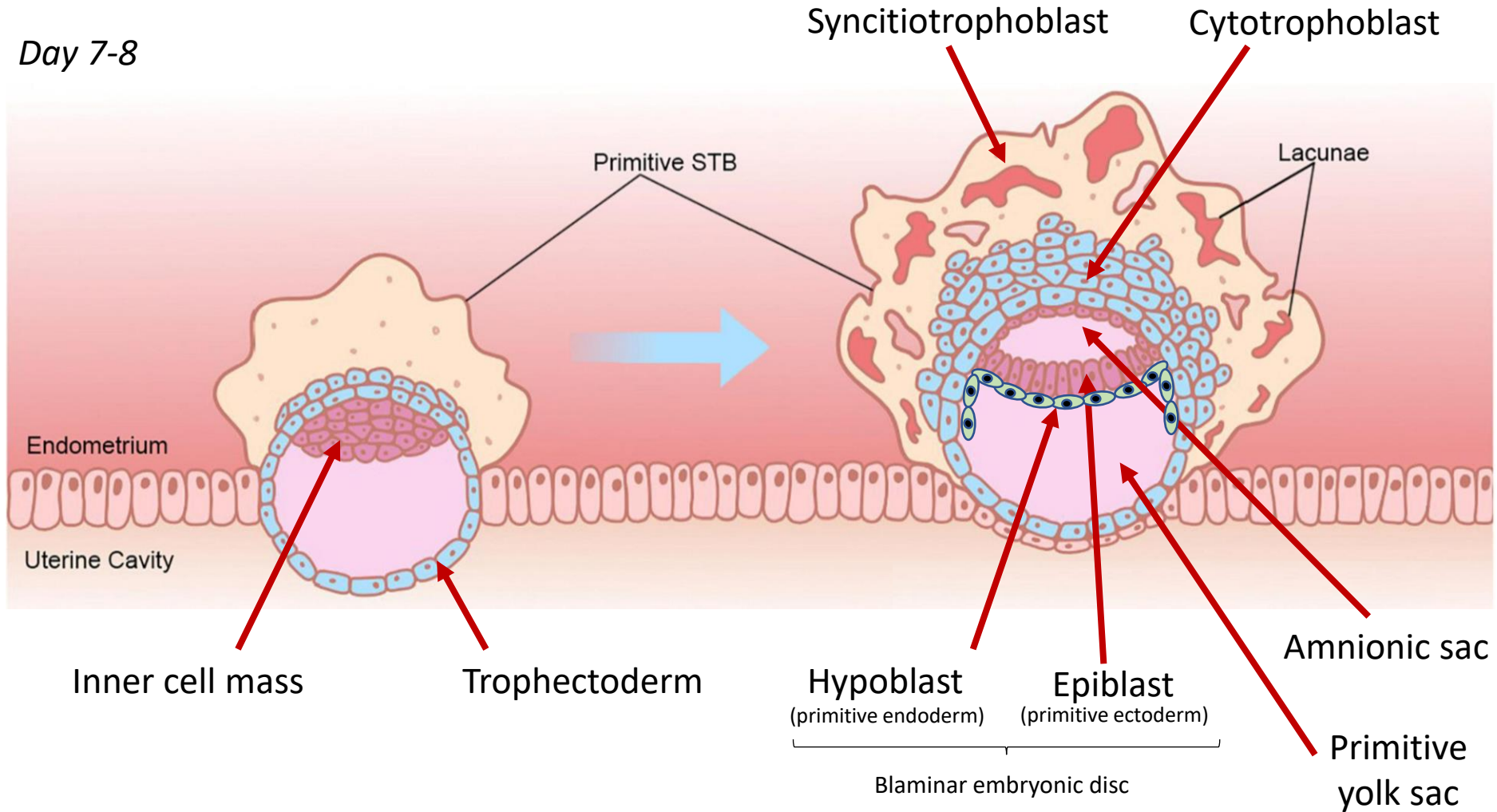
- D11 pc -12 wg
- nutrients obtained from maternal uterine gland secretions
- glycolysis, low  $O_2$  environment

## 3) Hemotrophic nutrition

- 12 wg to term
- nutrients obtained from maternal blood
- oxidative phosphorylation

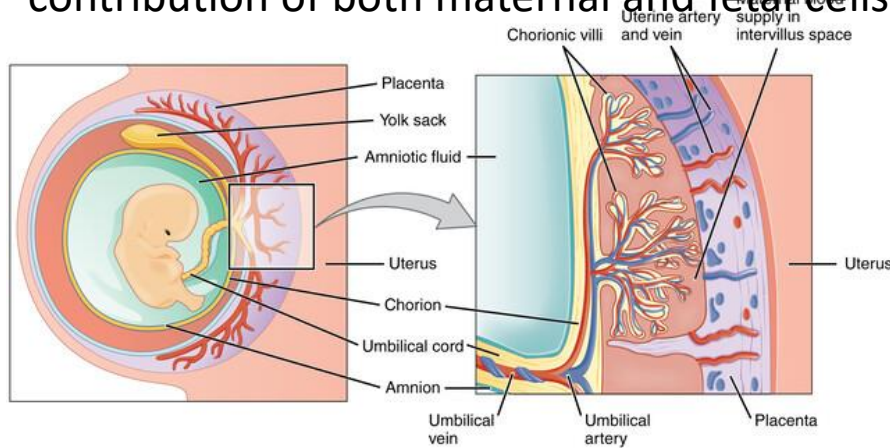
# Perimplantation stage

Day 7-8



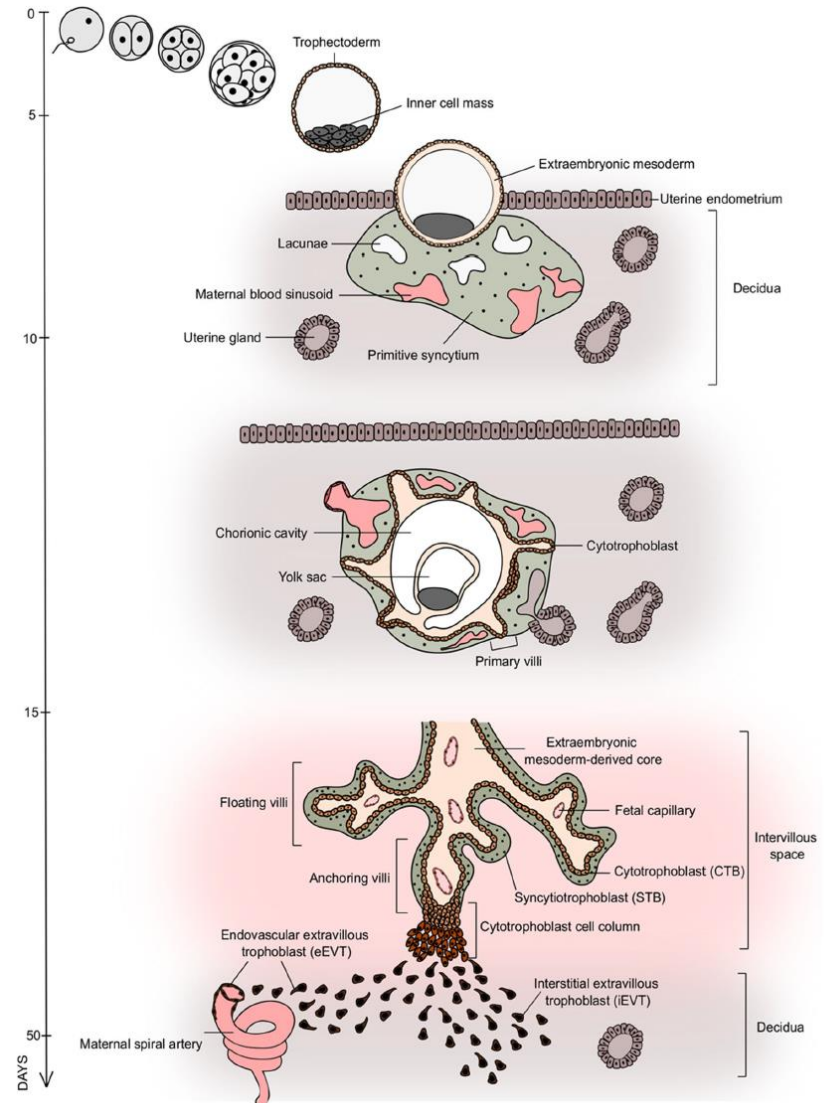
# Placentation

- establishing of a stable maternal-fetal interface in specialized ephemeric organ  
= **placenta**
- contribution of both maternal and fetal cells

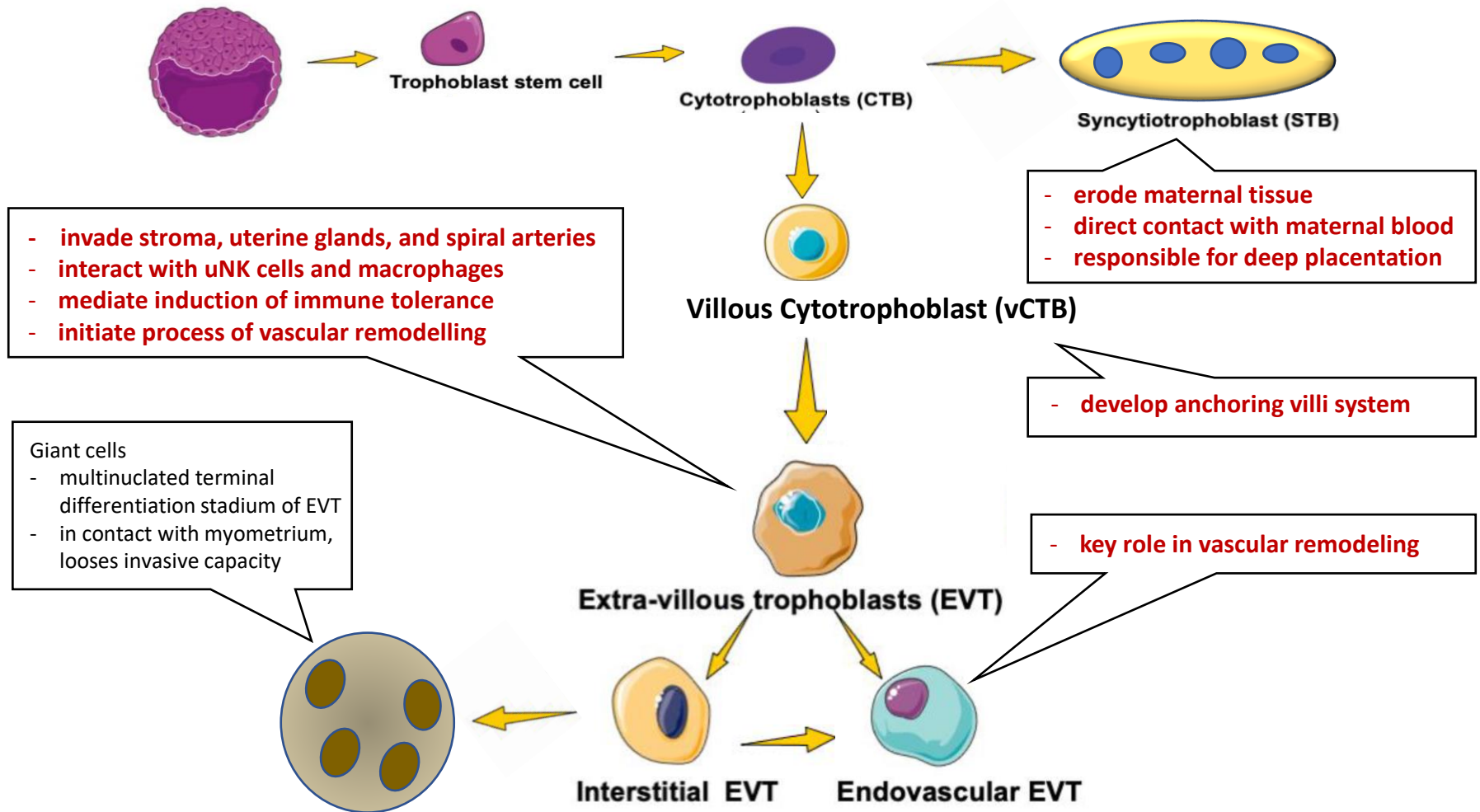


Key steps of placenta development:

- 1) Trophoblast differentiation and invasion
- 2) Developing of chorionic villi (branching and angiogenesis)
- 3) Angiogenesis and remodeling of maternal vasculature



# Trophoblast differentiation



# Development of chorionic villus

Carlson 2009

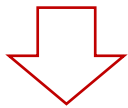
- **Villous cytotrophoblast**

- proliferative CTB cells make protrusions penetrating primitive syncytium



**primary villi**

- + branching and infiltration by **hypoblast-derived extraembryonic mesoderm (ExM)**

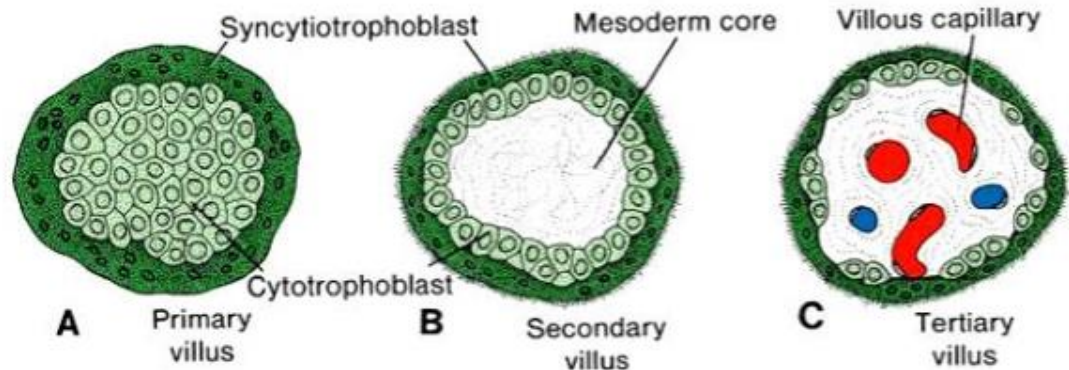
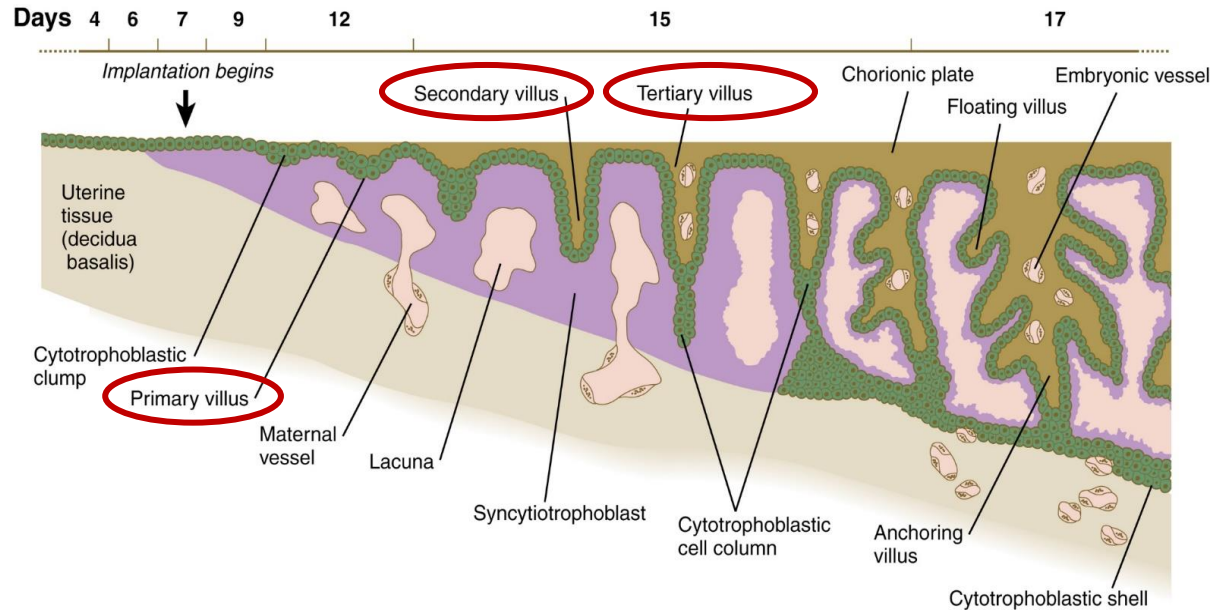


**secondary villi**

- + vascularization



**tertiary villi**

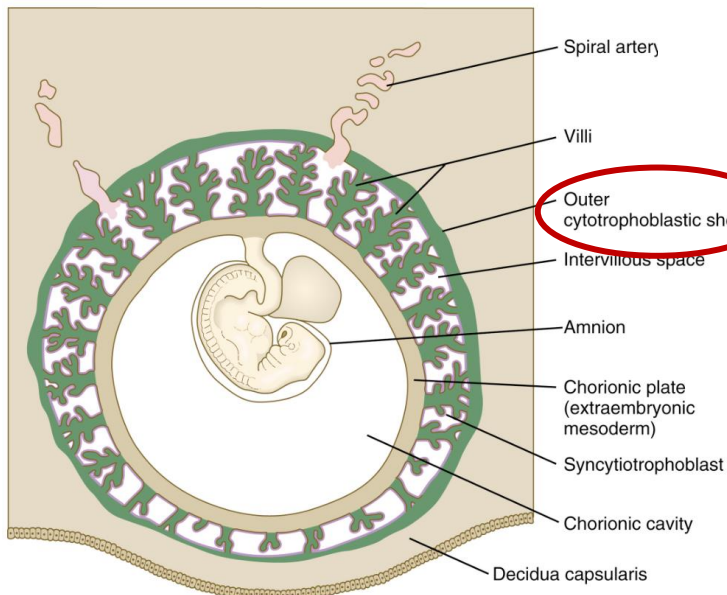
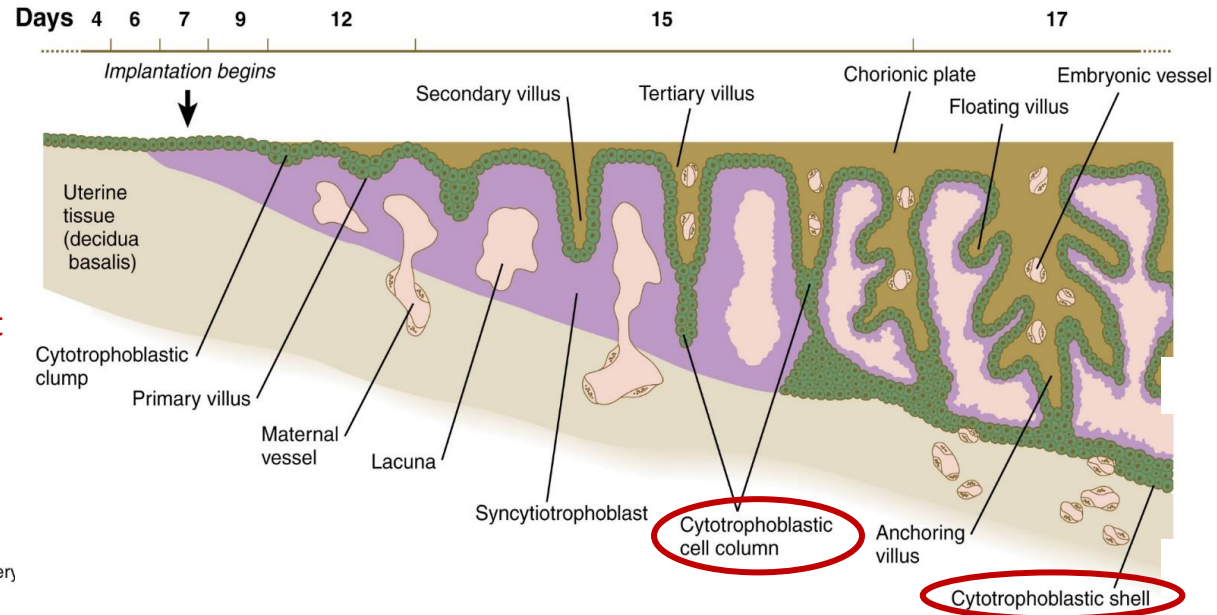


# Development of chorionic villus

Carlson 2009

- **Cytotrophoblastic column**

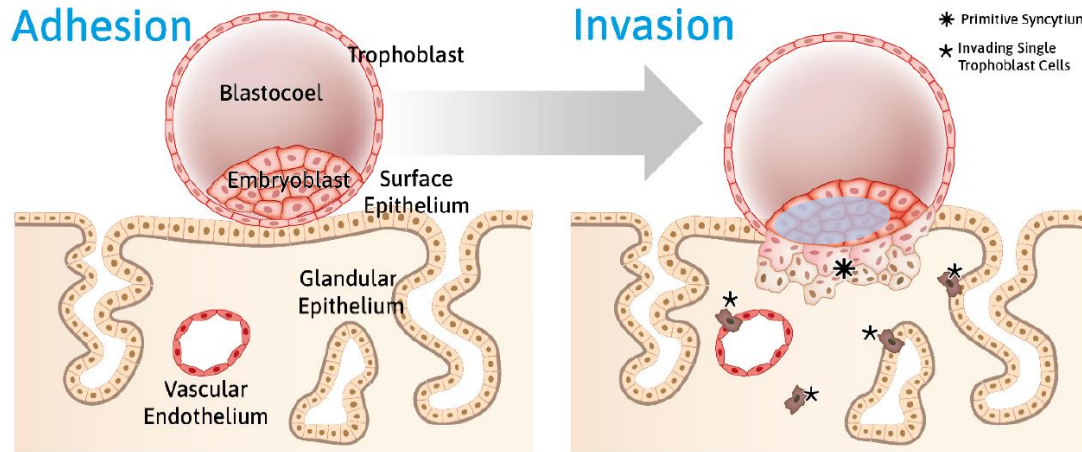
- rows of proliferative cells connecting tertiary villi to **cytotrophoblastic shell**
- cease mitosis and undergo endoreplicative cycles (polyploidization) at distal ends → **extravillous trophoblast (EVT)**



- **Cytotrophoblastic shell**

- continuous external CTB layer
- encapsulates fetus at maternal-fetal interface
- anchors embryo to decidua
- defects (thin, premature, disorganized structure) can lead to spontaneous miscarriage

