Lecture 4

Reproductive biology and Embryology

- Early embryo cleavages
- Implantation
- Somatic nuclear transfer– cloning
- Gastrulation
- Extraembryonal structures
- Fetal membranes

Brno, March 2018

Fertilization

Zygote formation and the first cleavages

~8-12 hrs → ~6-8 hrs → ~12-14 hrs → ~3-4 days

Sperm
- DNA
- Cenriol
- Oocyte-activating factor

Ooocyte
- DNA
- Mitochondria
- Cytoplasm

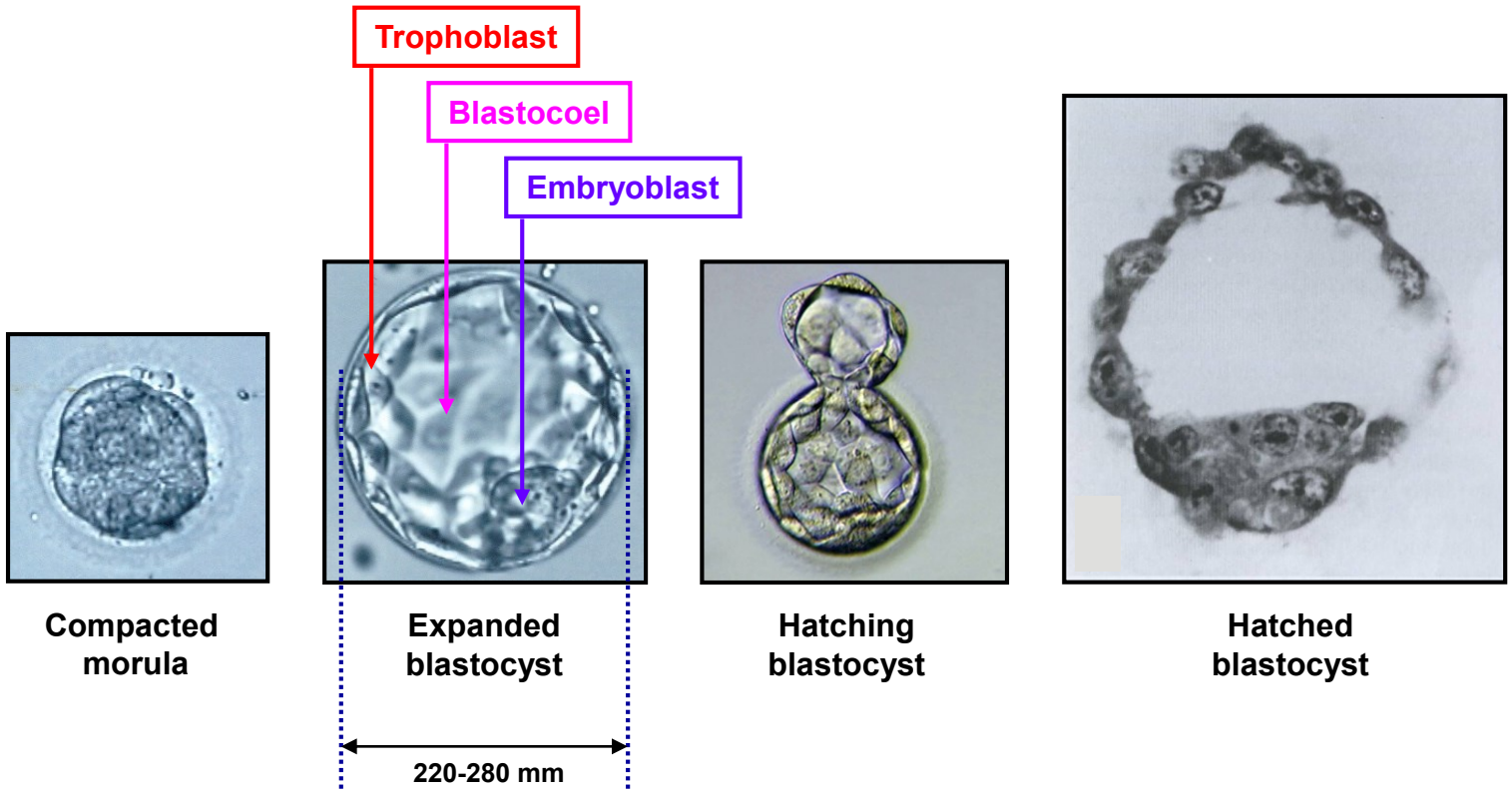
DNA synthesis

Zygote
pronuclei fused
(Syngamy)