# Development of chorionic villus

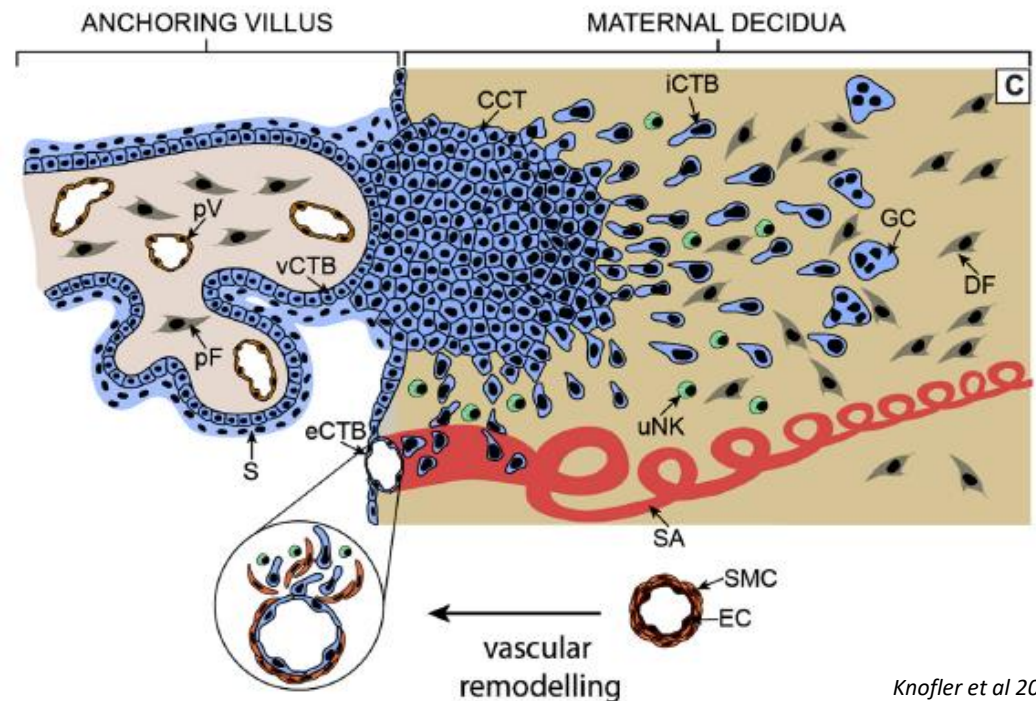


Sternberg et al 2021

- **Extravillous trophoblasts (EVTs)**

- ← differentiation of villous CTB

- single cells detaching from tips of anchoring villi (**cell column trophoblasts**)
- migrate through stroma and invade **uterine glands, and spiral arteries**



Knofler et al 2013



# Vascular remodeling

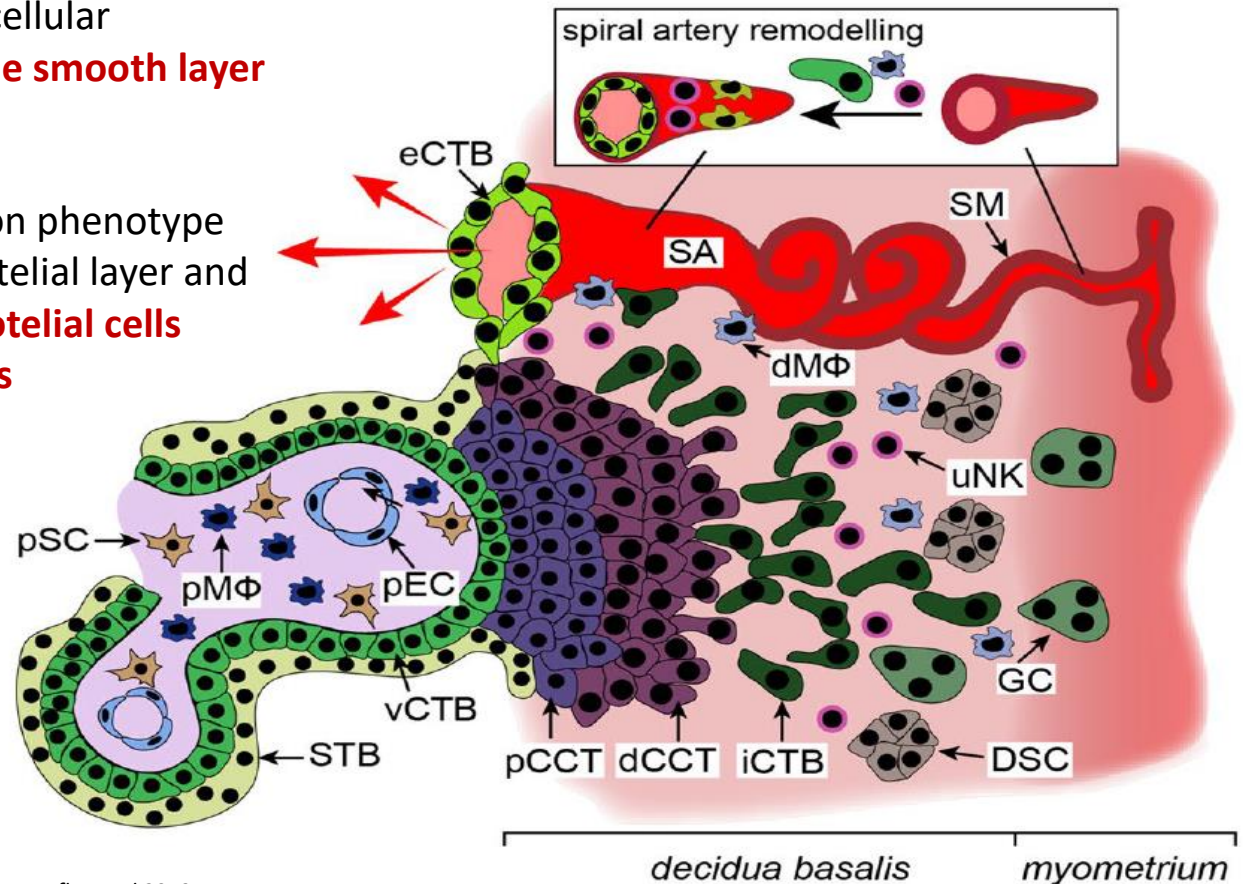
- transformation of narrow high-resistance maternal vessels to highly dilated low resistance conduits

## - iEVT

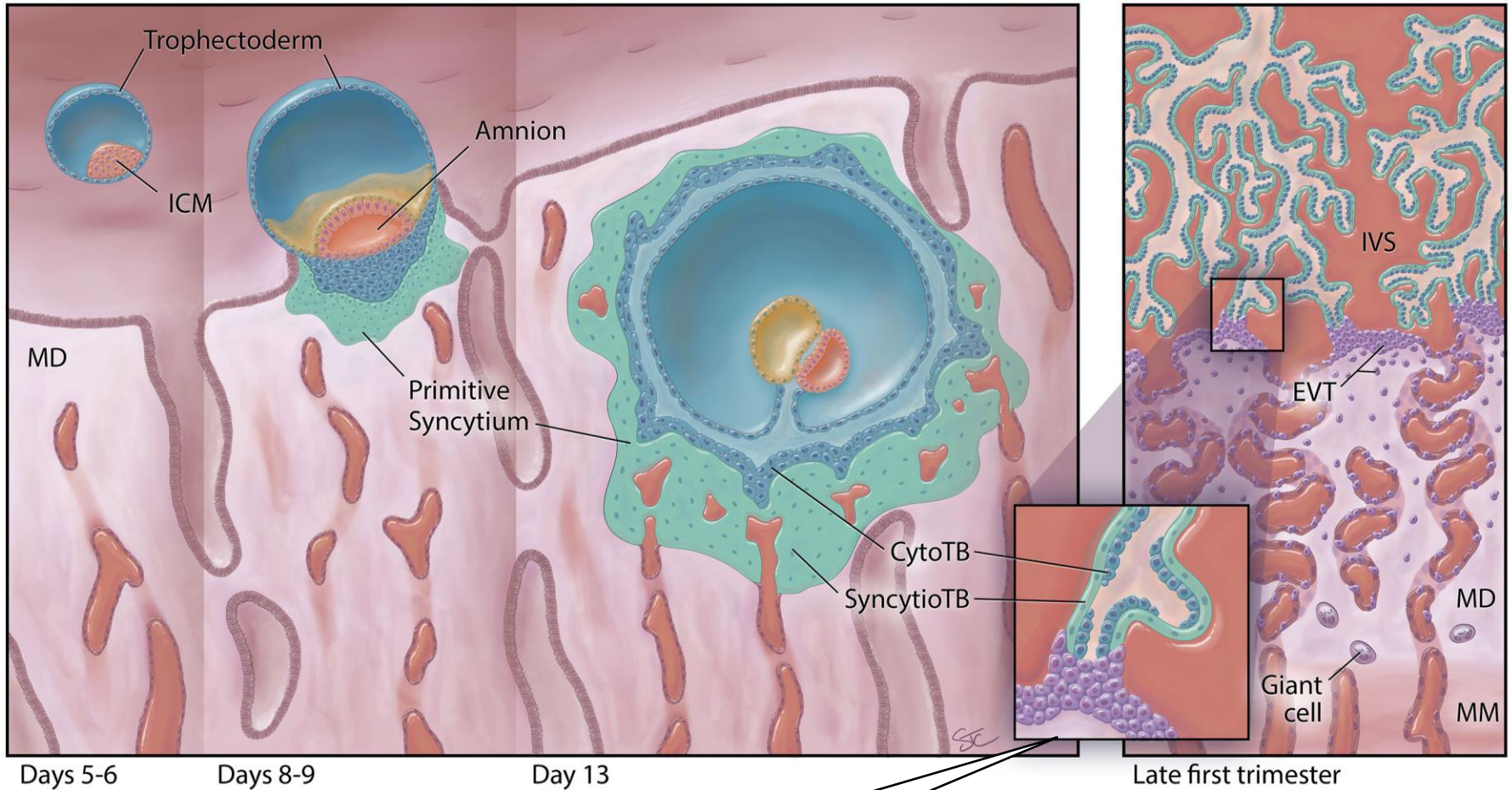
- induce apoptosis and cellular **dedifferentiation of the smooth layer**

## - eEVT

- adopt vascular adhesion phenotype
- interdigitate into endothelial layer and **replace maternal endothelial cells**
- **form trophoblast plugs**



# Vascular remodeling



## Trophoblast plugs

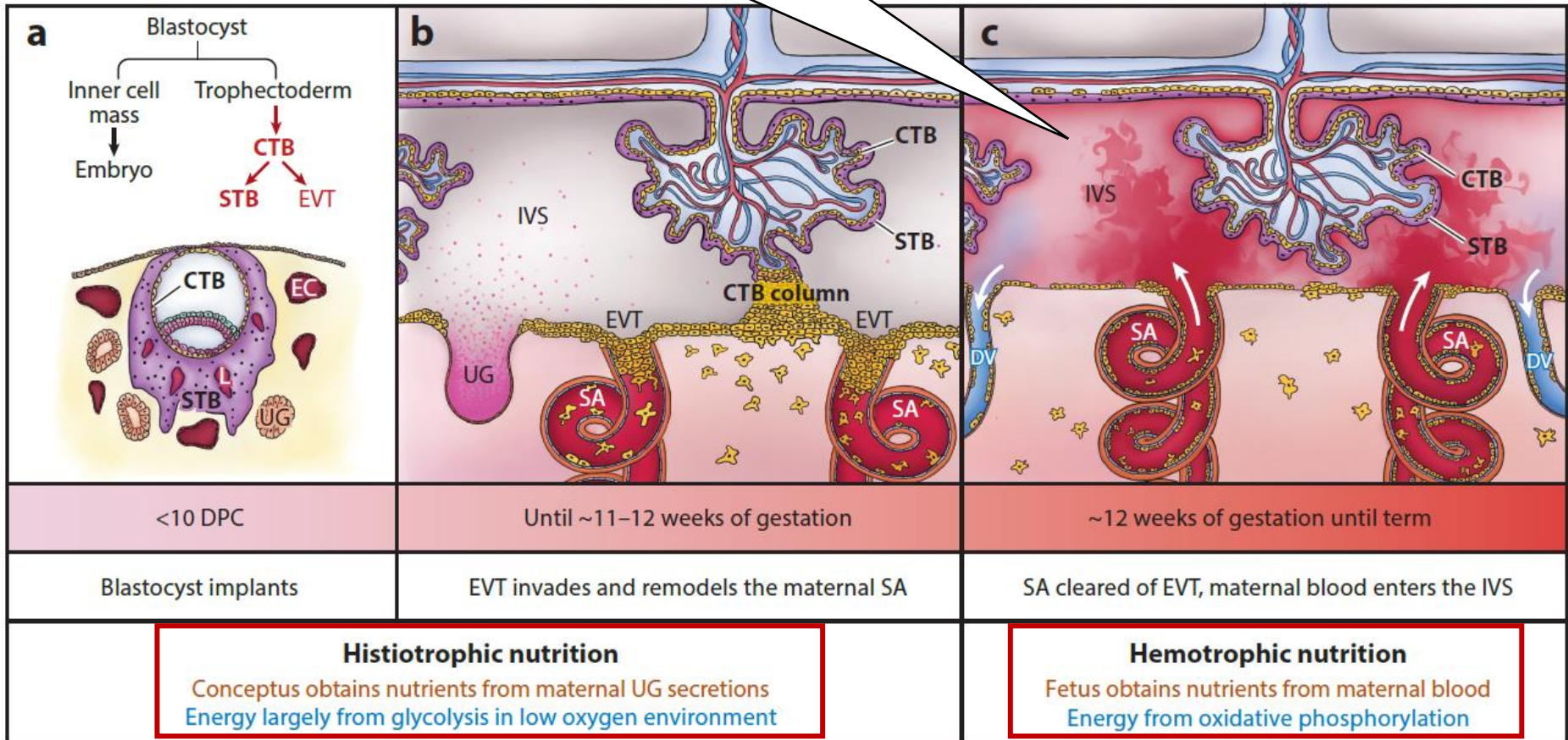
- Temporary occlusions of spiral arteries made by endovascular EVT
- low  $O_2$  environment



- Promotion of angiogenesis and vasculogenesis
- Enhancement of placenta development
- Prevention of ROS-induced damage

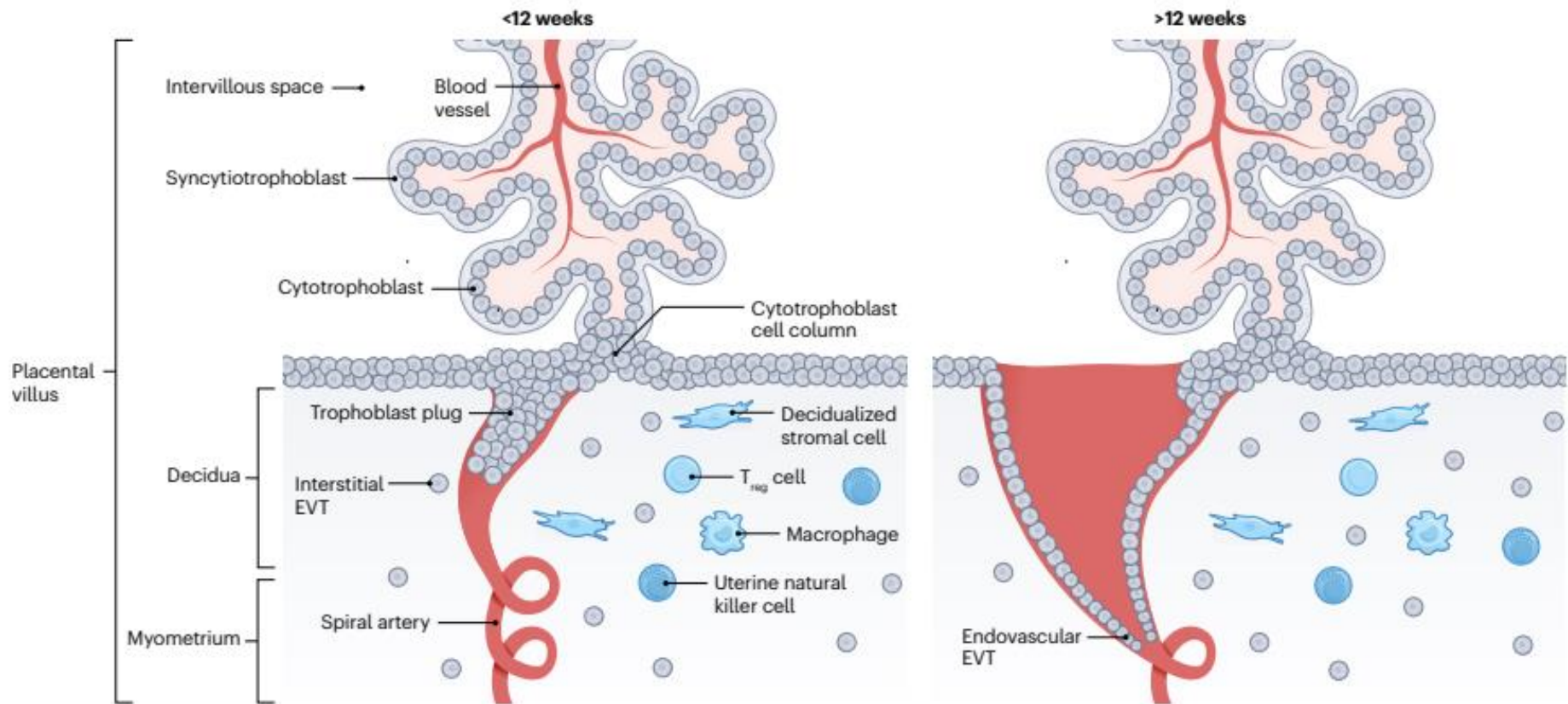
# Vascular remodelling

**Disintegration of trophoblast plugs**  
at the end of first trimester  
→ perfusion with highly oxygenated maternal blood



# Vascular remodeling

- during the first trimester of pregnancy, narrow, high resistance and low capacity uterine spiral arteries are transformed to wide, **high-flow and low resistance** vessels capable to provide hemotrophic nutrition during later stages of pregnancy



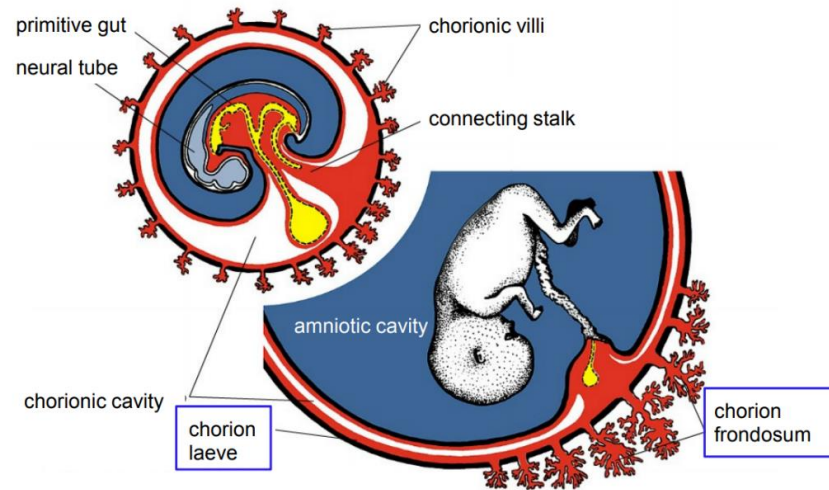
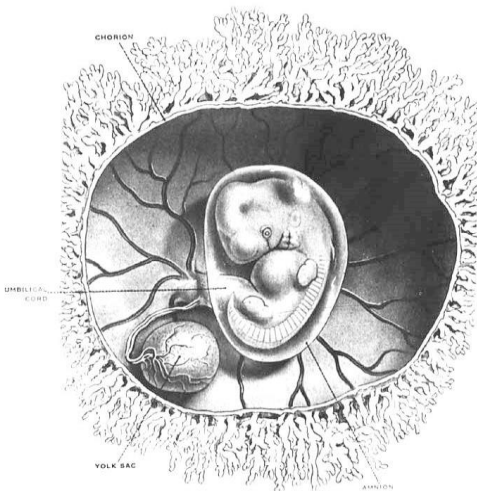
- vessel dilatation: 0.25 → 2-3 mm (3x ↑ O<sub>2</sub> tension)

# Chorion

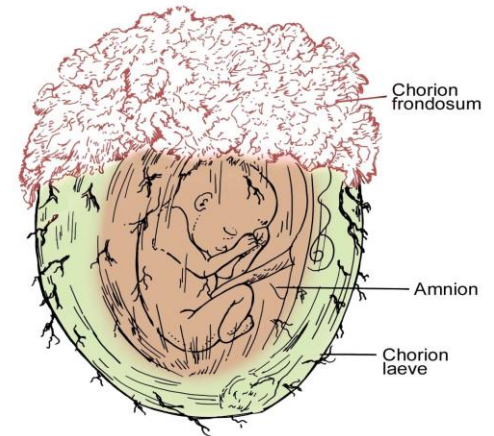
## = chorionic vesicle

- layer encapsulating embryo
- consist from trophoblast cells and extraembryonic mesoderm

5 wg



4th month

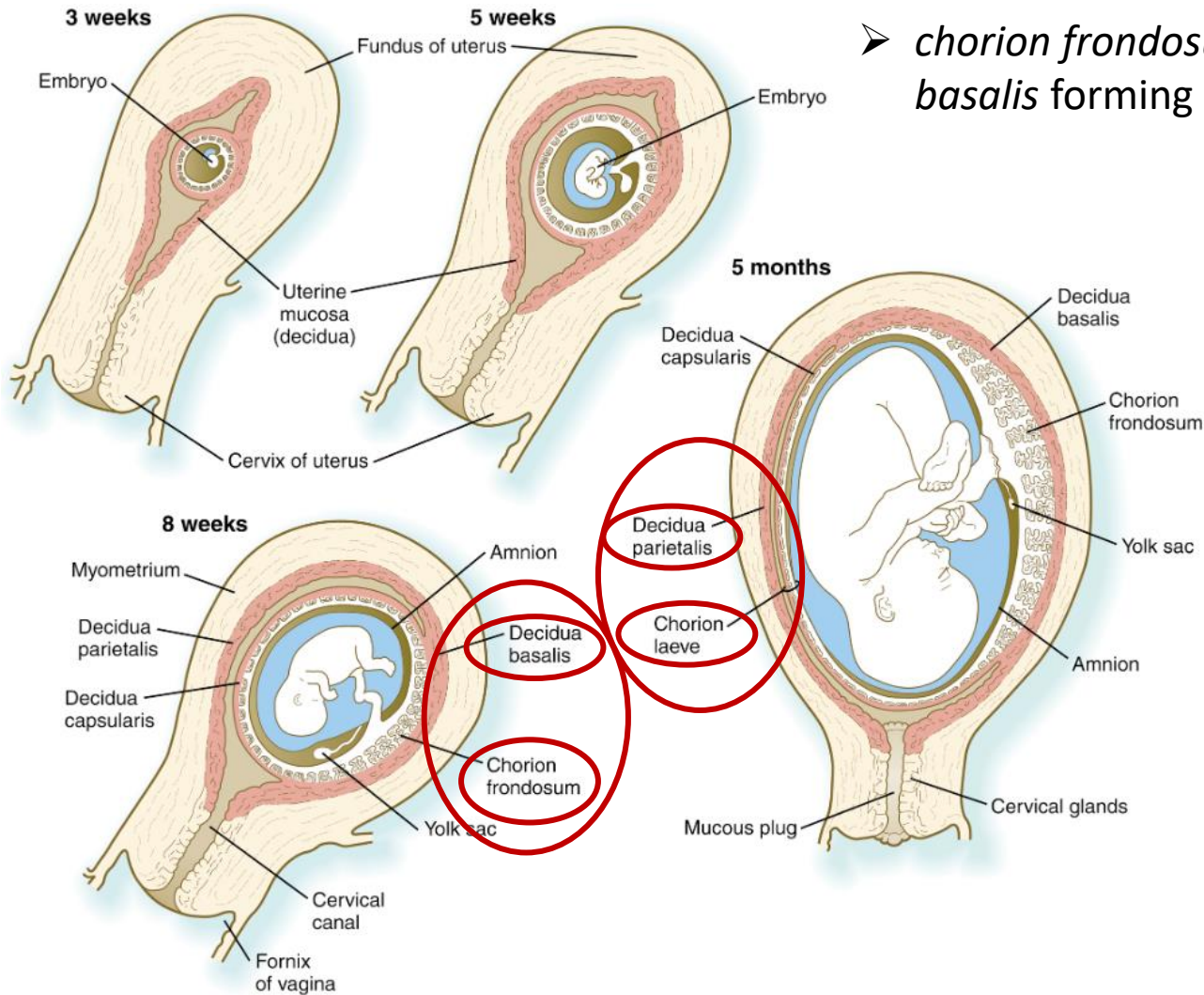


- primary and secondary villi project uniformly from entire outer surface
- tertiary villi formed asymmetrically, preferentially close to *decidua basalis*
  - villi at embryonic pole grow and branch (*chorion frondosum*)
  - villi at abembryonic pole atrophy (*chorion leave*)