Gap junctions

Oviduct → **Uterus**

Blastocyst formation

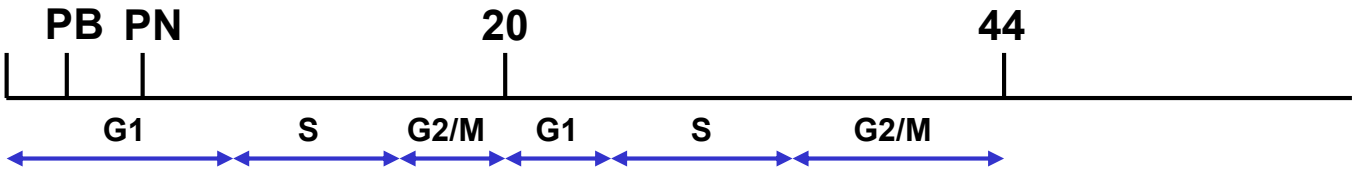


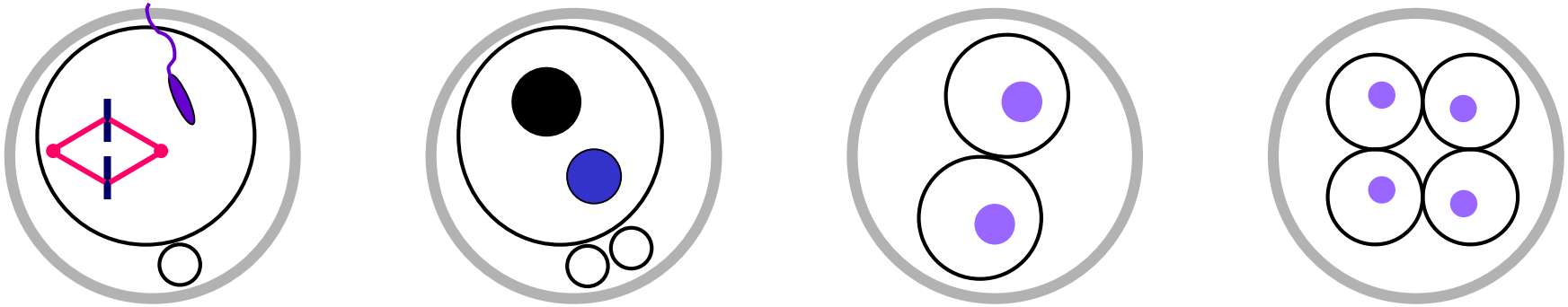
Day 5 – 6
(60 to 8O cells)

Early embryogenesis of human embryo

Dr. Zuzana Holubcová
REPROFIT, Brno

A potency of oocyte cytoplasm





Translation of maternal mRNA

Translation of zygotic mRNA

Zygotic transcription

Repression of transcription

Significance of „enhancers“

Activation of embryonal genome

Activation of embryonal genome

It is not a single discrete event
(first signs occur in zygote, in man it reaches its maximum in 4- to 8-cell embryo)

Two types of transcripts

Transcrips that replace degraded maternal mRNAs

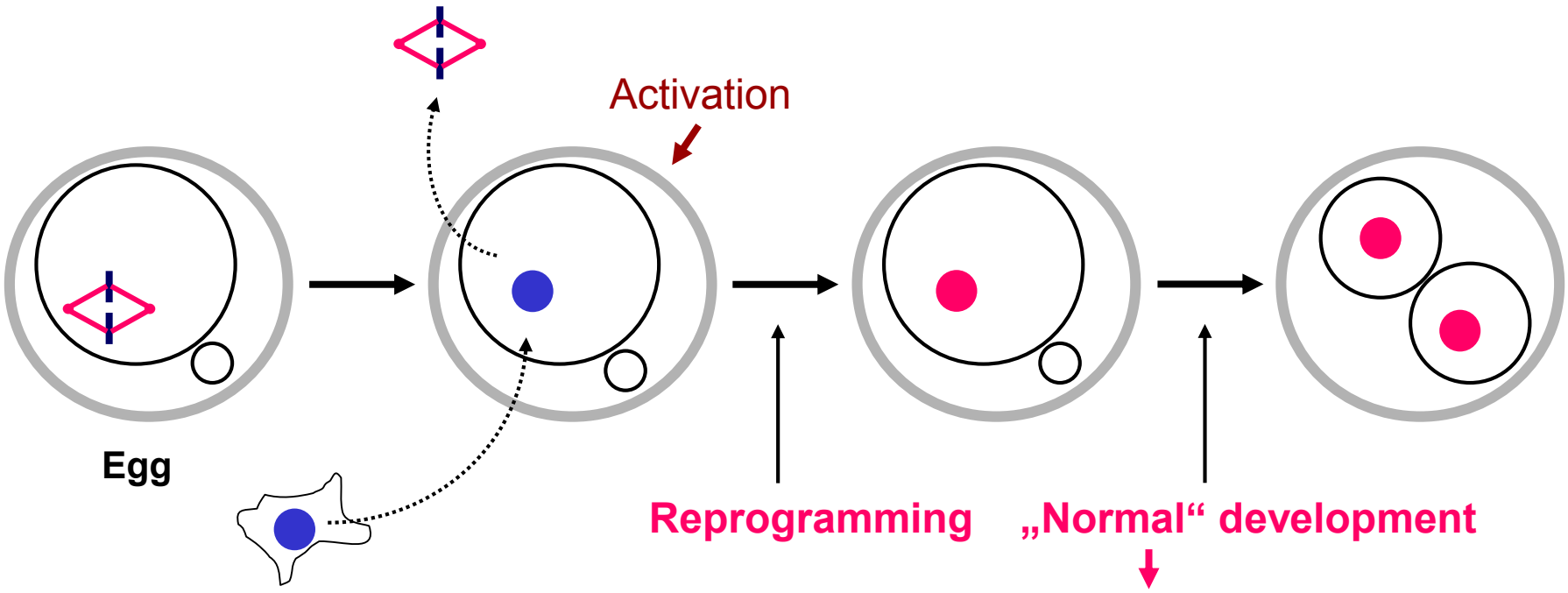
Novel transcripts that underlie new pattern of gene expression

It is „responsible“ for establishment of totipotency of blastomeres

&

It represents phenomenon known as genome REPROGRAMMING

Nuclear transfer (cloning) - principle



Effectivity of cloning is very low (1-3%)

Reprogramming is slow and most likely incomplete (as the result, gene expression is often abnormal)

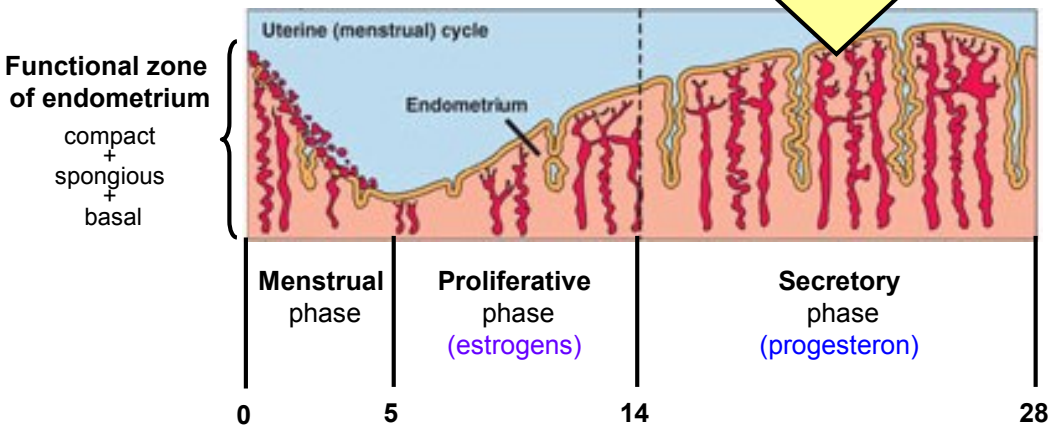
Effectivity of reprogamming depends on many factors (type of somatic cells, position in cell cycle phase, …)