# Chorion

➤ *chorion laeve* fuses with *decidua parietalis*

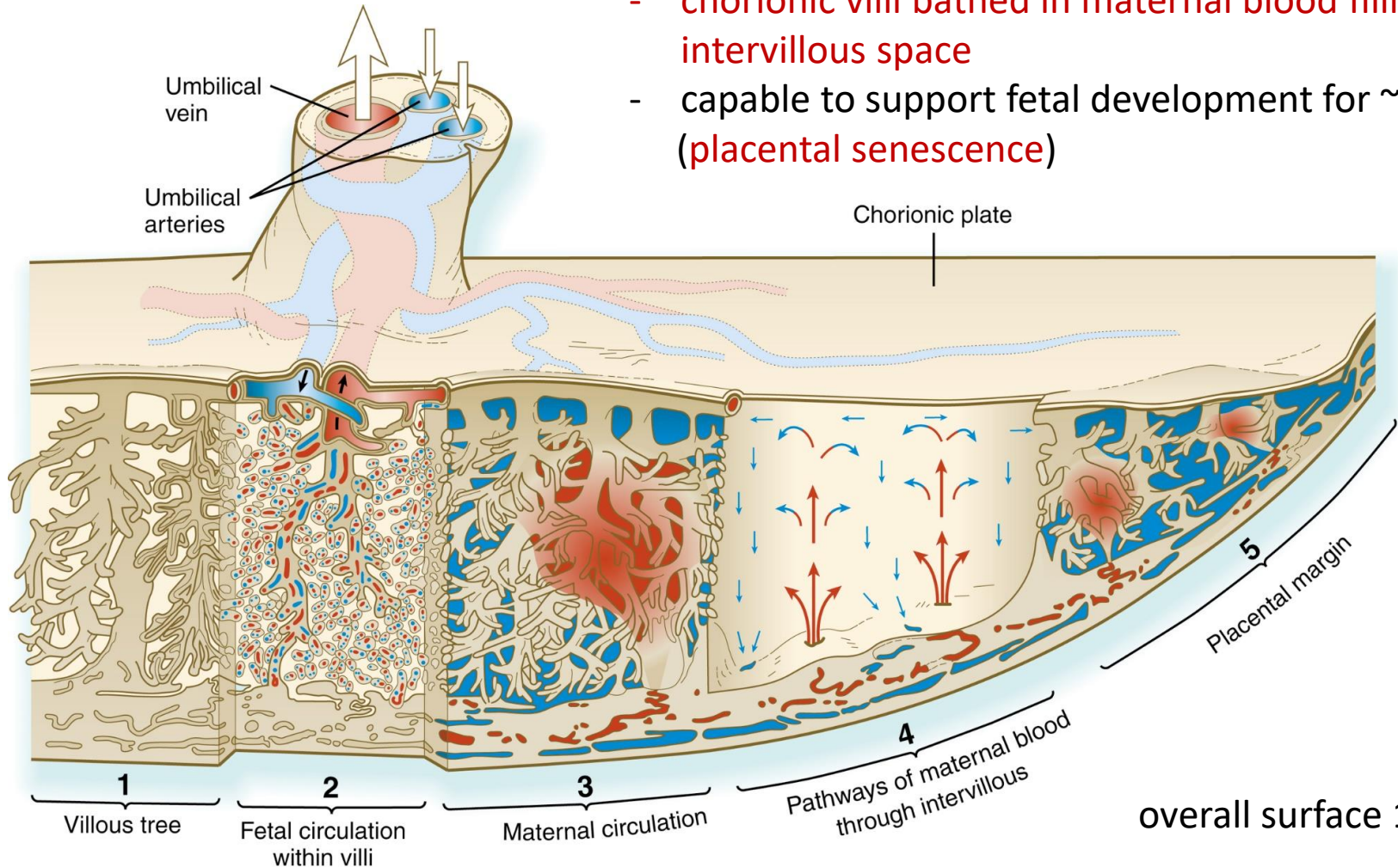
➤ *chorion frondosum* fuses with *decidua basalis* forming **discoid placenta**



# Uteroplacental circulation

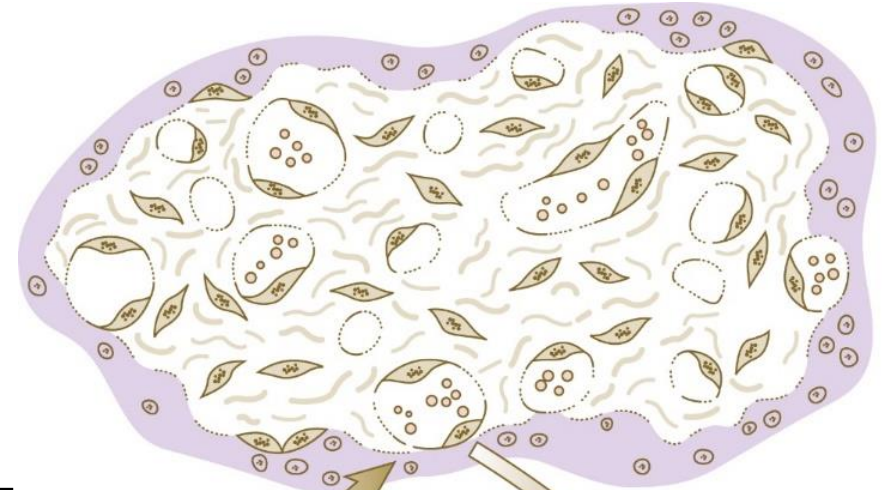
## ❖ Hemochorial placenta

- direct contact of endometrial/fetal tissue with maternal blood
- chorionic villi bathed in maternal blood filling intervillous space
- capable to support fetal development for ~ 42 gw (placental senescence)



# Function of placenta

- ❖ **Oxygenation**
- ❖ **Nutrition**
- ❖ **Excretion**
- ❖ **Immunity**
- ❖ **Endocrine function**
  - hCG, hPL
- ❖ **Anabolism**
  - (glycogen, cholesterol, fatty acids)

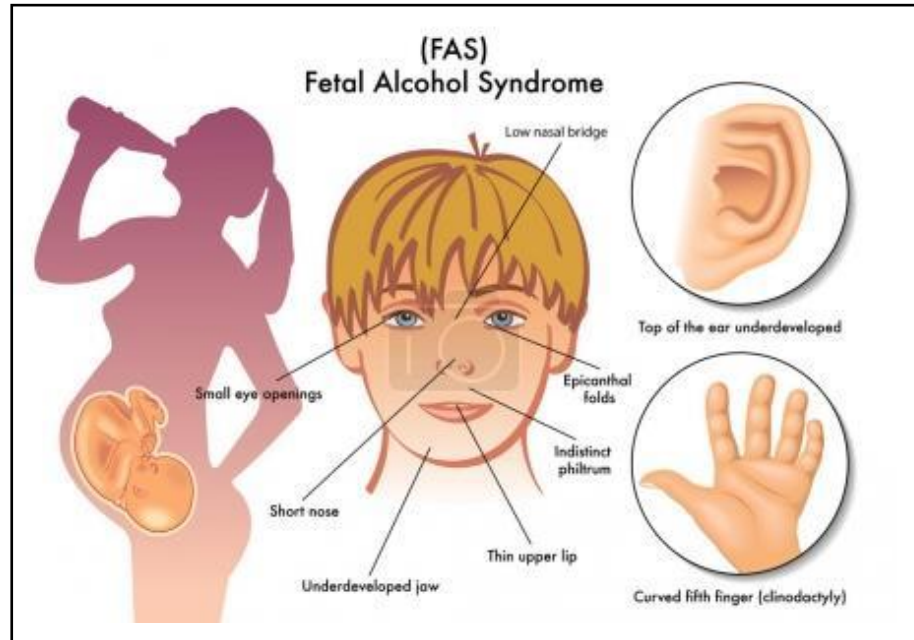


**From mother to fetus**

O<sub>2</sub>  
Water, electrolytes  
Nutrients  
Carbohydrates  
Amino acids  
Lipids  
Hormones  
Antibodies  
Vitamins  
Iron, trace elements  
Drugs  
Toxic substances  
Alcohol  
Some viruses

**From fetus to mother**

CO<sub>2</sub>  
Water, electrolytes  
Urea, uric acid  
Creatinine  
Bilirubin  
Hormones  
Red blood cell antigens



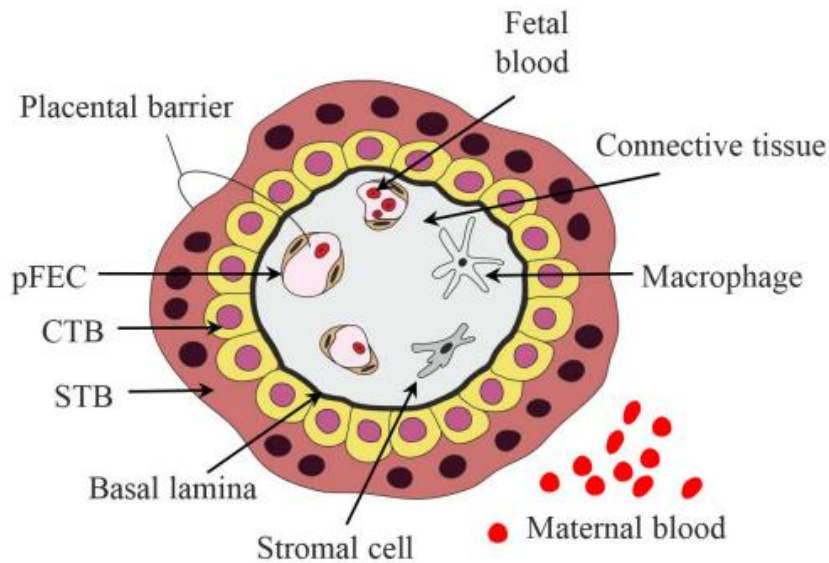


# Placental barrier

- prevents direct contact of maternal and fetal blood cells

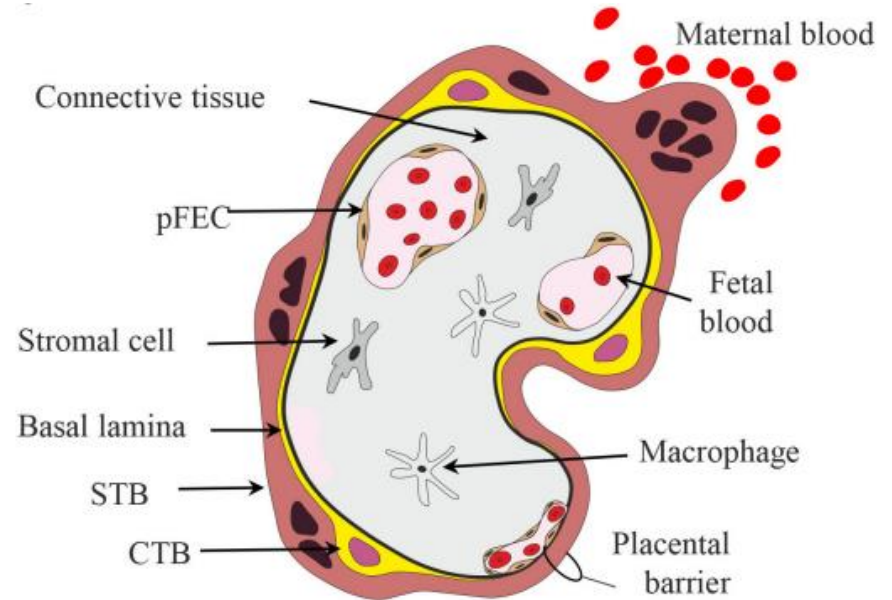
## 1st trimester

- basal lamina (ECM)
- cytotrophoblast (CTB) forms a continuous layer beneath the syncytiotrophoblast (SCT)



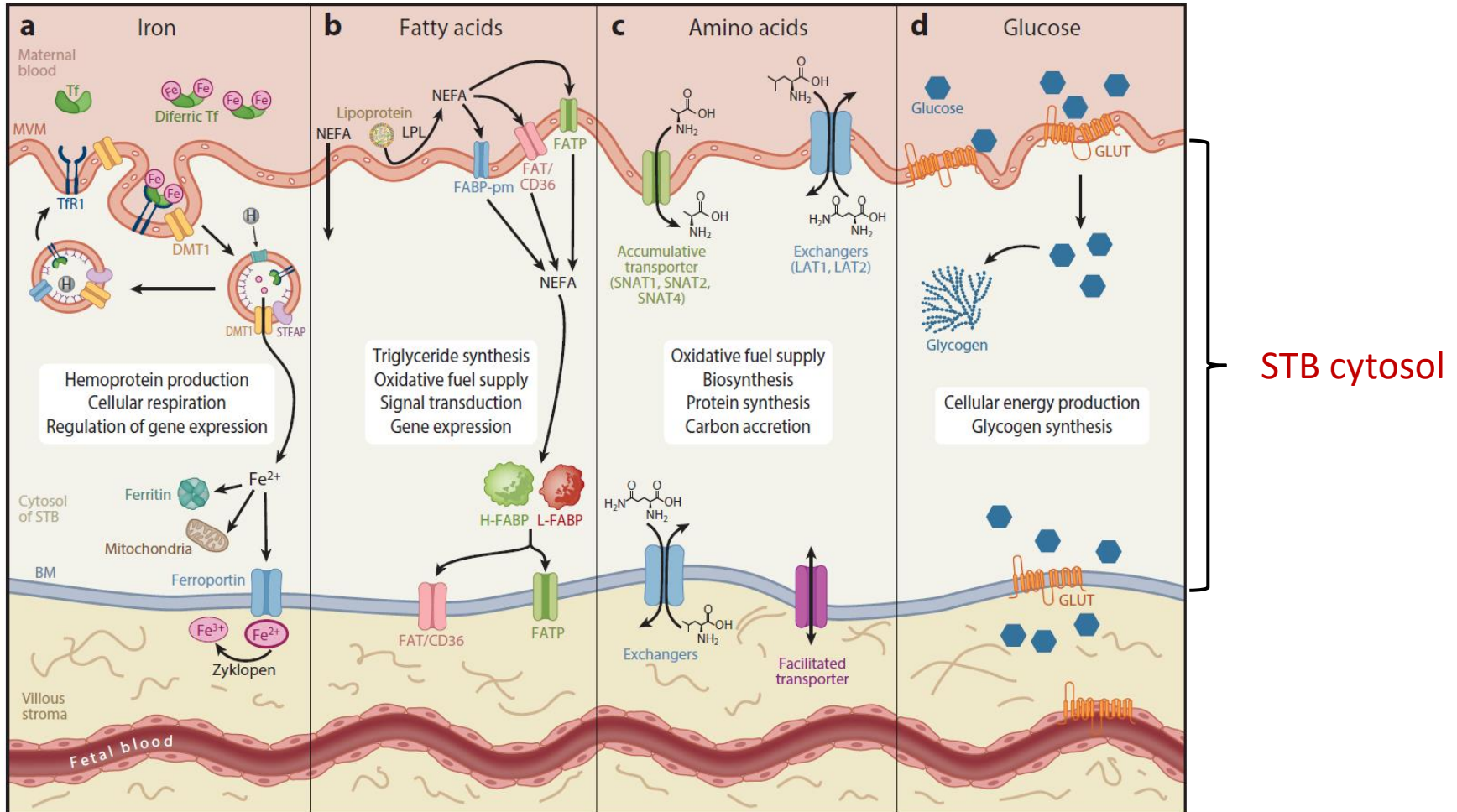
## 3rd trimester

- basal lamina (ECM)
- discontinuous CTB layer beneath thin STB cytoplasmic layer



# Placental barrier

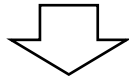
- transcellular gradient transfer across STB syncitium via **facilitated diffusion**



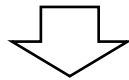
STB cytosol

# Placental barrier

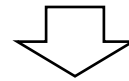
defects of placental vasculature



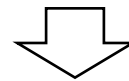
paracellular transfer



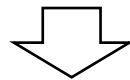
small numbers of fetal cells can escape to maternal circulation



mother develops antibodies against allogenic epitopes



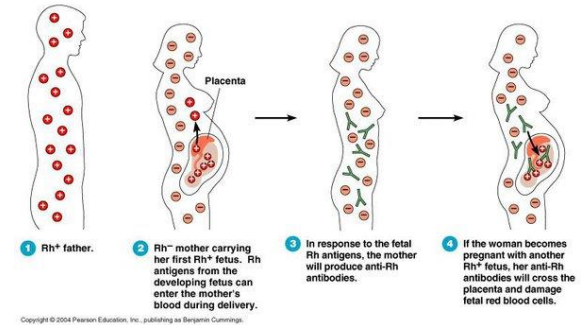
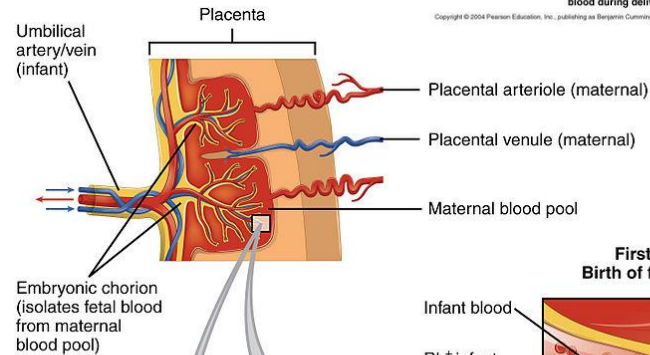
fetal blood cell's hemolysis



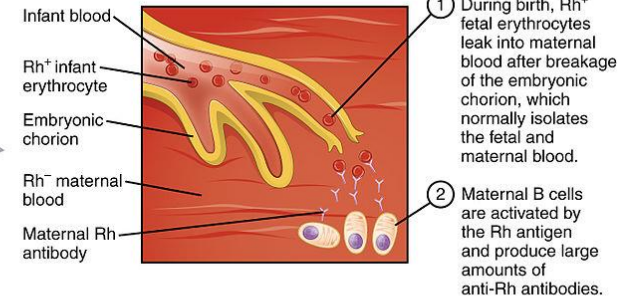
**Erythroblastosis fetalis  
= Hemolytic Disease  
of the Newborn (HDN)**

(e.g. Rh factor, Kell)

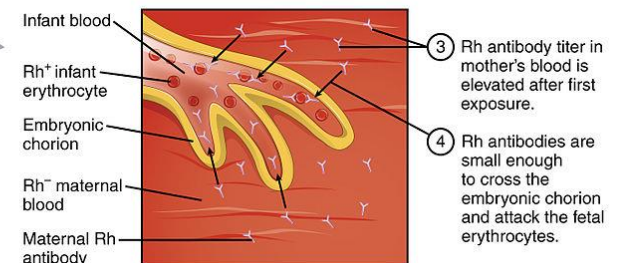
hemorrhage during previous delivery



**First exposure:  
Birth of first Rh<sup>+</sup> infant**

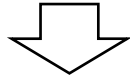


**Second exposure:  
Rh<sup>+</sup> fetus**

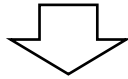


# Placental barrier

- defects in placental vasculature

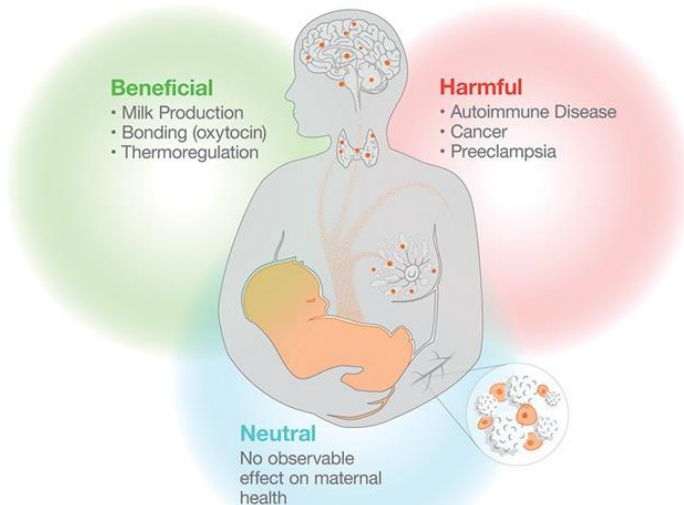


small numbers of fetal cells can escape to maternal circulation

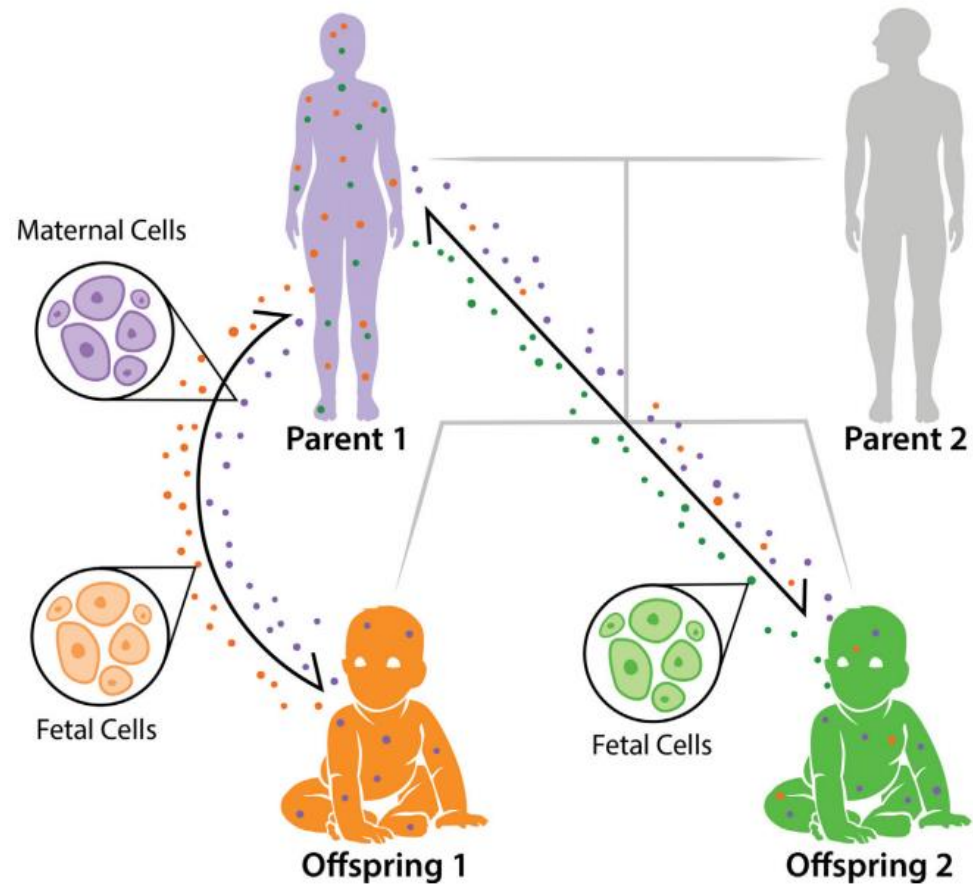


## Microchimerism

= presence of cells from one individual in another genetically distinct individual