Blastocyst implantation

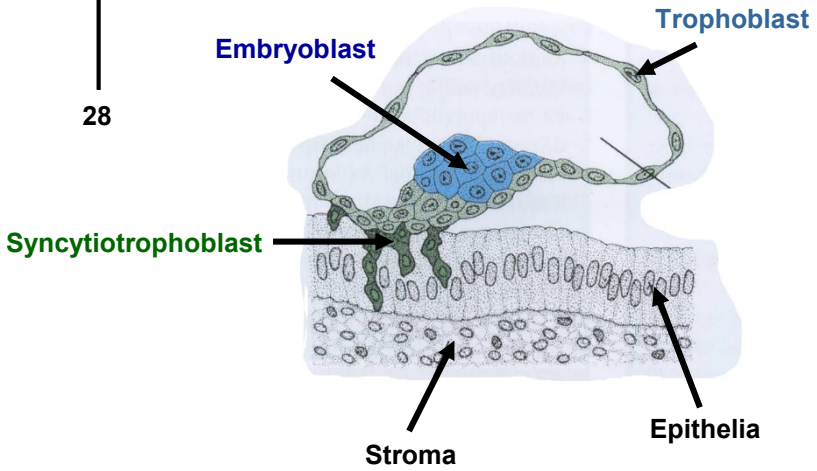
Molecular players

Selectin on trophoblast + glycoproteins on epithelia
Integrins + Laminin, Fibronectin
IGF1, IGF2, VEGF
Metaloproteinases + their tissue inhibitors

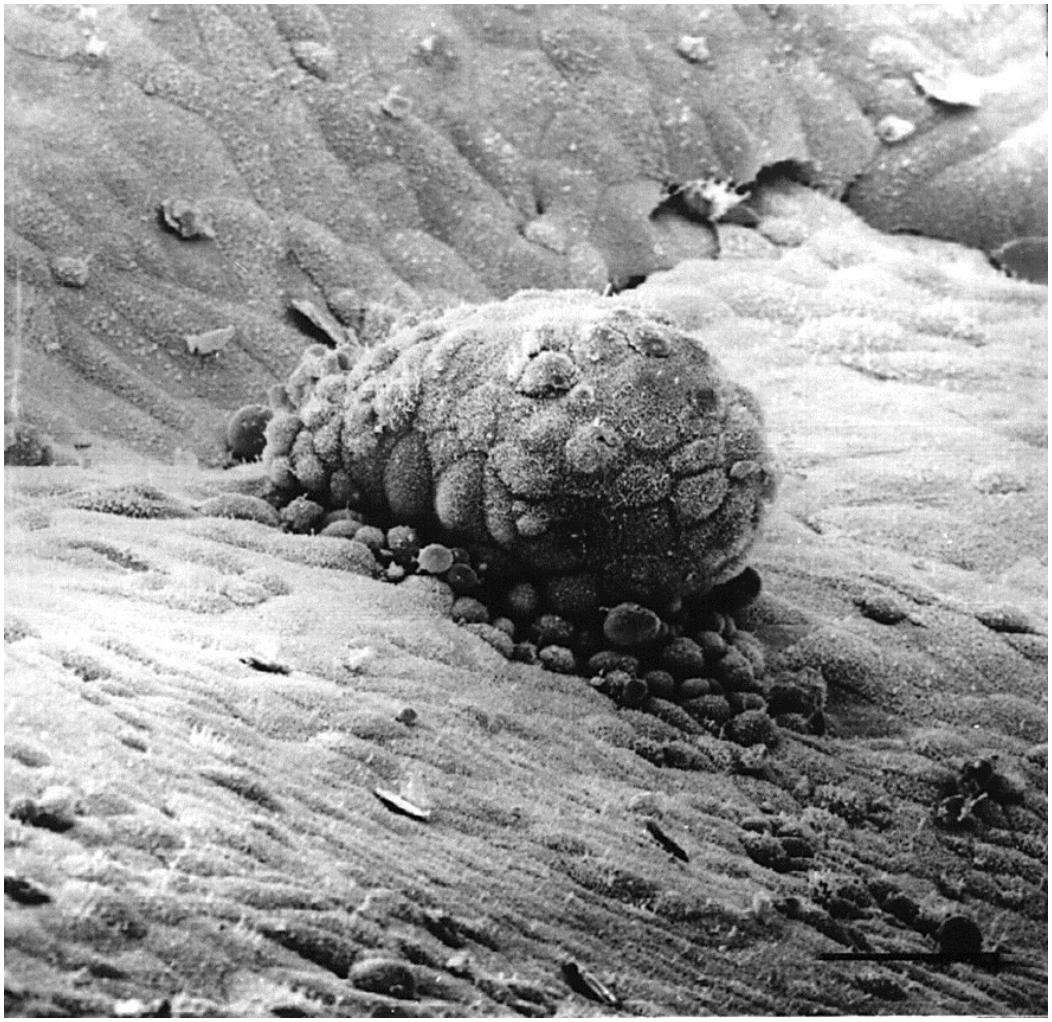
Progesteron
hCH
Prostaglandins



Ovulation
followed by
Fertlizaion



Blastocyst implantation

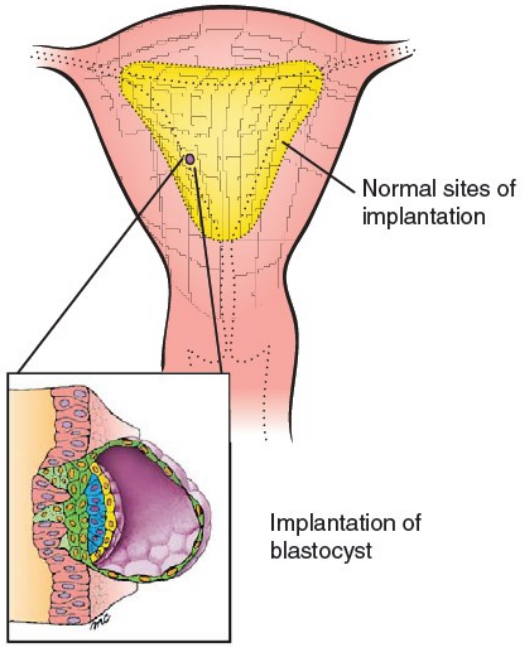


http://myselfishgenes.blogspot.hu/2013/05/what-happens-to-my-embryos-if-they-do.html

Blastocyst impantation – place of implantation

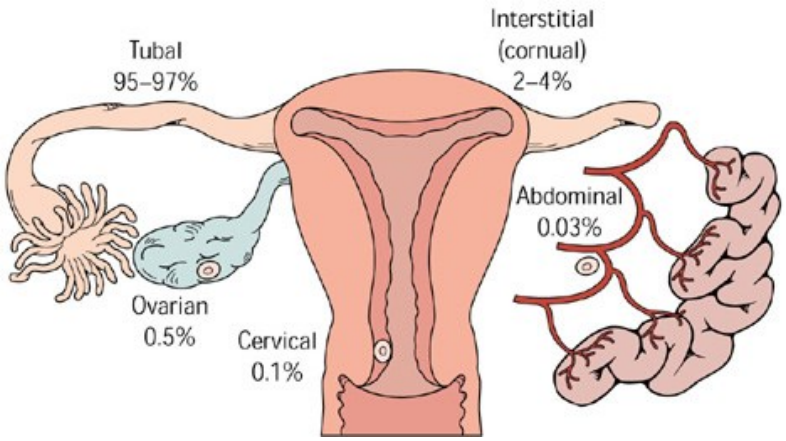
Normal

(posterior / anterior wall of uterus)