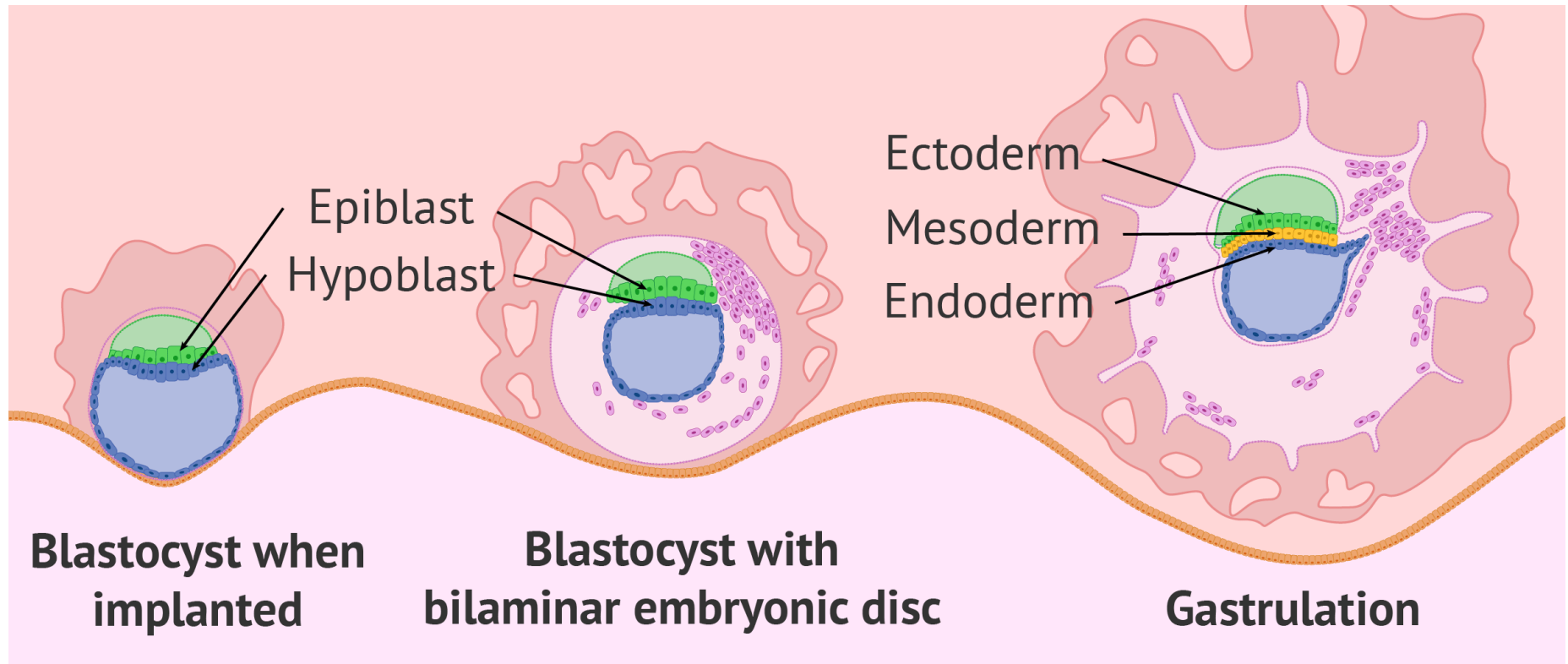
Fetal Microchimerism & Maternal Health



Boddy et al 2015

- bi-directional cell trafficking between fetus and mother during pregnancy
- positive and negative effects

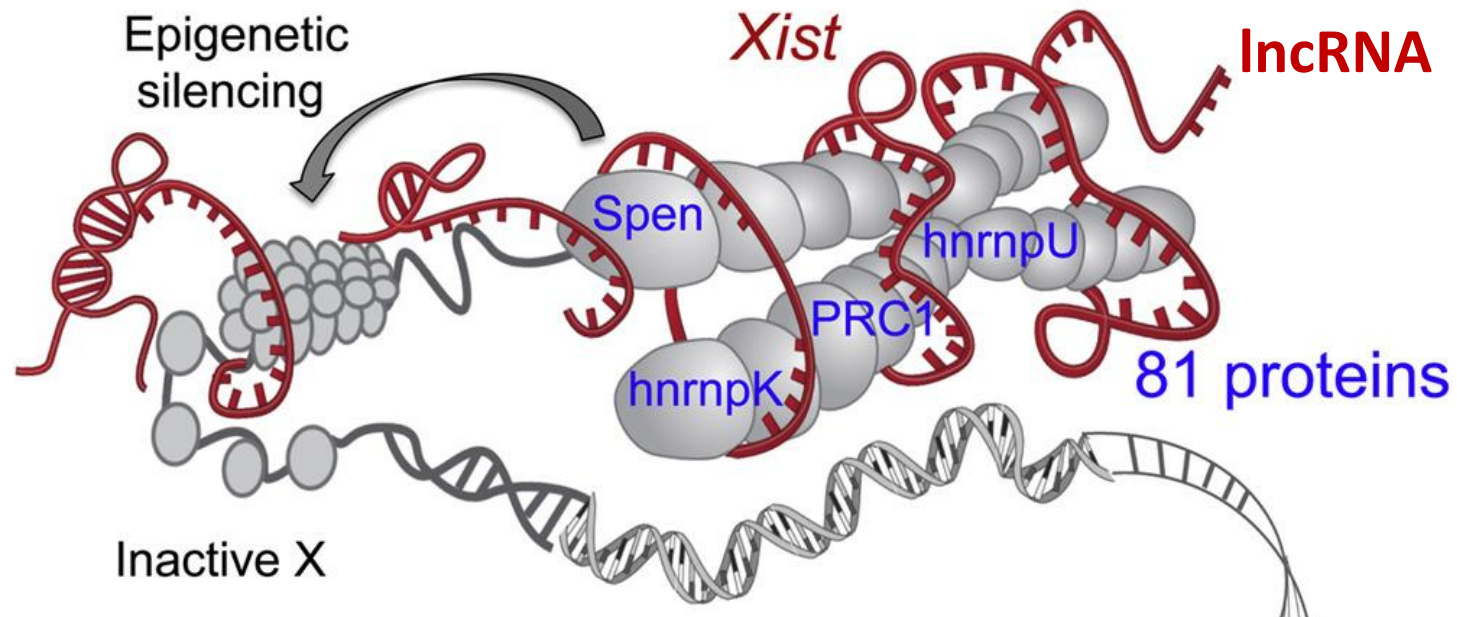
# Peri-implantation embryo



# Lyonization

## = X-Chromosome inactivation

- one of the X chromosomes in female embryos is packed into transcriptionally inactive heterochromatin
- demethylation of **X-inactivation center on X-chromosome (XIST)**
  - translation of long non-coding RNA (**lncRNA**) that coats the entire X chromosome and associates with chromatin remodelling proteins inducing **epigenetic silencing** of genes encoded on **one of the two X chromosomes in female cells**

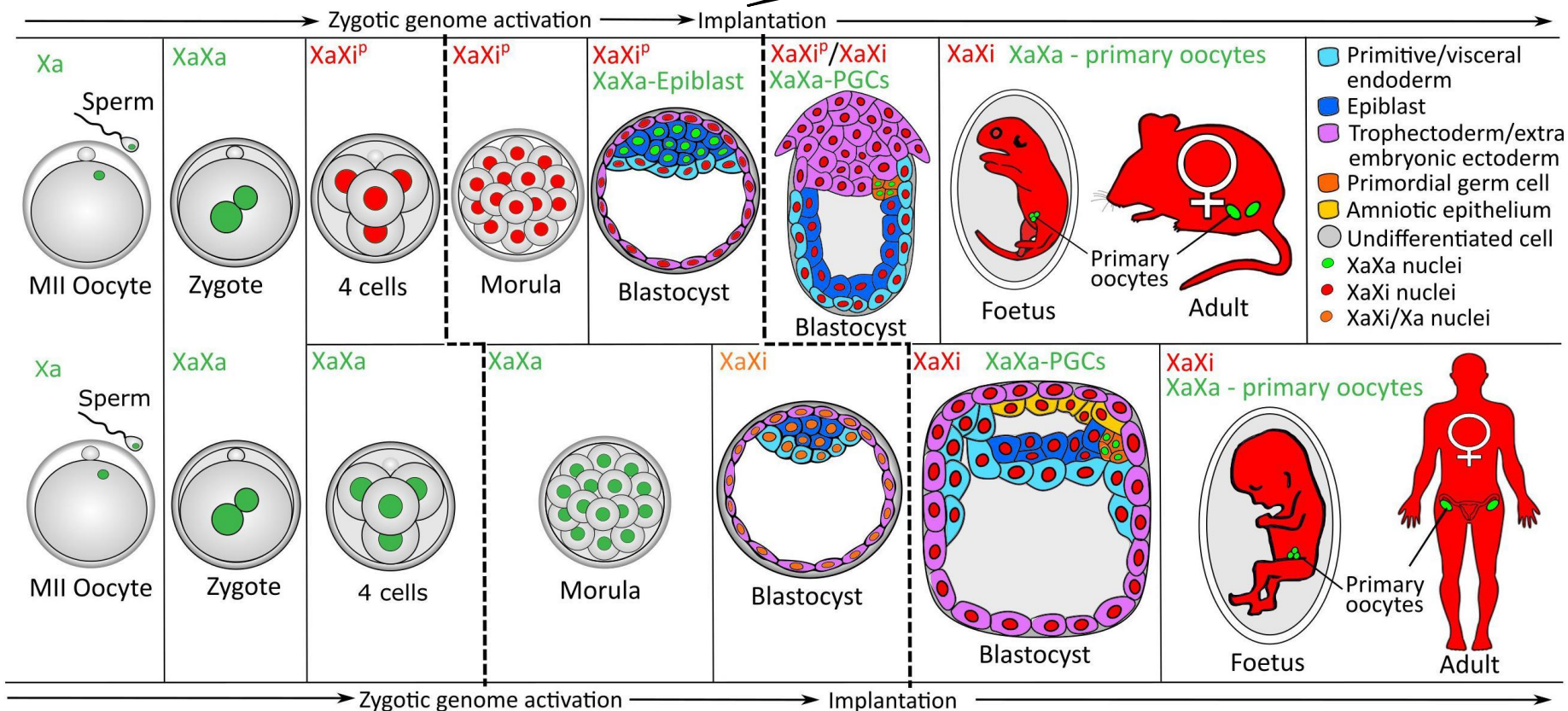


# Lyonization

## = X-Chromosome inactivation

- occurs **randomly** in peri-implantation female embryos
- in PGCs X chromosome reactivate

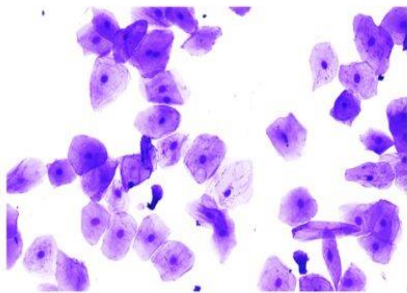
Transient reactivation in ICM of preimplantation mouse blastocyst



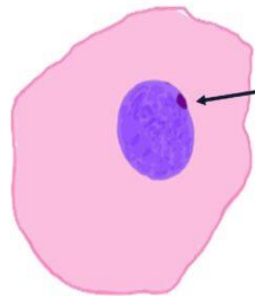
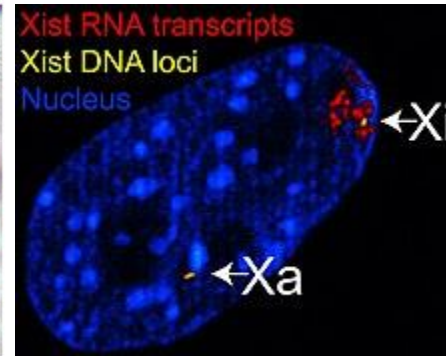
# Lyonization

## = X-Chromosome inactivation

- dosage compensation of X-coded genes between female and male cells
- influences severity of X-linked diseases in females

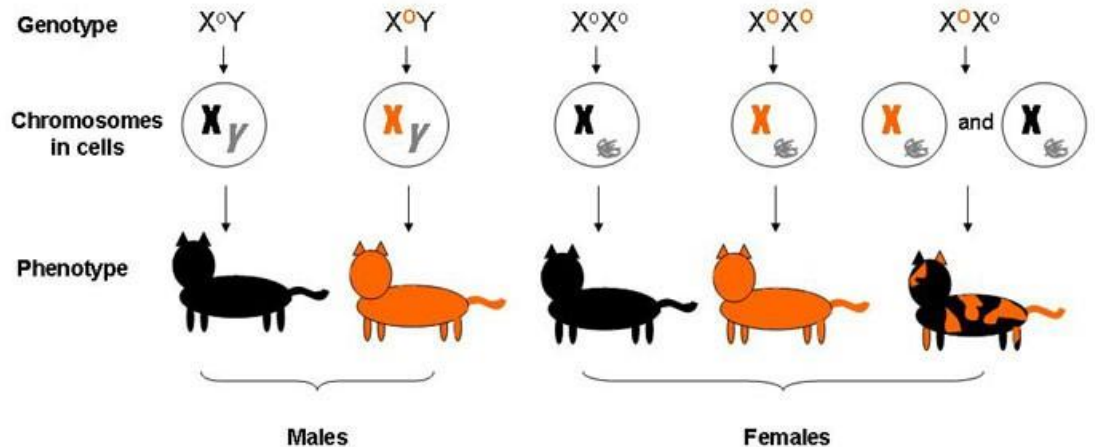


Squamous cells in the buccal / smear



Barr Body

Seen in 30% to 60% of the female somatic cells

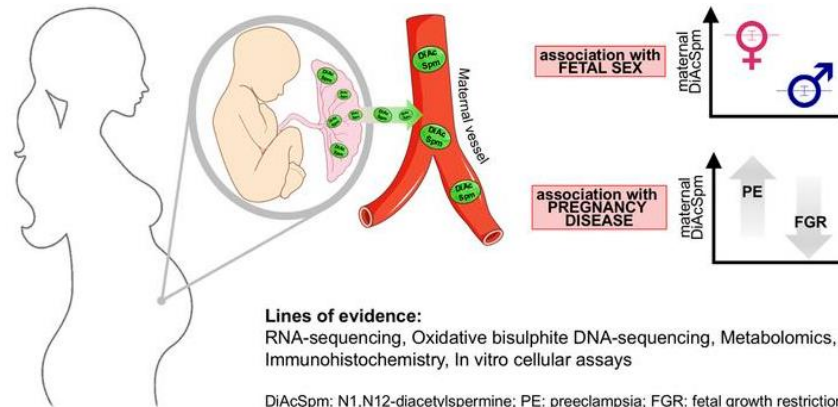




# Lyonization

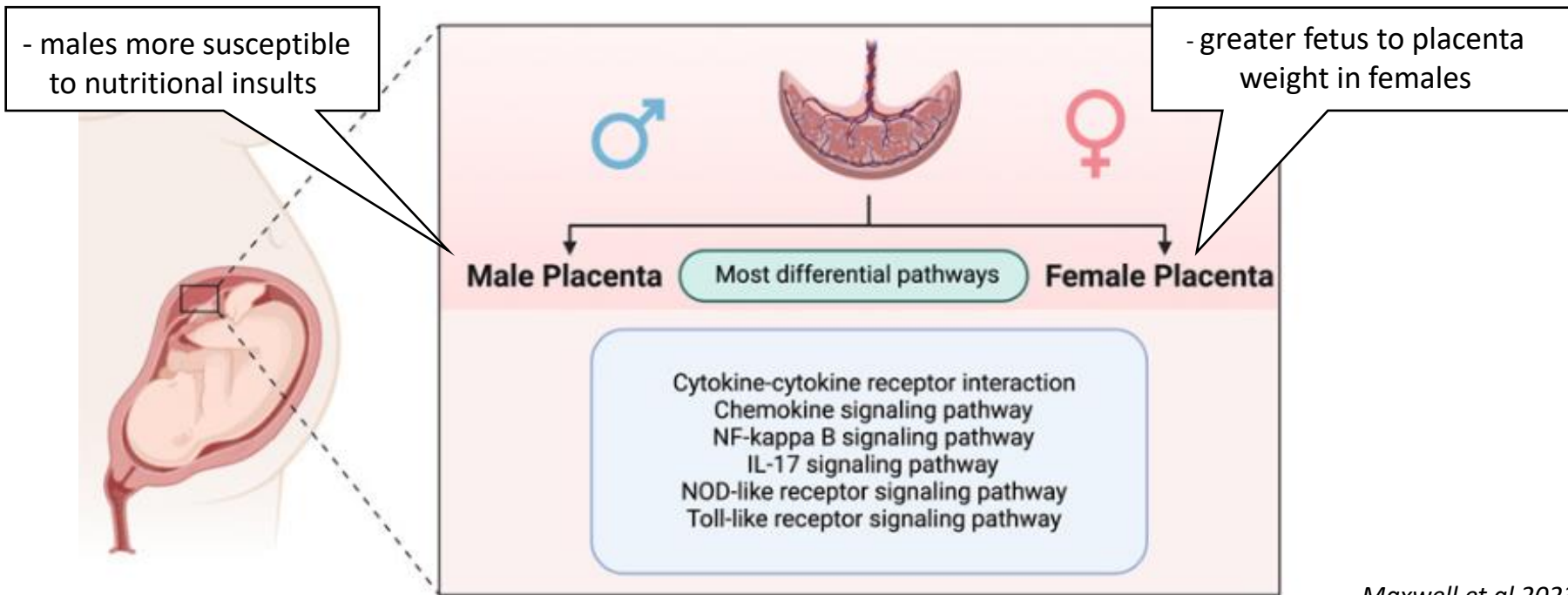
## = X-Chromosome inactivation

- some genes are known to escape X inactivation
  - increased gene expression in females relative to males
  - e.g. sex-dependent expression of SMS gene and synthesis of antioxidant spermine