Abnormal

(0,25 až 1%of all implantations)



Early development – The second week (1)

Completion of implantation + Further embryo development

Trophoblast

- Furtner invasion into endometrium
- Damage to capilaries and glands
- Engulfment of apoptosing cells (Fas/Fas ligand)

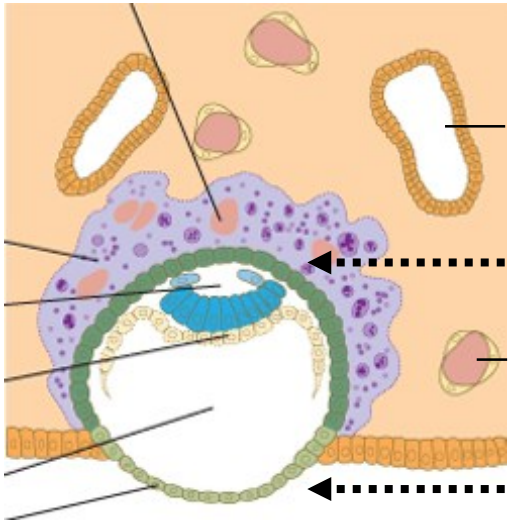
Cytotrophoblast

- Mitoticaly dividing cell (single layer)
- Supply for syncytiotrophoblast

Syncytiotrophoblast

- Fused cells (nondividing, multinucleated)
- Produces hCG (stimulates CL)

Status at day 8



Early development – The second week (2)

Decidual reaction of endometrium

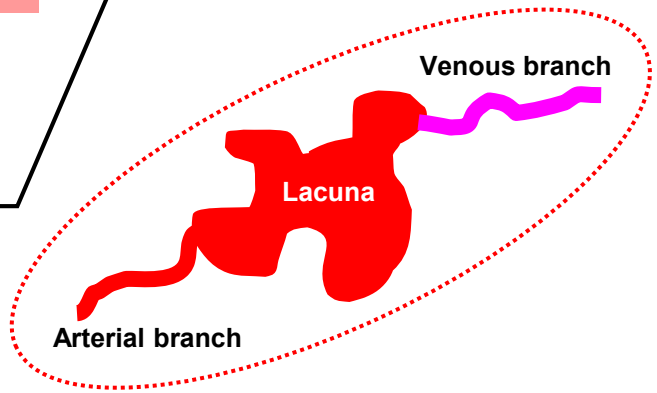
- Vessel number/density increases
- Fibroblasts differeniate into glycogen- and lipid-containing cells