→ **sexual dimorphism** in placental function

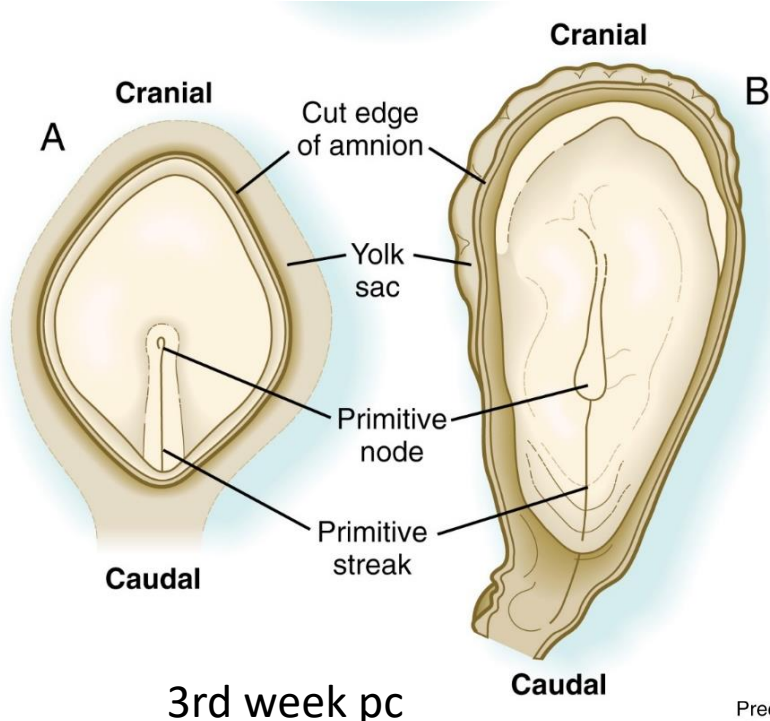
*Gong et al 2018*



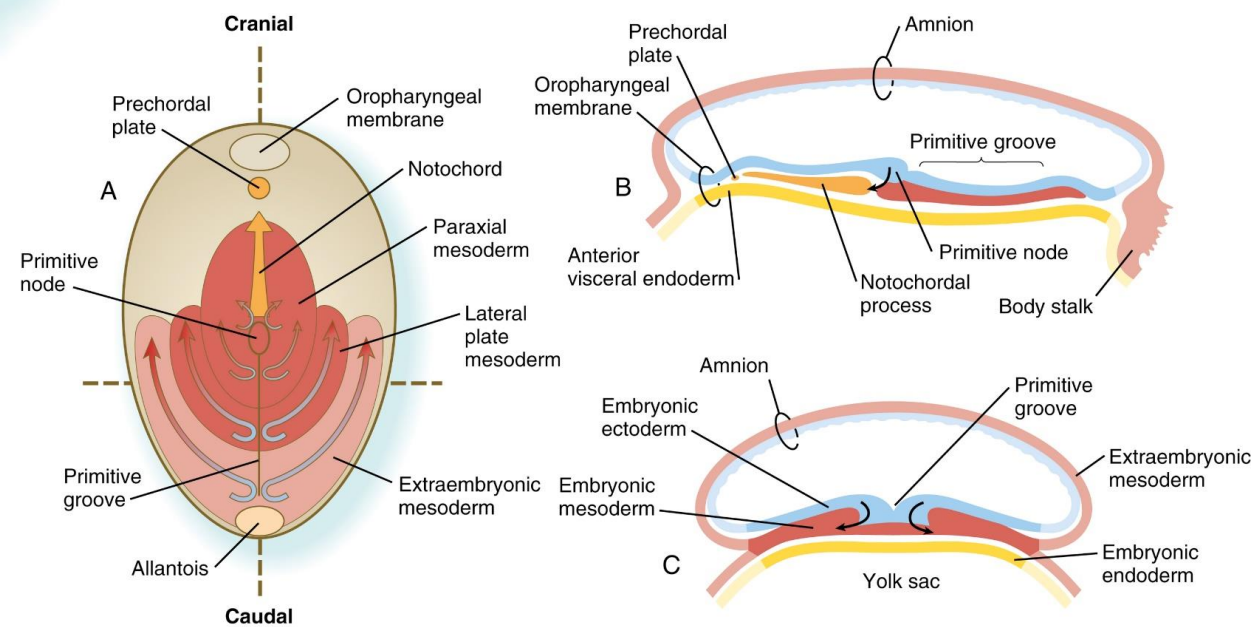
*Maxwell et al 2023*

# Gastrulation

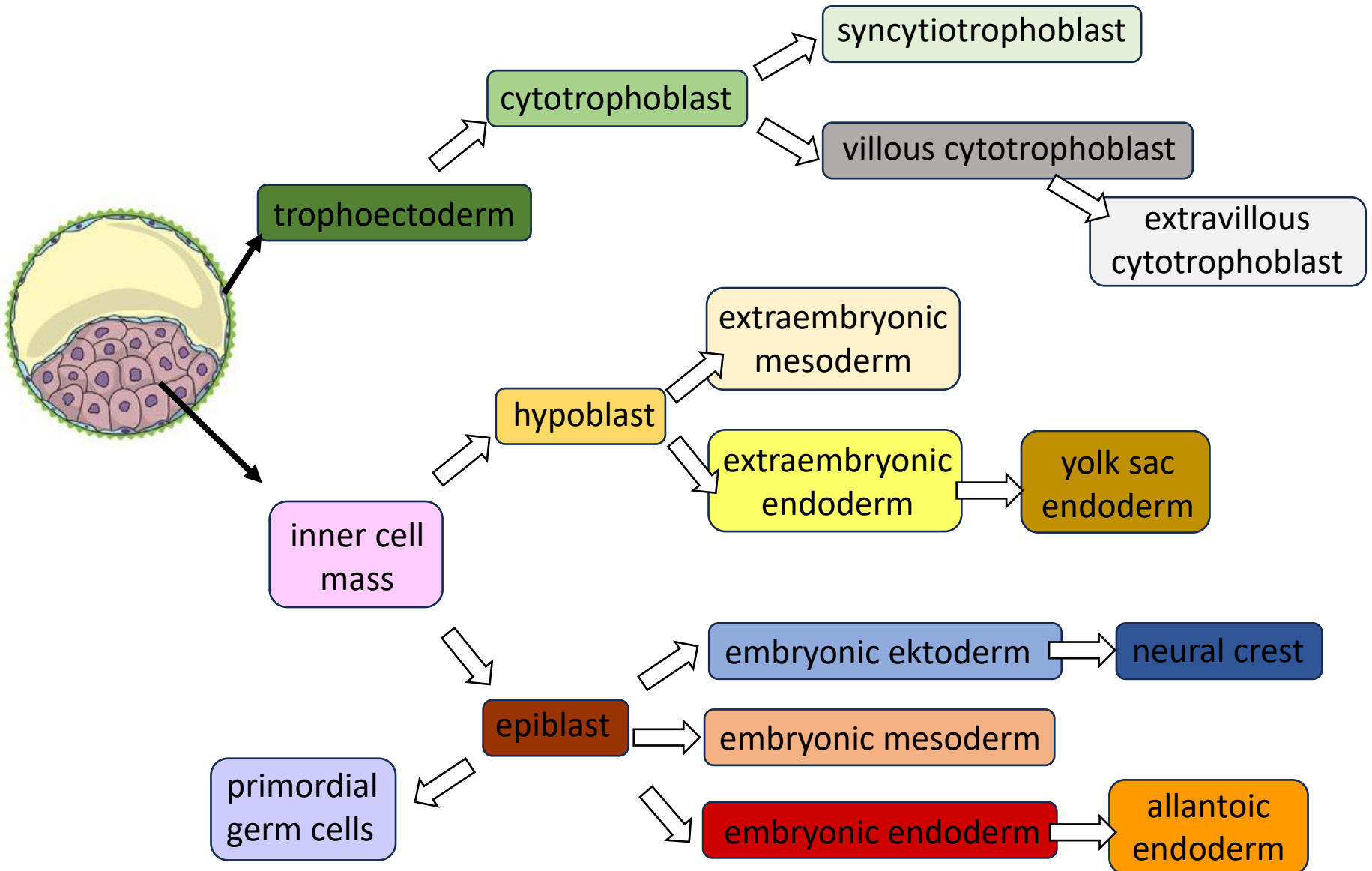
- Establishment of 3 germ layers



3rd week pc

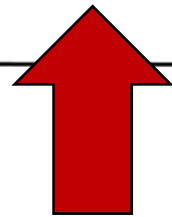
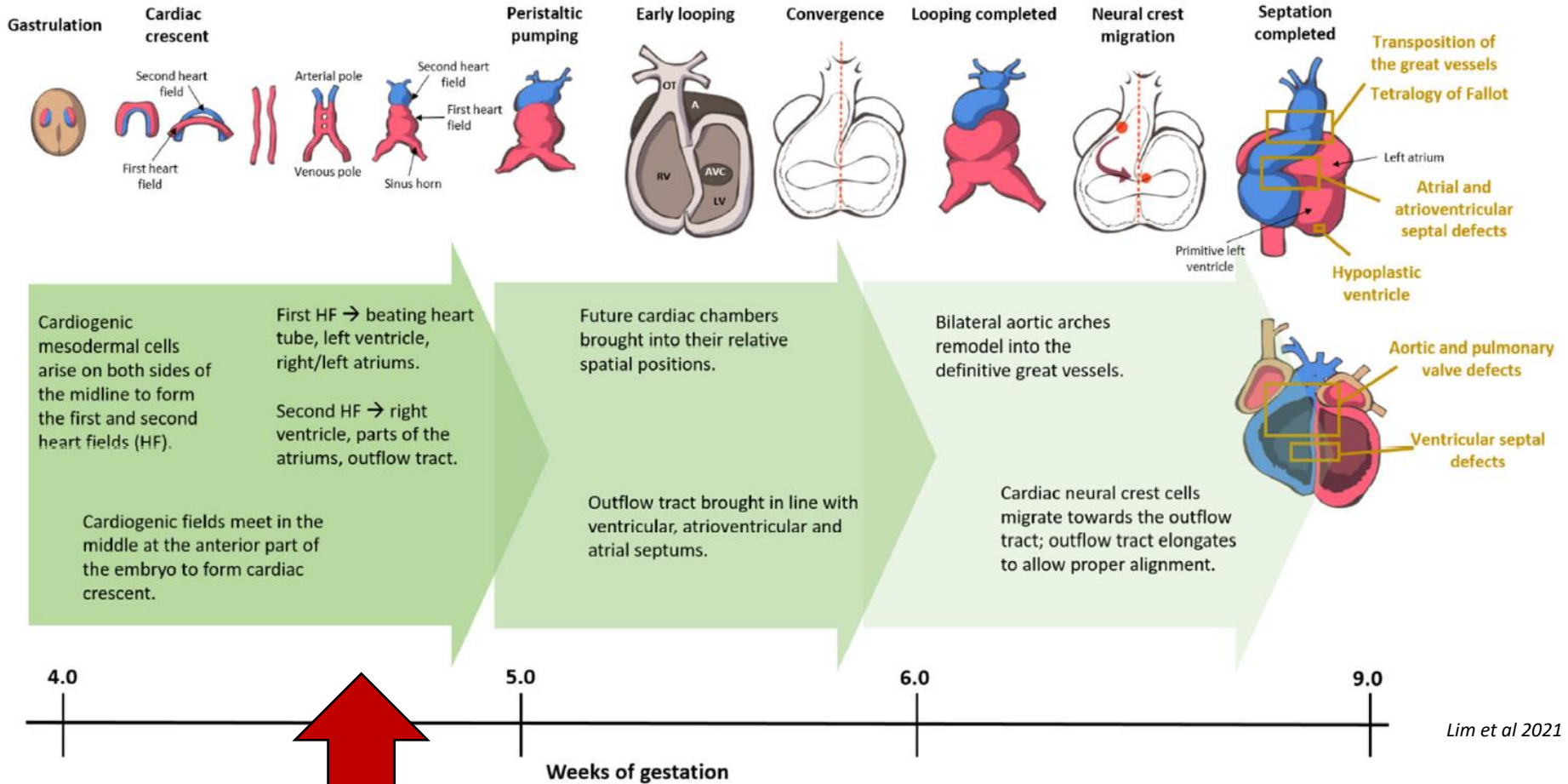


# Early derivatives

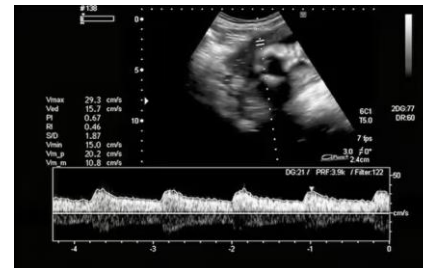




# Clinical pregnancy



„heart“ beat  
detection by ultrasound

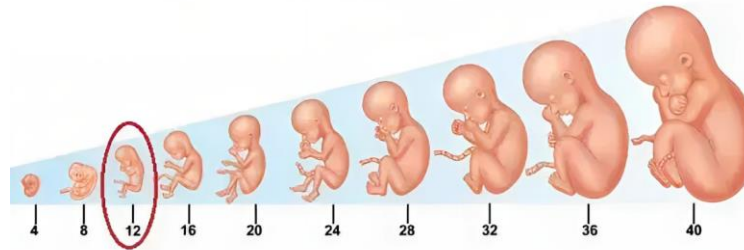


# Ongoing clinical pregnancy



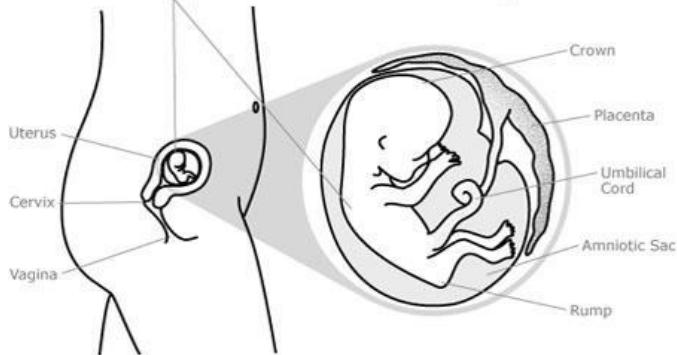
12 Weeks Pregnant

- placenta and organ systems fully formed



## 12 WEEKS AFTER LAST MENSTRUAL PERIOD

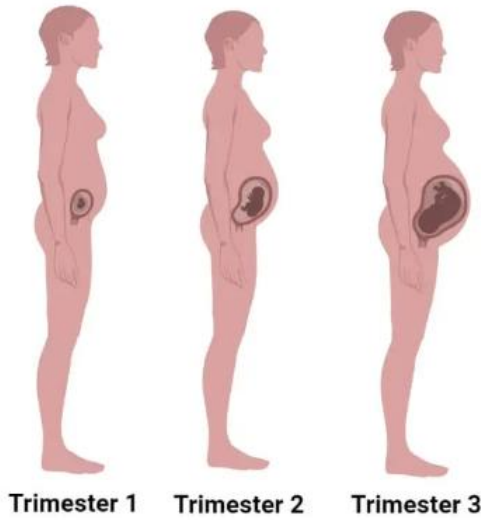
Fetus (actual size 2 – 3 inches from crown to rump)



## 12 Weeks Pregnant Symptoms

- 1 Reduced Morning Sickness
- 2 Weight gain
- 3 Food Aversions and Cravings
- 4 Urinary Incontinence
- 5 Leg or Foot Cramps
- 6 Mood Swings
- 7 Sensitivity to smell
- 8 Bloating

# Live birth



## Fetal Development



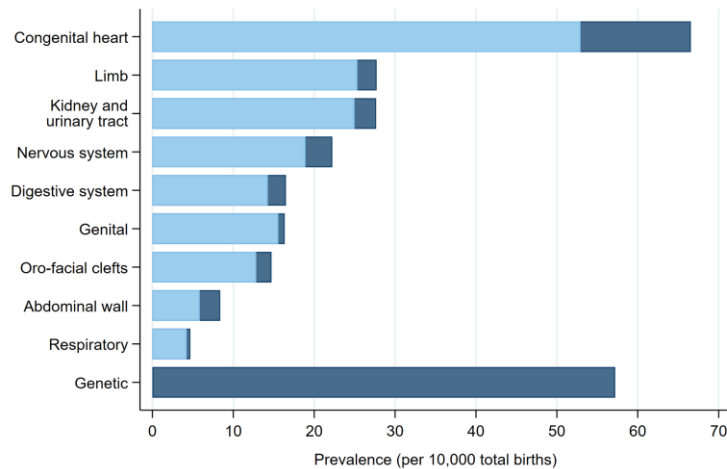
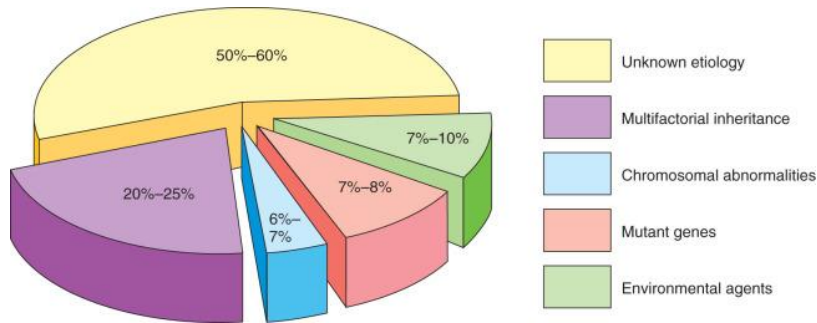
- gestational age 38-42 weeks
- ~ 50 cm, 3-3.5 kg
- eutrophic
- signs of fetal maturity

MAKE ROOM FOR BABY

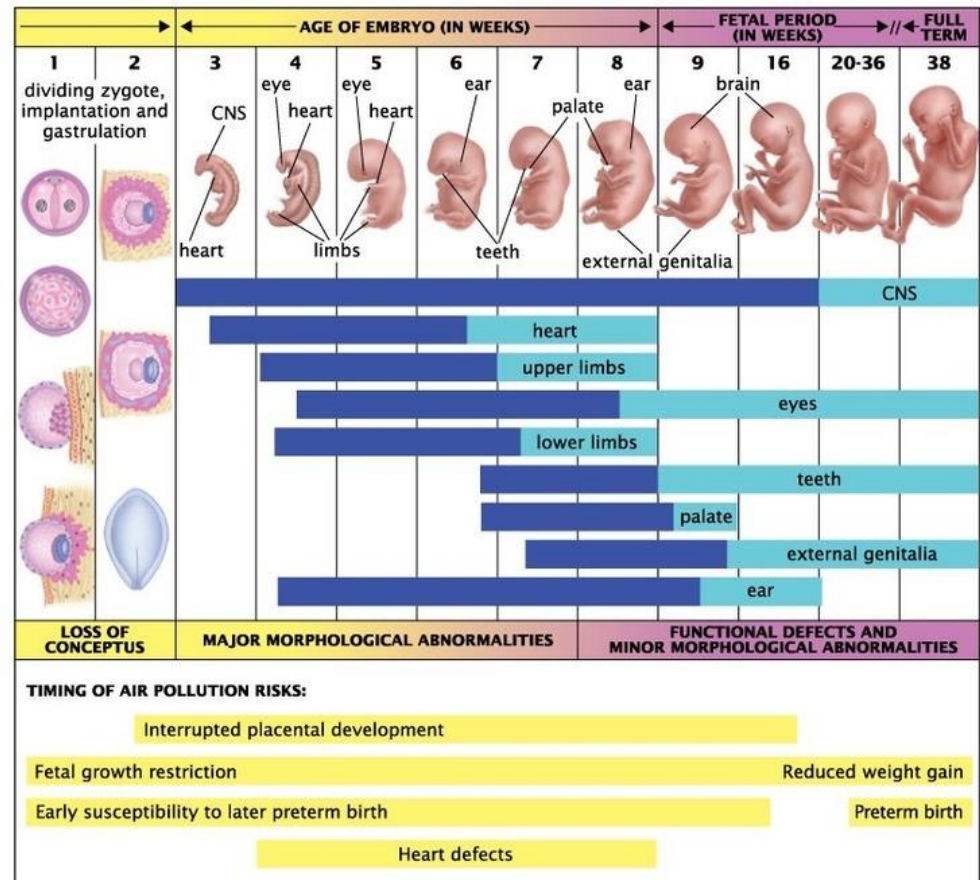


# Congenital anomalies

- major anomalies present in ~3% of liveborn infants
- induced by genetic and/or environmental factors



Legend: ■ Non-genetic ■ Genetic



### TIMING OF AIR POLLUTION RISKS:



Note: Blue bars indicate time periods when major morphological abnormalities can occur, while light blue bars correspond to periods at risk for minor abnormalities and functional defects.



# Prevention of congenital anomalies

## Estimated RISK

with respect to maternal age

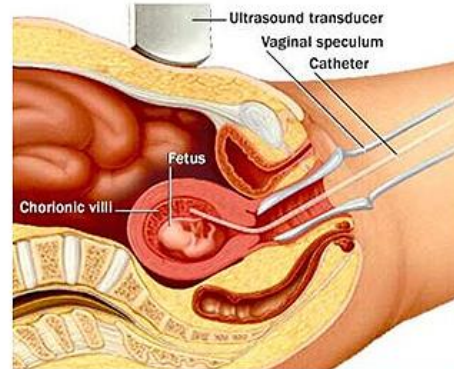
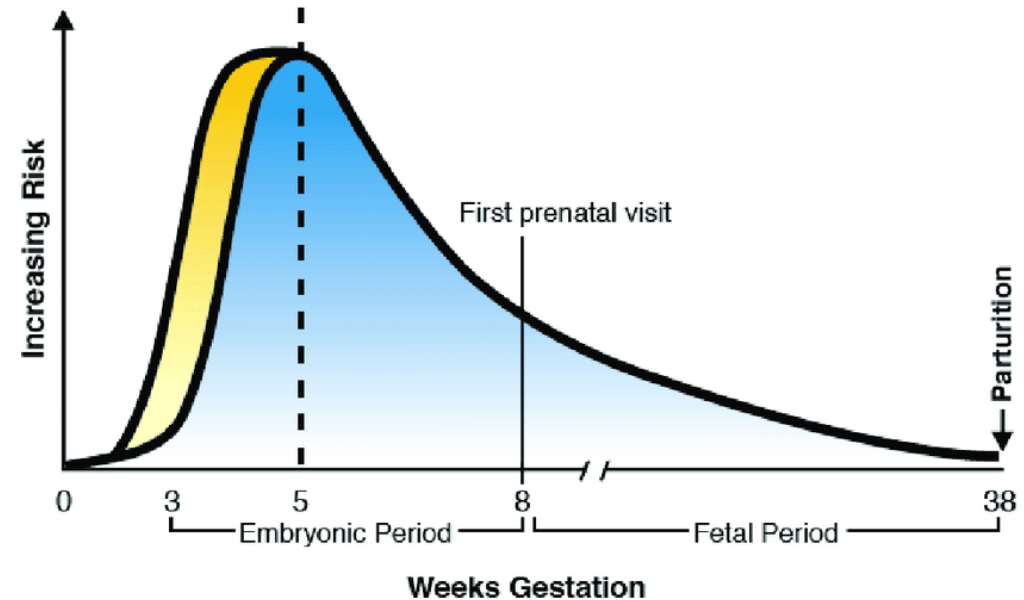
### ❑ Non-invasive diagnostic methods

- Anamnesis
- Ultrasound
- Biochemical testing
  - hCG, AFP, PAPP-A, eE3
- cfDNA

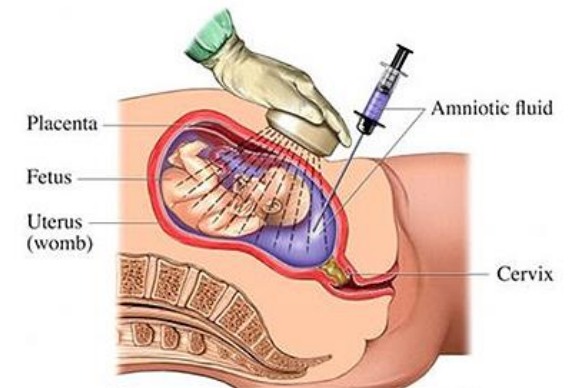
### ❑ Invasive diagnostic methods

- PGT
- Chorionic villous sampling
- Amniocentesis
- Fetal blood sampling
- Induced abortion

## Risk of Birth Defects Being Induced



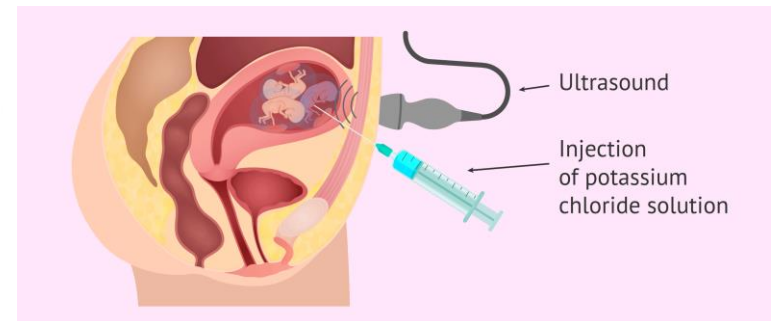
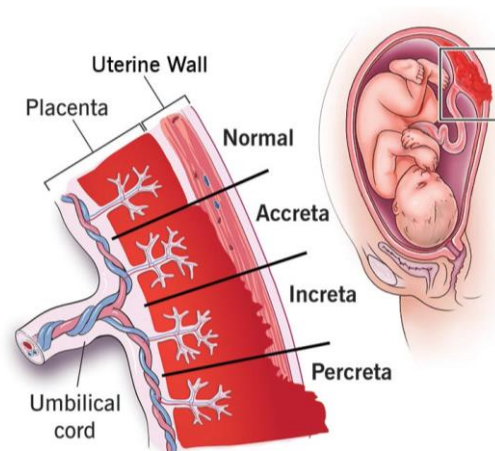
11-13 wg



15-18 wg

# Pregnancy complications linked to placenta pathology

- Pre-eclampsia
- Gestational trophoblastic diseases
- Pregnancy loss after 12 wg
  - clinical miscarriage
  - silent/missed miscarriage
- Fetal growth restriction
- Fetal macrosomia
- Preterm labour
- Still birth
- Ceasarian section
- Placenta accreta

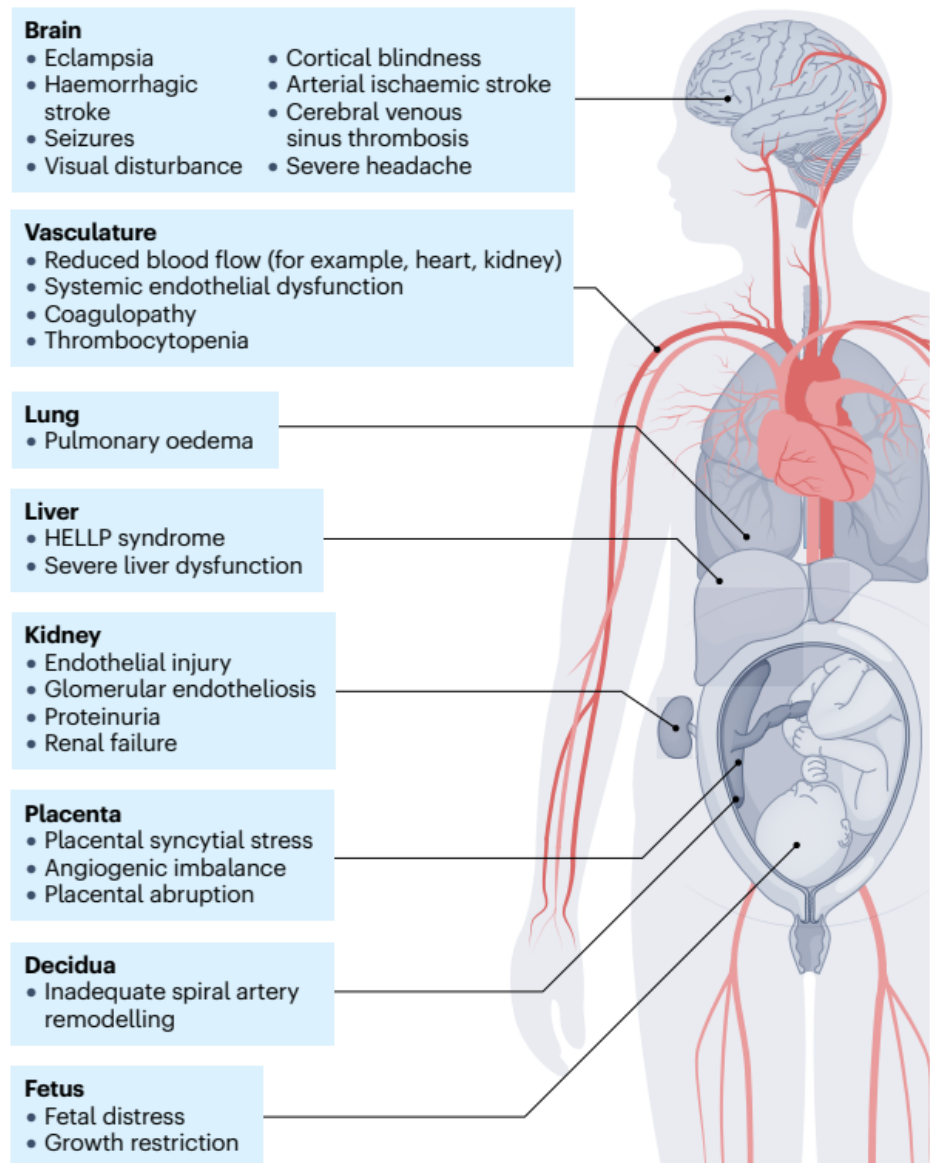


„fetoreduction“

= elective reduction of number of fetuses in high risk multifetal monochorial pregnancies

# Pre-eclampsia

- life-threatening disease of pregnancy caused by **placenta dysfunction**
- exclusive to humans, 1-5% pregnancies (20+wg)
- leading cause of maternal and neonatal morbidity
- survivors have long-term complications and high risk of later development of metabolic and cardiovascular diseases
- complex multisystem disease
- manifested by
  - sudden-onset hypertension
  - proteinuria
  - edema
- complications
  - **HELLP syndrome** = **H**emolysis , **E**levated **L**iver enzymes and **L**ow **P**latelet count
  - **Eclampsia** - seizures and coma
  - Haemorrhagic stroke
  - Placental abruption
  - Renal failure
- **preterm pre-eclampsia with early onset** < 34 wg
- **preterm pre-eclampsia with late onset** ≥ 34 wg
- **term pre-eclampsia** ≥ 37 wg
- **post-partum pre-eclampsia** - diagnosed after delivery



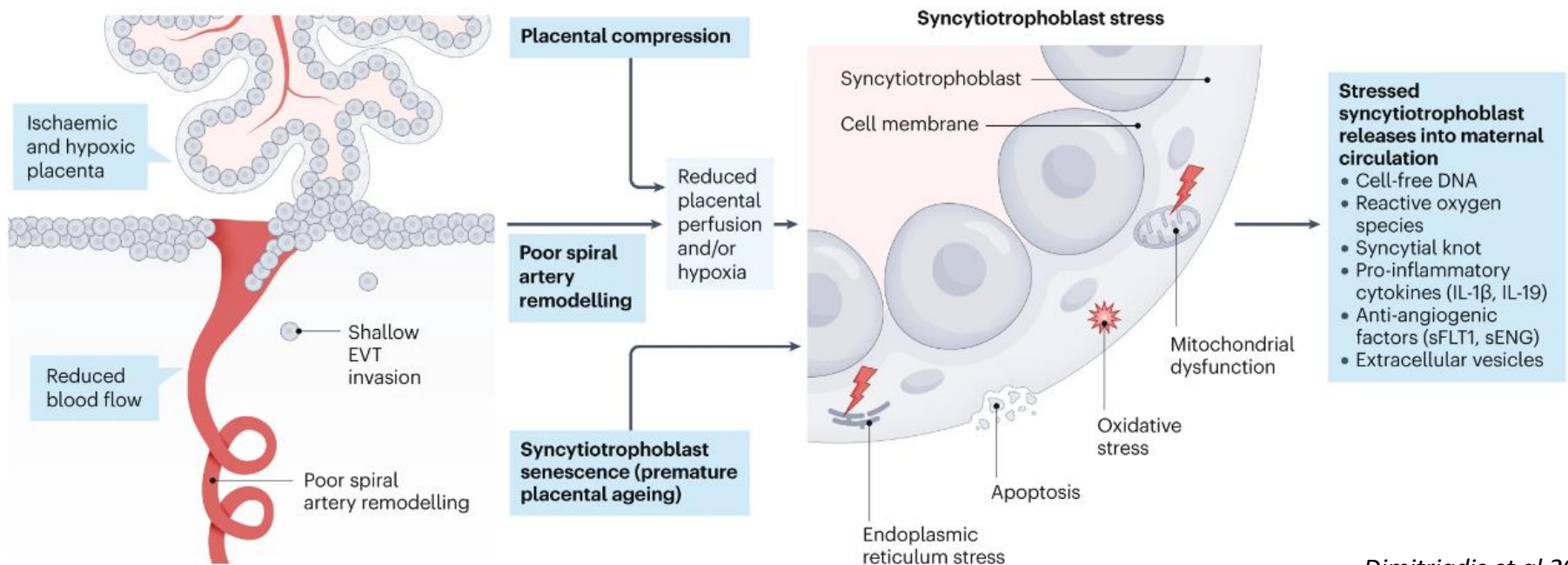
# Pre-eclampsia

- **caused by placenta dysfunction** caused by defective or insufficient (“shallow”) placentation

## ❖ Proposed mechanisms:

- ? poor invasion and/or premature senescence of the trophoblast
- ? STB stress
- ? inadequate spiral artery remodelling
- ? incomplete plugging and premature onset of blood perfusion
- ? immune imbalance at maternal-fetal interface
- ? excessive blood clotting

placental malperfusion (ischemia and reperfusion injury),  
disregulated release of placental factors, angiogenic imbalance, maternal endothelia dysfunction, reduced vasodilatation and systemic inflammation

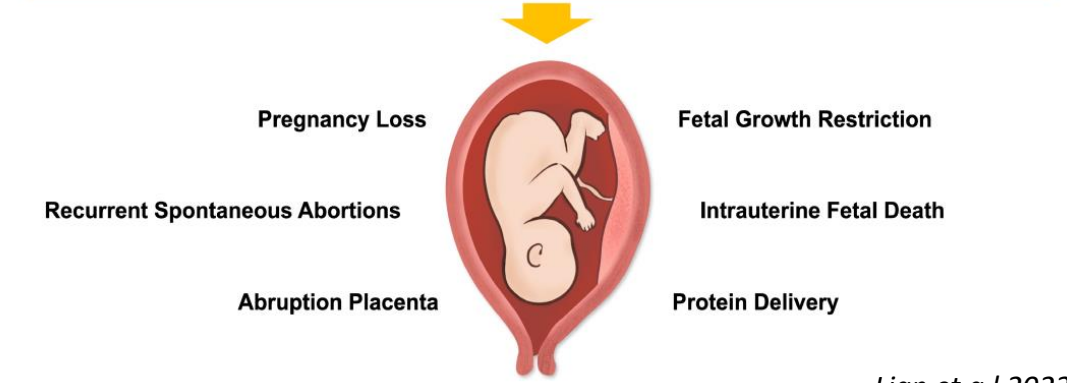
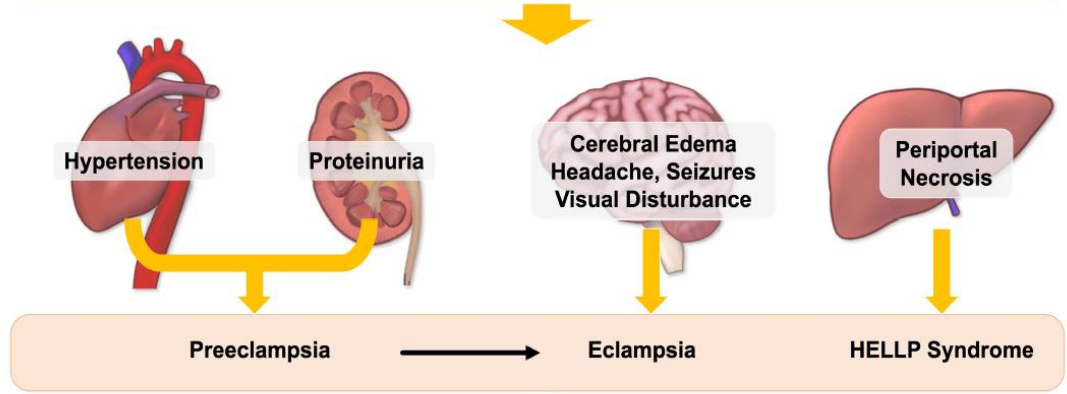


# Pre-eclampsia

Exact etiology?



Role of miRNAs



- Major Risk Factors**
- Obesity
  - Gestational diabetes mellitus
  - Multifetal gestation
  - Antiphospholipid syndrome
  - Kidney disorders
  - Chronic hypertension
  - Anemia.
  - High pre-pregnancy BMI

- Other risk factors**
- Systemic lupus erythematosus
  - Prepregnancy BMI >25
  - Nulliparity
  - Chronic kidney disease
  - Genetic susceptibility (mother, father)
  - Trisomy 13 fetus

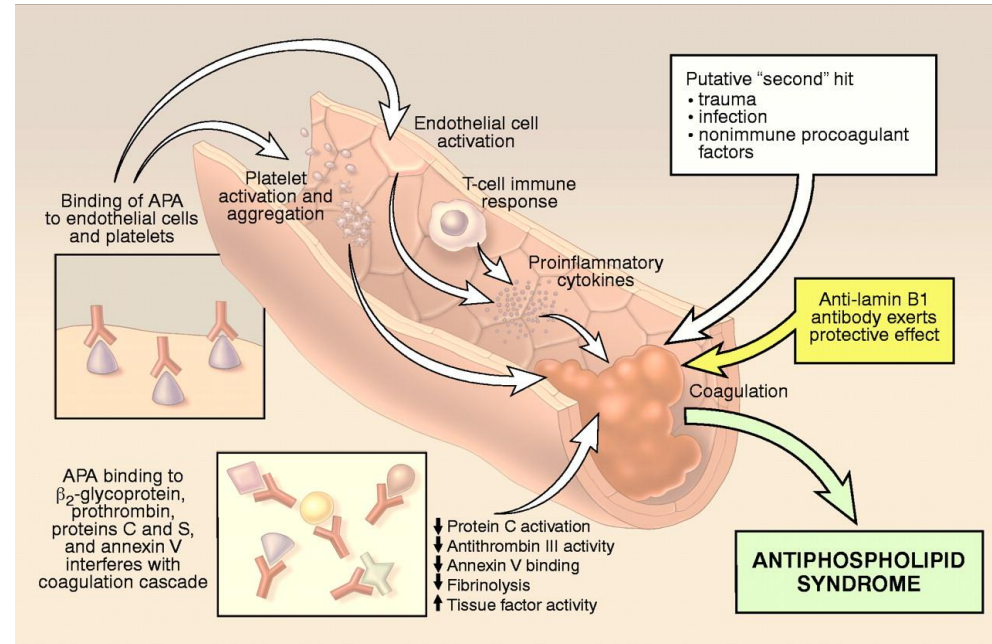
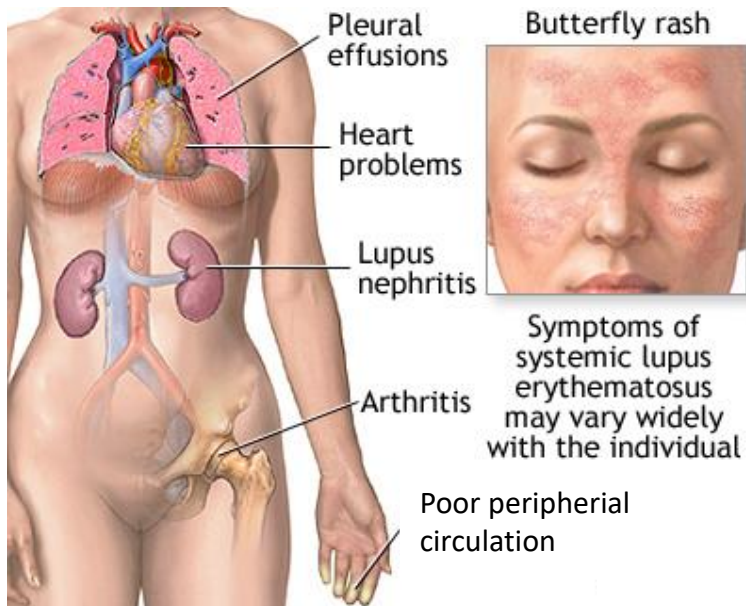
- Minor Risk Factors**
- Advanced maternal age
  - Stillbirth history
  - Nulliparity
  - Placental abruption,
  - Atherosclerosis,
  - Use of assisted reproductive technologies (ART)
  - Family history of preeclampsia



# Pre-eclampsia

## ❖ Antiphospholipid syndrome (APS)

- autoimmune disorder associated with pregnancy complication including pre-eclampsia
- caused by antiphospholipid antibodies (APA) raising risk of blood clotting

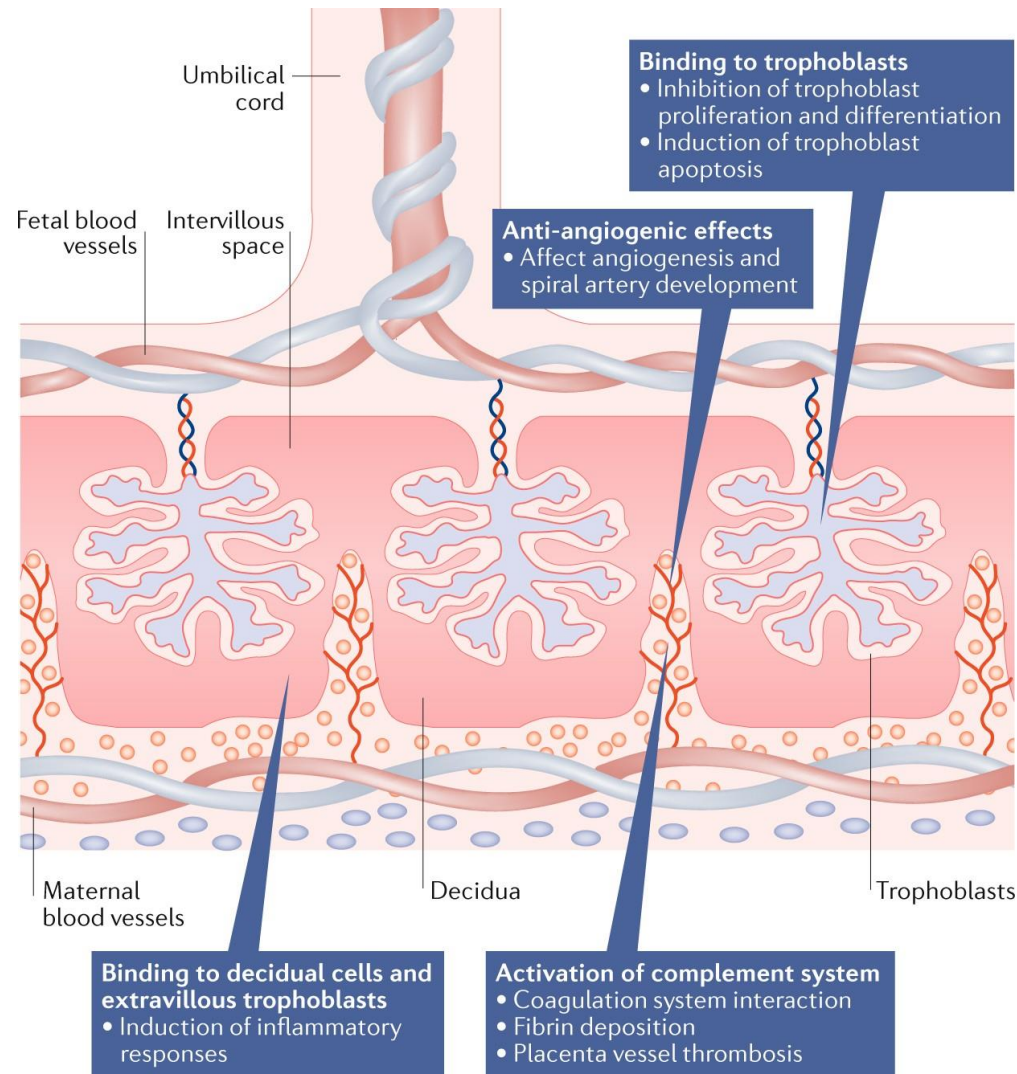


## ❖ Systemic Lupus Erythematosus (SLE)

- autoimmune disease in which immune system mistakenly attacks nuclear and cytoplasmic antigens in the body
- multisystem inflammation and increased risk of infertility and pregnancy complication including preeclampsia

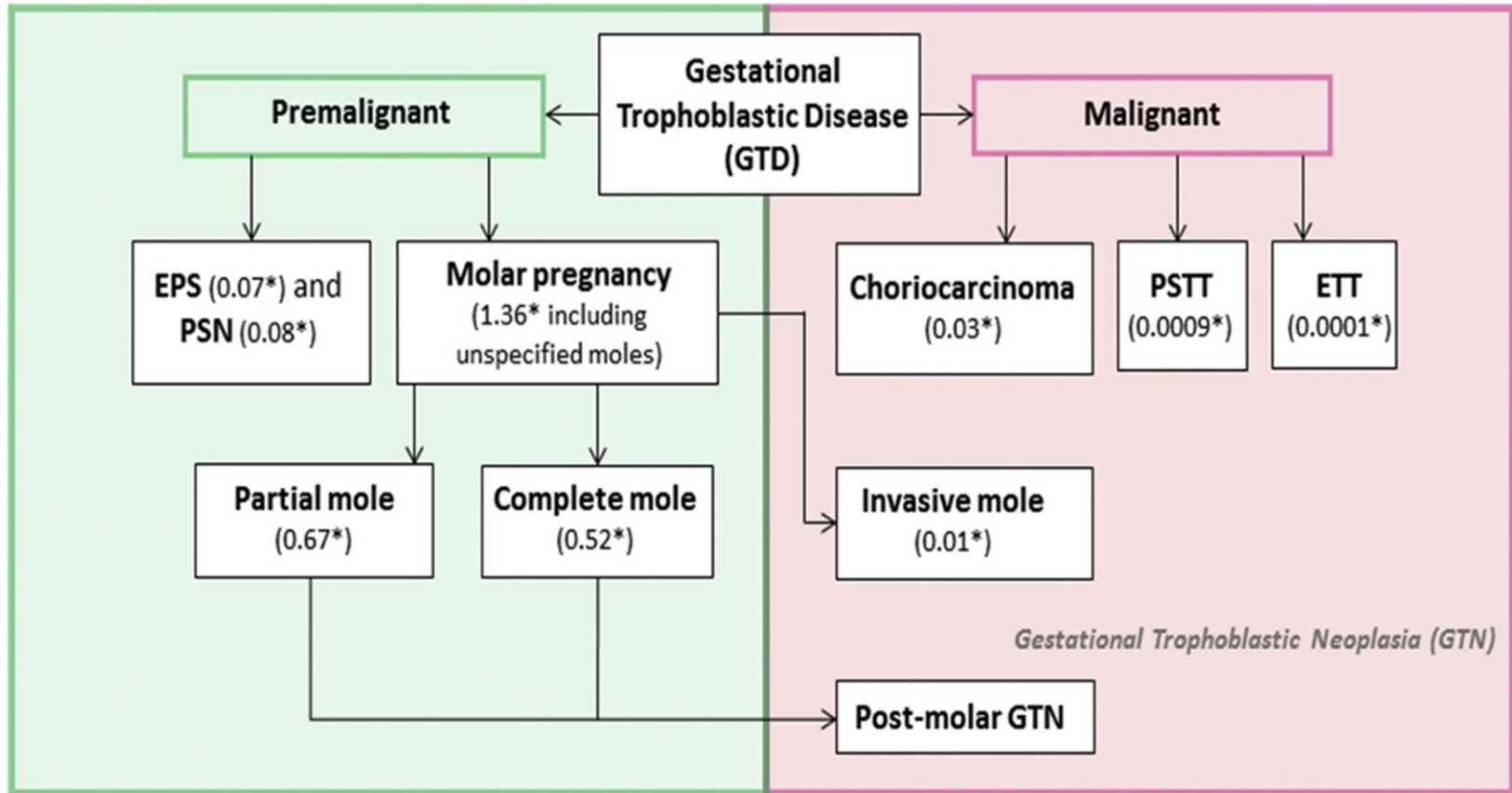
# Pre-eclampsia

- autoantibody effect on fetal maternal interface



# Gestation Trophoblastic diseases

- heterogenous group of pregnancy-related disorders arising from **abnormal proliferation of placental trophoblast**



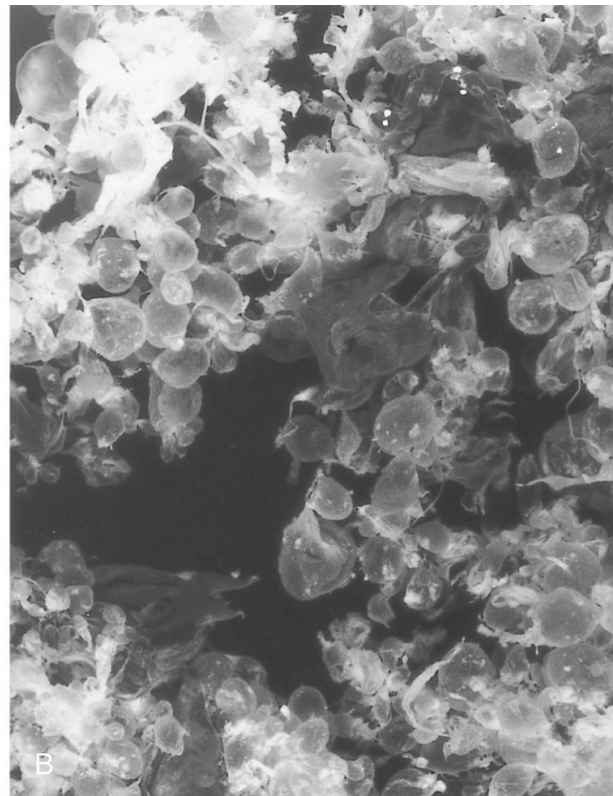
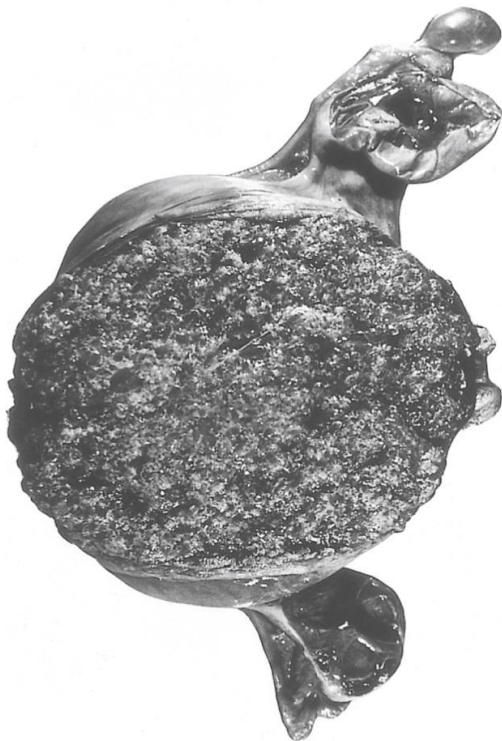
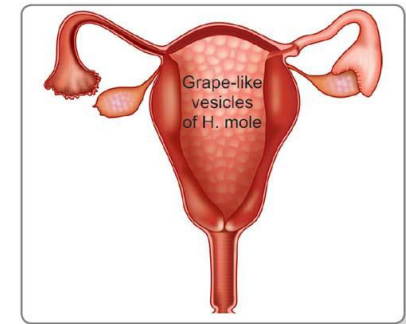
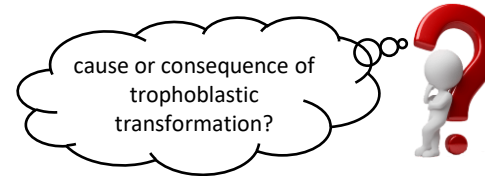
EPS, exaggerated placental site; ETT, epithelial trophoblastic tumour; PSN, placental site nodule; PSTT, placental-site trophoblastic tumour. \* Incidence rates per 1000 deliveries per year in the Netherlands between 1994-2013 (Figure drafted by M Frijstein based on Eysbouts et al. Gynecol Oncol. 2016)



# Molar pregnancy

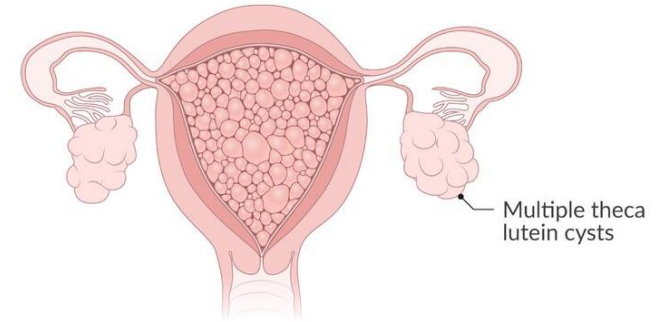
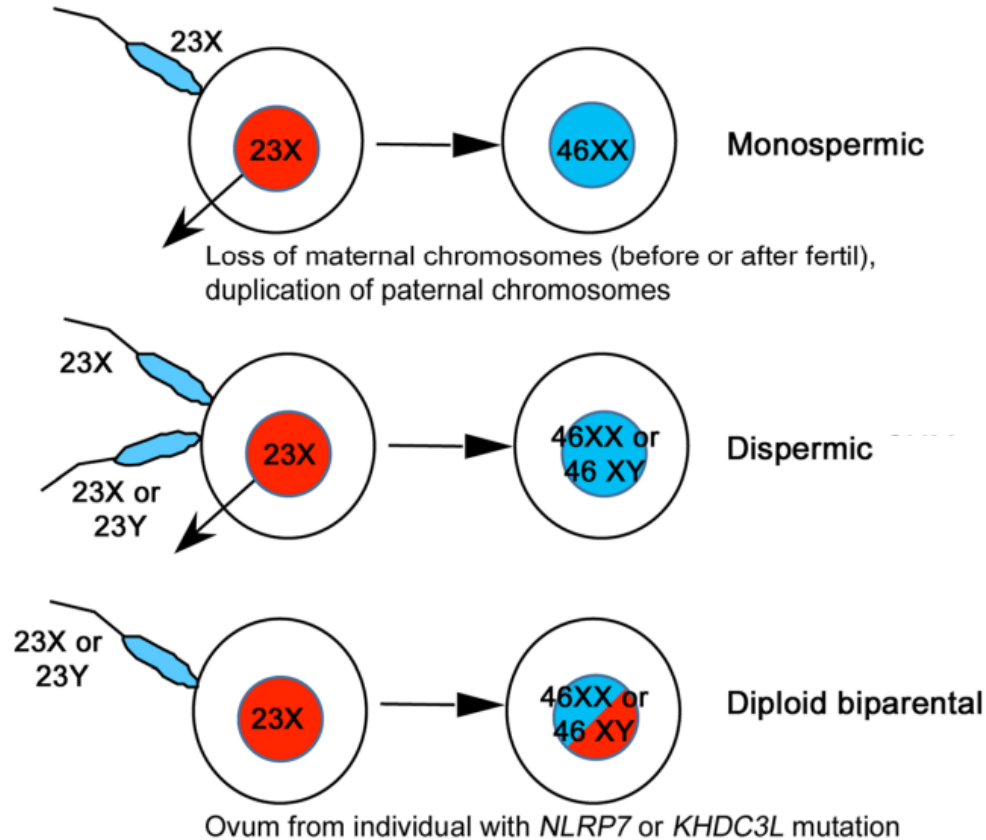
## = Hydatidiform mole (*mola hydatidosa*)

- overproliferation and dysmorphysim of chorionic villi
- vascular obliteration and nodular swellings
- embryo is absent or non-viable

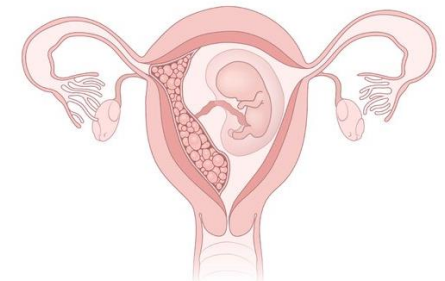
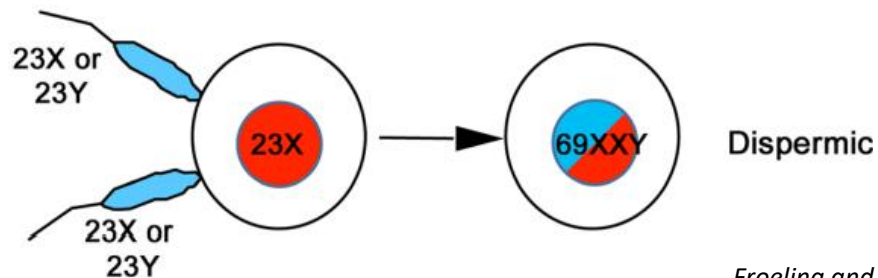


# Molar pregnancy

## Complete Mole

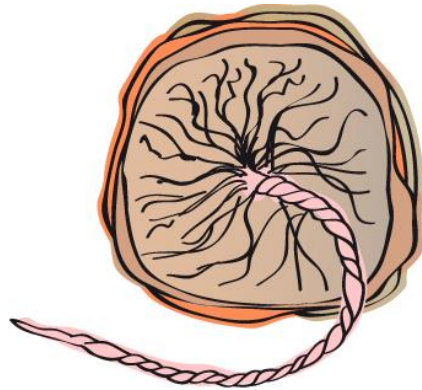


## Partial Mole



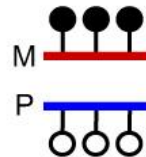
# Molar pregnancy

- imbalance of genomic imprinting, dominance of growth-promoting paternal genes

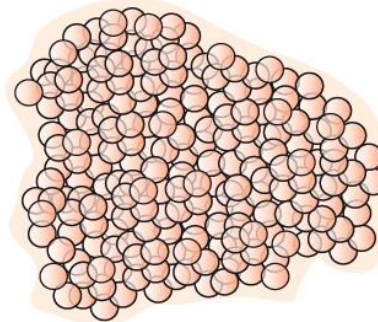
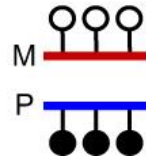


Control Placenta

Oocyte-derived methylation

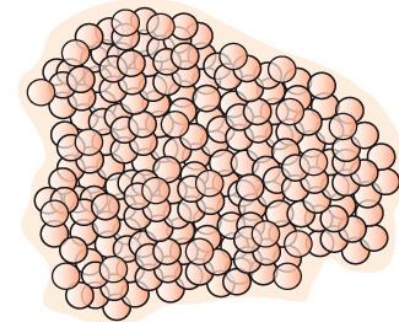
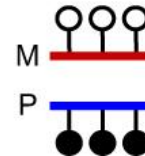
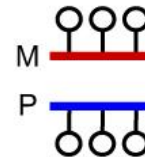


Sperm-derived methylation

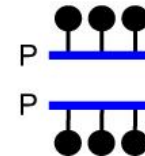
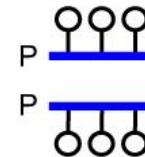


KHDC3L-mutated  
or

*NLRP7*-mutated  
molar tissue



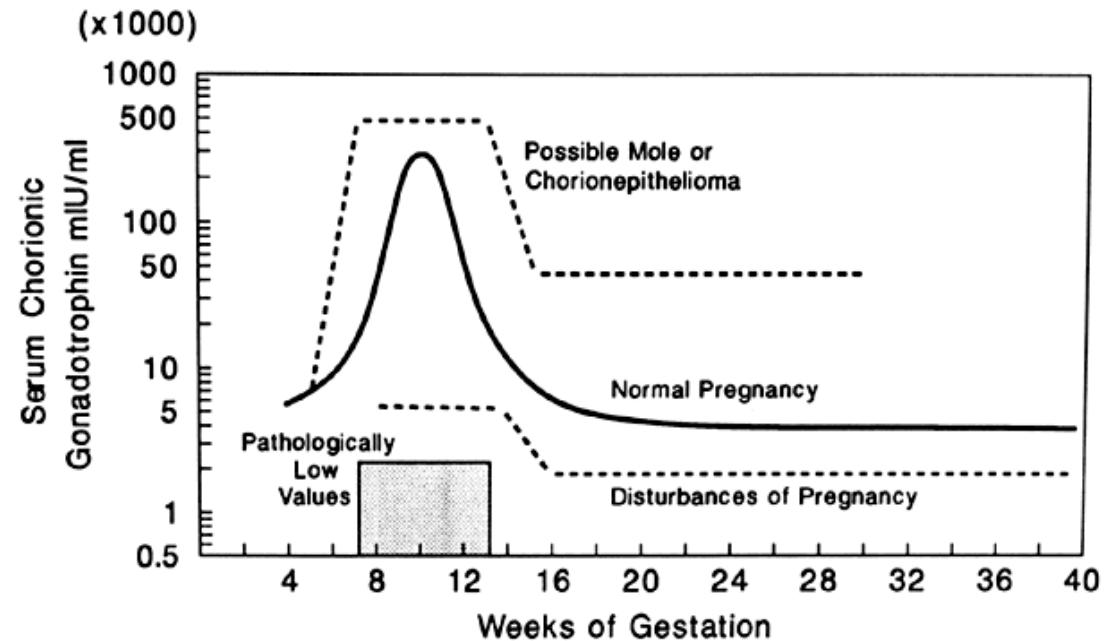
Androgenetic derived  
molar tissue



# Molar pregnancy

## ➤ Clinical manifestation:

- Vaginal bleeding
- Uterus enlargement
- Extremely elevated hCG
- Hyperemesis Gravidarum
- Theca lutein cyst
- Hyperthyreoidism
- Tachycardia
- High blood pressure



**RISK of developing post molar tumor !**



Curettage



Hysterectomy



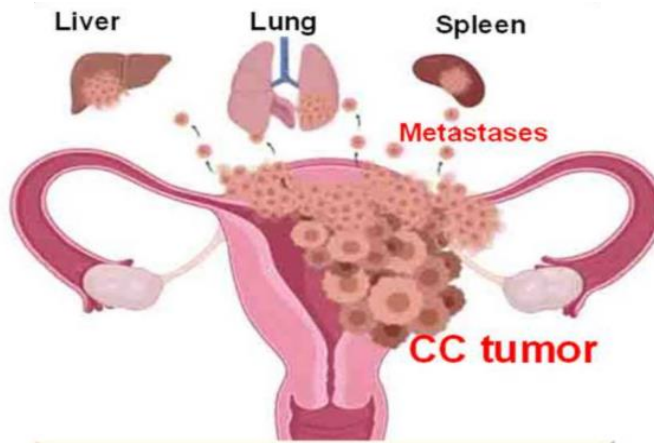
Control of hCG hormone



Chemotherapy

# Choriocarcinoma

- **Malignant trophoblastic cancer**
- consists of CTB and SCT cells at the absence of chorionic villi
- Elevated production of hCG
- Metastasis spreading typically to lungs

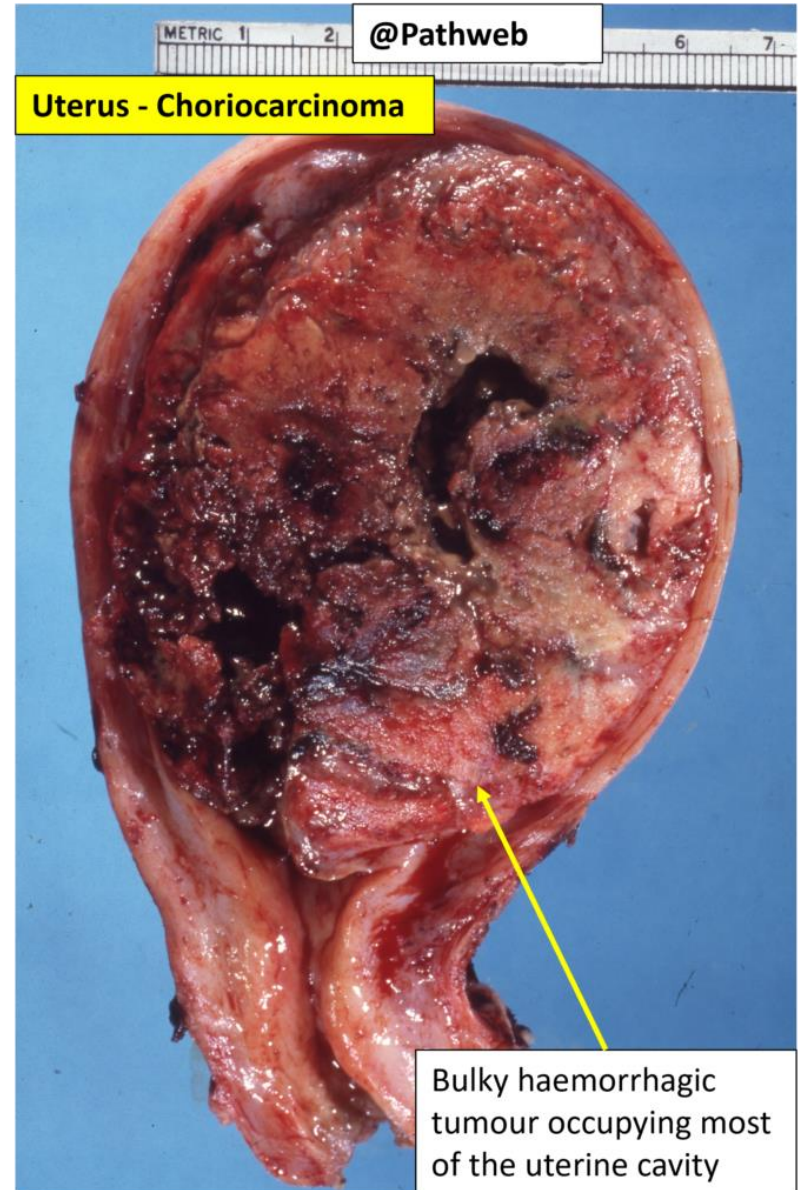


Preceded by

- ← molar pregnancy
- ← abortion
- ← normal gestation
- ← ectopic pregnancy
- ← de novo

= gestational  
choriocarcinoma

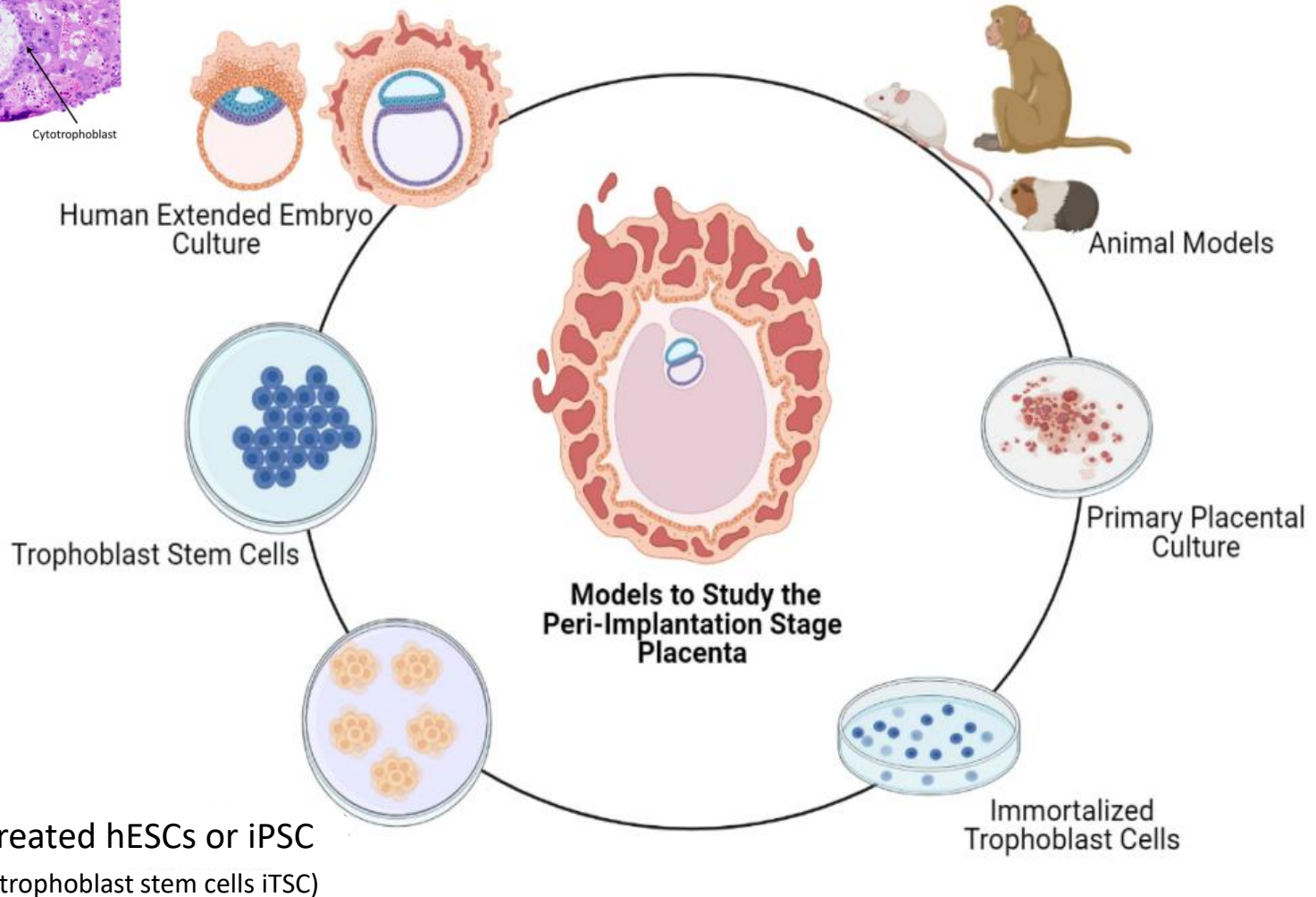
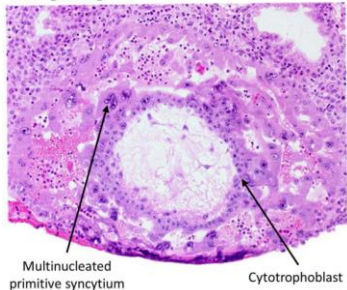
= germ cell tumour



# Research of peri-implantation development

## Fixed hysterectomy samples

Carnegie Stage 5c

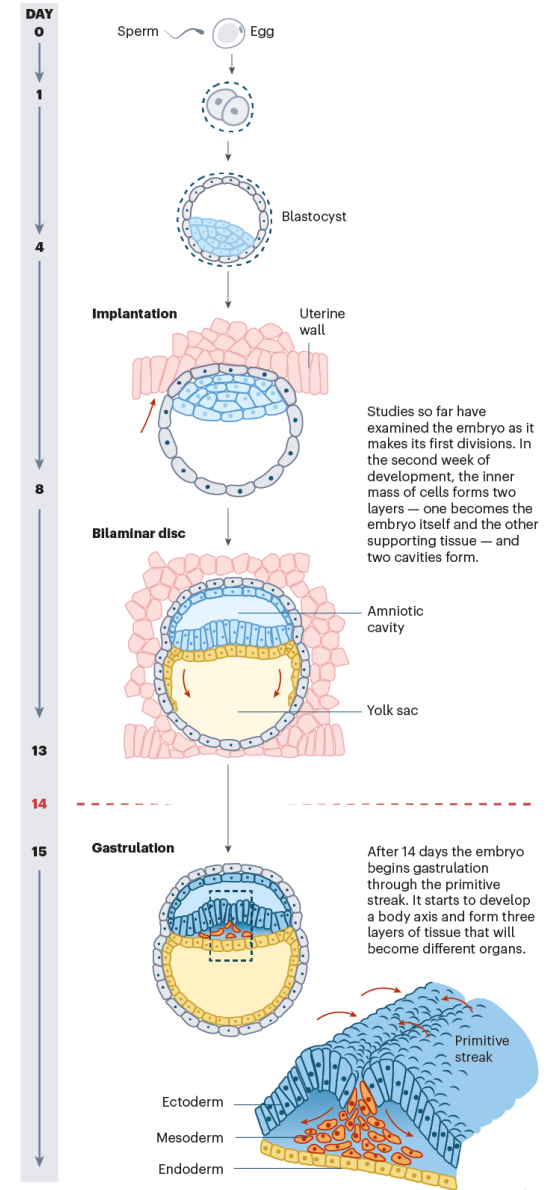


# Research of peri-implantation development

## „14 day rule“

1979 - Ethics Advisory Board of the US Department of Health, Education and Welfare

- embryos shall not be kept alive in vitro longer than 14 days after fertilisation or the stage of development that is equivalent to when embryos finish implantation



# Research of peri-implantation development

## „14 day rule“

- introduction of culture system capable to support embryonic development past implantation stage



Magdalena Zernicka-Goetz

## NATURE PROTOCOLS

D8.5 pc



Bedzhov et 2014

nature cell biology

D13 pc



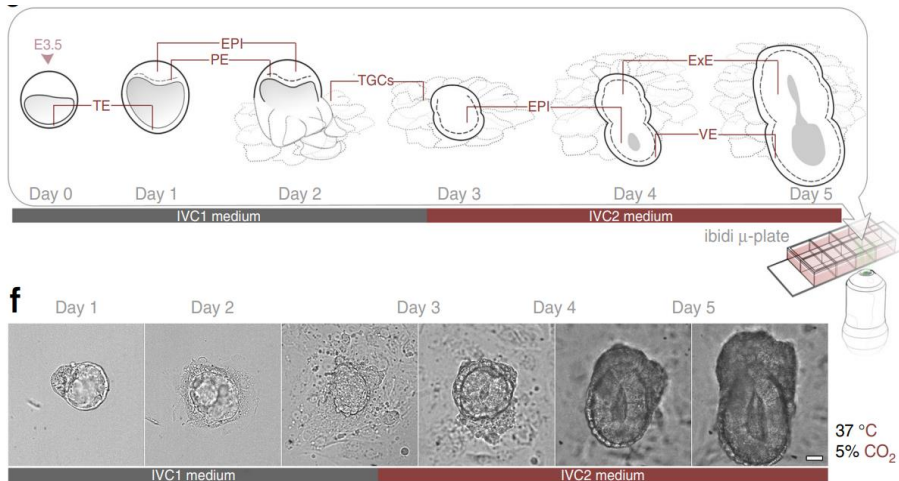
Shahbazi et 2016

### PROTOCOL

## In vitro culture of mouse blastocysts beyond the implantation stages

Ivan Bedzhov<sup>1-3</sup>, Chuen Yan Leung<sup>1-3</sup>, Monika Bialecka<sup>1-3</sup> & Magdalena Zernicka-Goetz<sup>1,2</sup>

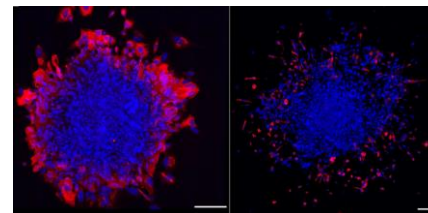
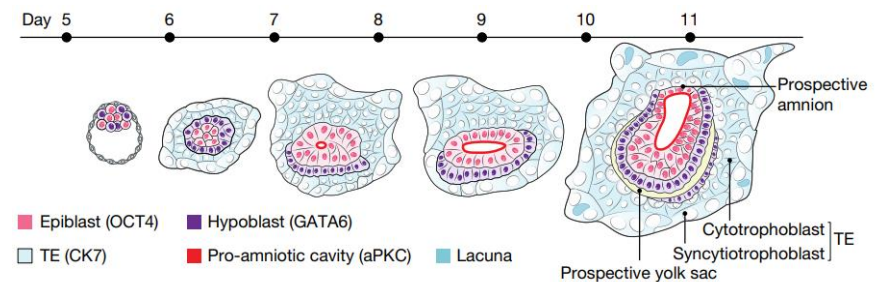
<sup>1</sup>Department of Physiology, Development and Neuroscience, Downing Site, University of Cambridge, Cambridge, UK. <sup>2</sup>Wellcome Trust/Cancer Research UK Gurdon Institute, University of Cambridge, Cambridge, UK. <sup>3</sup>These authors contributed equally to this work. Correspondence should be addressed to M.Z.-G. (mz205@cam.ac.uk).



- ECM coated dishes, no endometrium!

Self-organization of the human embryo in the absence of maternal tissues

Marta N. Shahbazi<sup>1,5</sup>, Agnieszka Jedrusik<sup>1,5</sup>, Sanna Vuoristo<sup>1,5</sup>, Gaelle Recher<sup>1,6</sup>, Anna Hupalowska<sup>1</sup>, Virginia Bolton<sup>2</sup>, Norah M. E. Fogarty<sup>3</sup>, Alison Campbell<sup>4</sup>, Liani G. Devito<sup>2</sup>, Dusko Ilic<sup>2</sup>, Yakoub Khalaf<sup>2</sup>, Kathy K. Niakan<sup>3</sup>, Simon Fishel<sup>4</sup> and Magdalena Zernicka-Goetz<sup>1,7</sup>



CGB, HLA-G

- recapitulation of key features of peri-implantation embryo development



# Research of peri-implantation development

## „14 day rule“

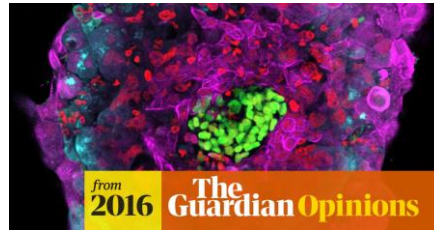
**nature**  Menu

NATURE | COMMENT

### Embryology policy: Revisit the 14-day rule

Insoo Hyun, Amy Wilkerson & Josephine Johnston

04 May 2016



2021



- drop of 14 day limit

## New ISSCR guidelines

**Conditionally permitted human embryos** to be cultured in vitro beyond 14 days post-fertilization



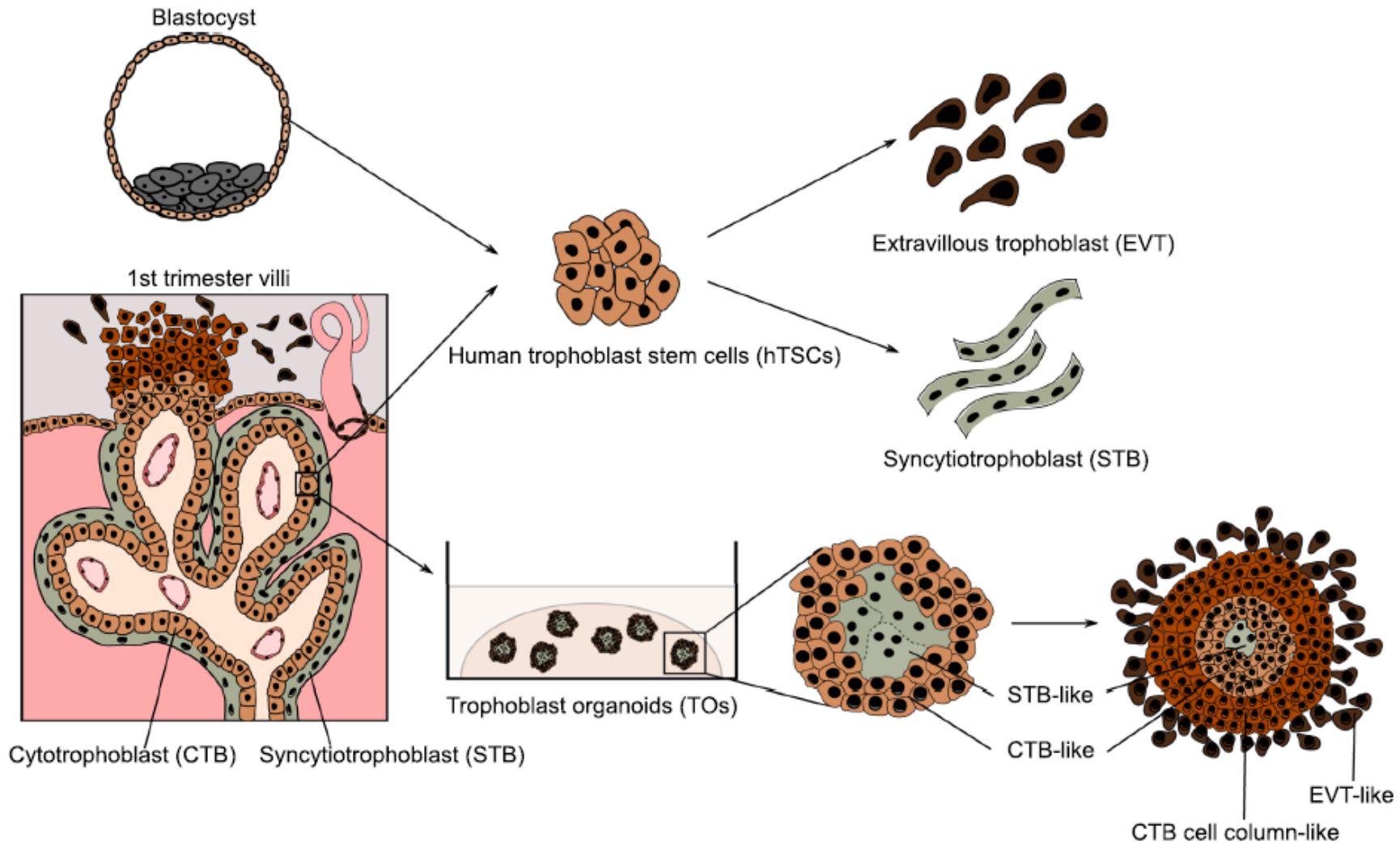
- |               |  |
|---------------|--|
| <b>Reason</b> | <ul style="list-style-type: none"><li>▶ Advances understanding of life events and disease mechanisms</li><li>▶ Technically possible to culture for 14 days or longer</li><li>▶ Research on embryo-like structures* made 14-day rule obsolete</li></ul> |
| <b>Issue</b>  | <ul style="list-style-type: none"><li>▶ Inconsistent regulations may complicate international cooperation.</li><li>▶ At what stage should the in vitro cultures of human embryos be terminated?</li></ul>  |

**Public discussion among citizens is needed**



Need to decide how to proceed with research on embryos and embryo-like structures

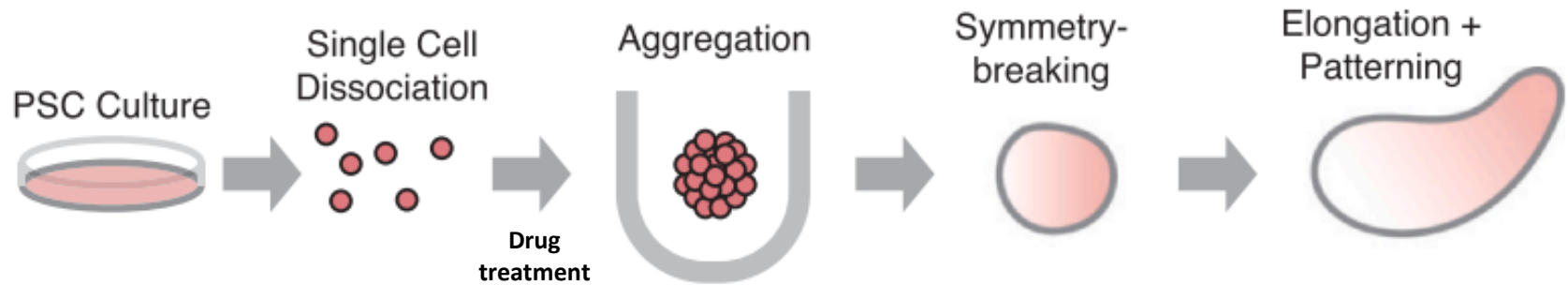
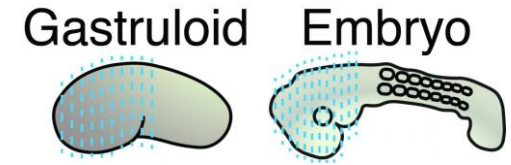
# Research of peri-implantation development



# Research of peri-implantation development

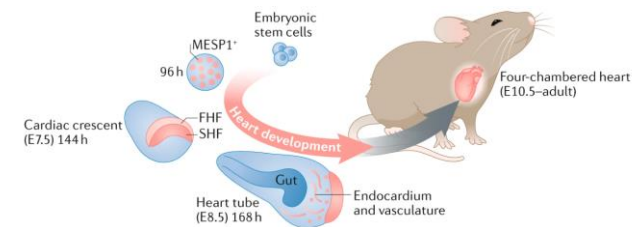
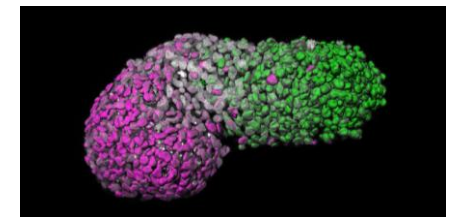
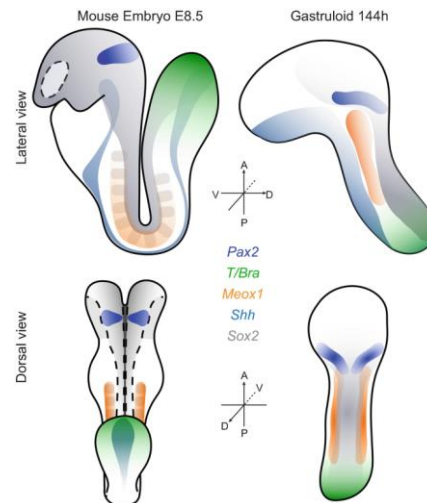
## ❖ Gastruloids

- stem cell-based models recapitulating gastrulation process
- 2D/3D structures generated by aggregation of pluripotent mouse/human stem cells



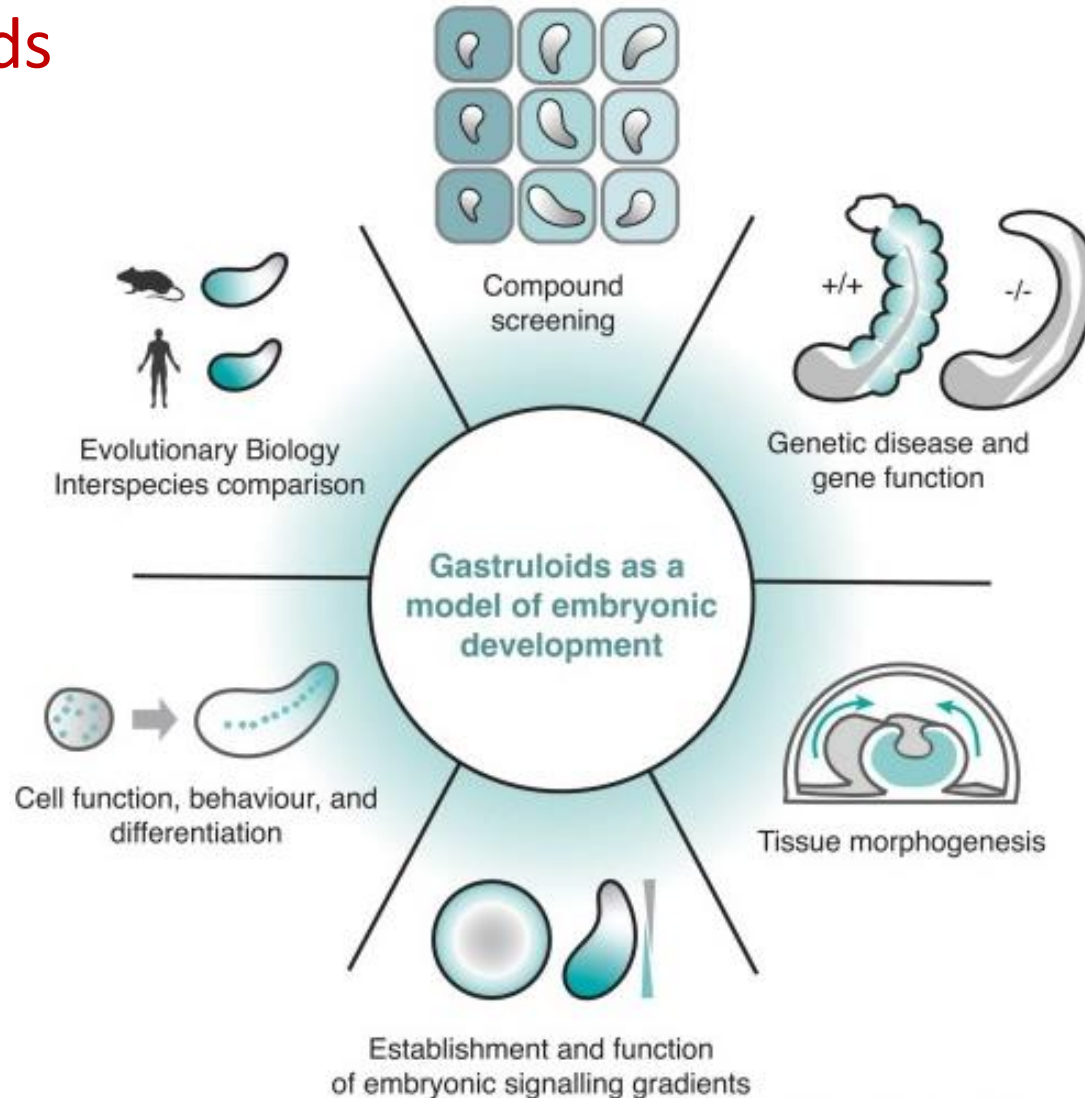
*Sullivan and Santos 2023*

- **mimic** features of early mammalian development and generate embryonic cell types
- trackable and scaleable culture



# Research of peri-implantation development

## ❖ Gastruloids



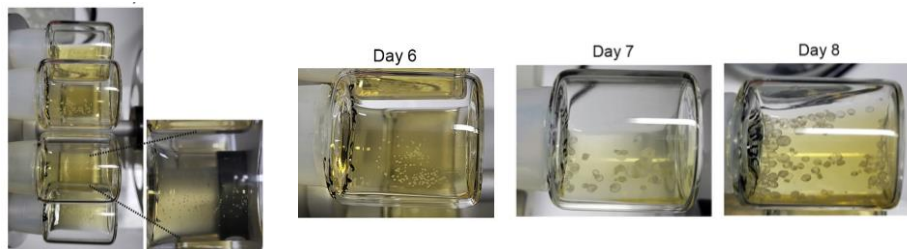
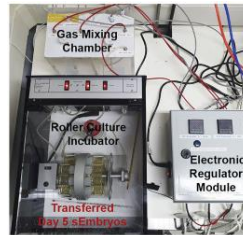
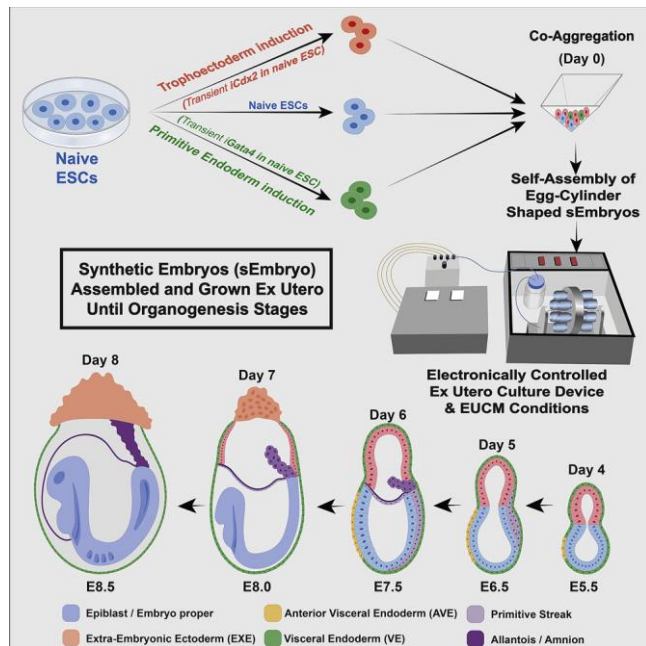
# Ex utero development

CellPress  
OPEN ACCESS

Cell

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

Shadi Tarazi,<sup>1,8,\*</sup> Alejandro Aguilera-Castrejon,<sup>1,8,10,\*</sup> Carine Joubran,<sup>1,8</sup> Nadir Ghanem,<sup>2</sup> Shahd Ashoukhi,<sup>1</sup> Francesco Roncato,<sup>1</sup> Emilie Wildschutz,<sup>1</sup> Montaser Haddad,<sup>3</sup> Bernardo Oldak,<sup>1</sup> Elidet Gomez-Cesar,<sup>1</sup> Nir Livnat,<sup>1</sup> Sergey Viukov,<sup>1</sup> Dmitry Lokshtanov,<sup>1</sup> Segev Naveh-Tassa,<sup>1</sup> Max Rose,<sup>1</sup> Suhair Hanna,<sup>4</sup> Calanit Raanan,<sup>5</sup> Ori Brenner,<sup>5</sup> Merav Kedmi,<sup>6</sup> Hadas Keren-Shaul,<sup>7</sup> Tsvee Lapidot,<sup>3</sup> Itay Maza,<sup>7,8,\*</sup> Noa Novershtern,<sup>1,9,10,\*</sup> and Jacob H. Hanna<sup>1,10,11,\*</sup>



ARTICLE

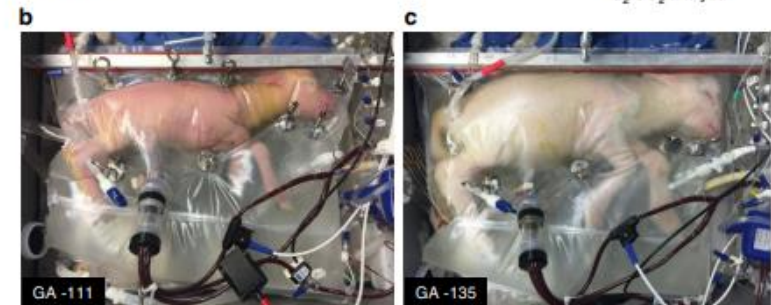
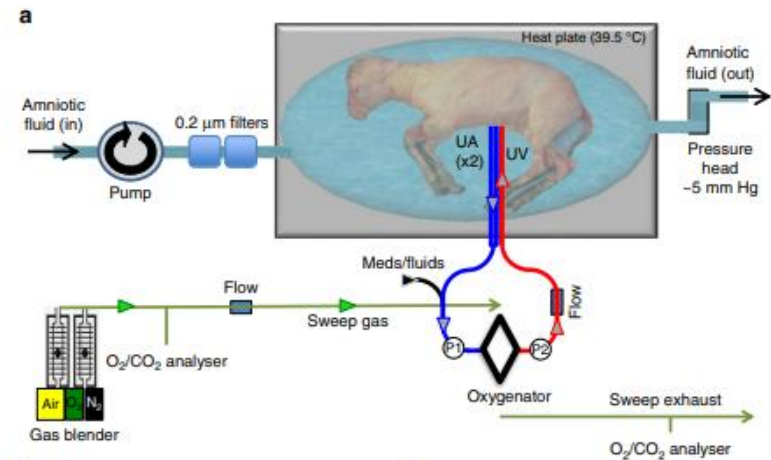
Received 25 Apr 2016 | Accepted 2 Mar 2017 | Published 25 Apr 2017

DOI: 10.1038/ncomms15112

OPEN

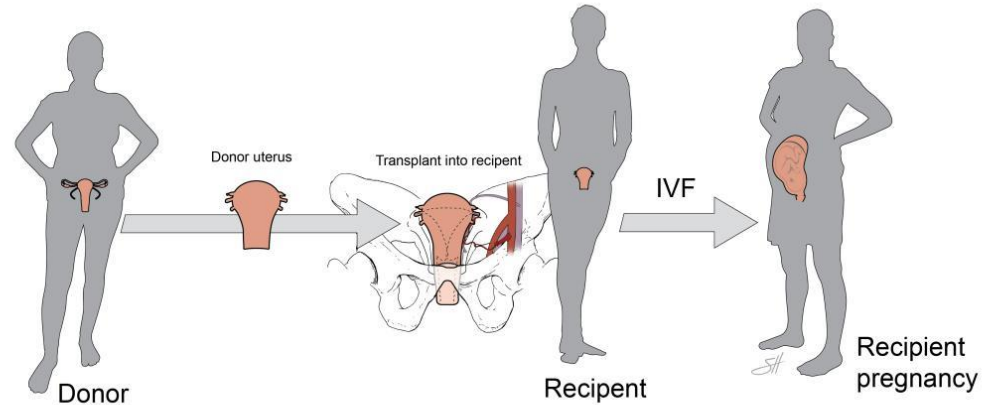
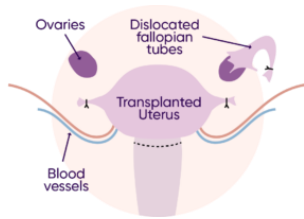
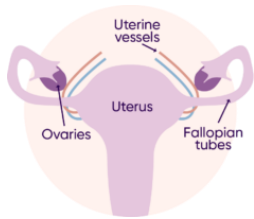
## An extra-uterine system to physiologically support the extreme premature lamb

Emily A. Partridge<sup>1,\*</sup>, Marcus G. Davey<sup>1,\*</sup>, Matthew A. Hornick<sup>1</sup>, Patrick E. McGovern<sup>1</sup>, Ali Y. Mejaddam<sup>1</sup>, Jesse D. Vrecenak<sup>1</sup>, Carmen Mesas-Burgos<sup>1</sup>, Aliza Olive<sup>1</sup>, Robert C. Caskey<sup>1</sup>, Theodore R. Weiland<sup>1</sup>, Jiancheng Han<sup>1</sup>, Alexander J. Schupper<sup>1</sup>, James T. Connelly<sup>1</sup>, Kevin C. Dysart<sup>2</sup>, Jack Rychik<sup>3</sup>, Holly L. Hedrick<sup>1</sup>, William H. Peranteau<sup>1</sup> & Alan W. Flake<sup>1</sup>



# Uterus transplantation

- uterus agenesis
- anatomical malformations
- previous hysterectomy



## HOW DOES A UTERUS TRANSPLANT WORK?

- 1** The uterus is removed from a deceased or living donor.
- 2** It is resupplied with blood.
- 3** The organ is transplanted into the recipient.
- 4** The recipient starts a course of immunosuppressants.
- 5** A fertilized egg is placed into the uterus via IVF.
- 6** The patient undergoes a C section at 37-40 weeks gestation.
- 7** The patient can undergo one more pregnancy if she wishes. If not, the uterus is surgically removed.
- 8** They stop taking immunosuppressants.

IKE  
M