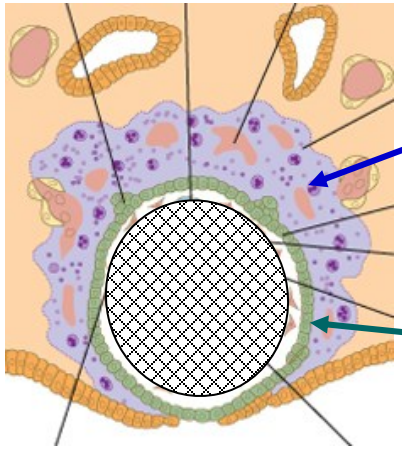
Fused lacunae create a network

+

Uterine vessels erode

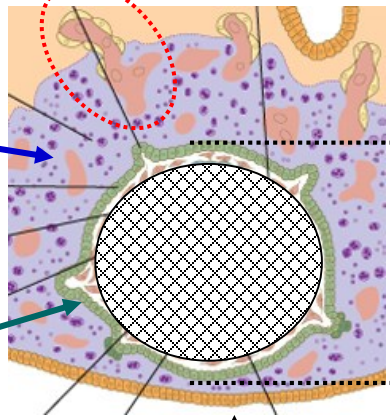
Uteroplacental circulation





Syncytiotrophoblast
Sponge-like appearance

Cytotrophoblast

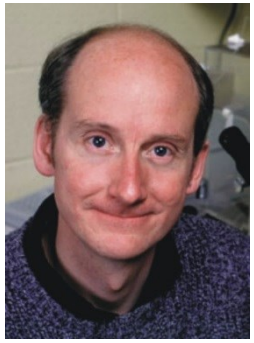


2-3 mm

Epithelization of the implantation site

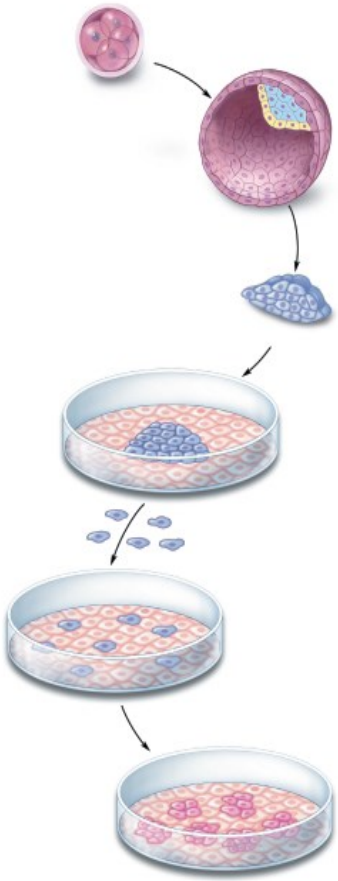
Status at day 10

Status at day 12

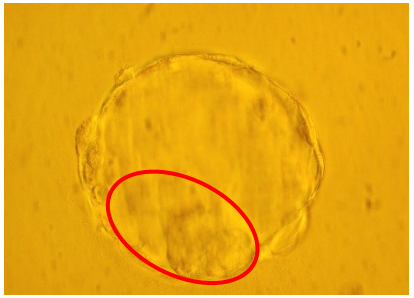


Human Embryonic Stem (hES) Cells

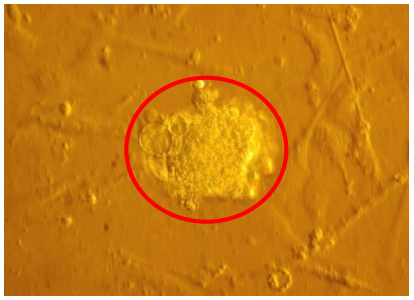
(Thompson et al, 1998)



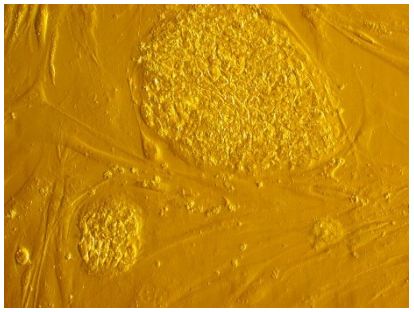
Early embryo at blastocyst stage



Isolated embryoblast (ICM - Inner Cell Mass)



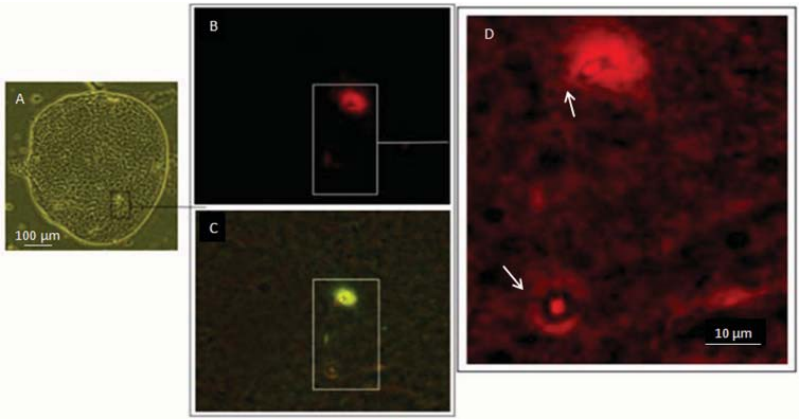
Isolated embryoblast after placing to *in vitro* conditions (+ feeder cells + FGF2)



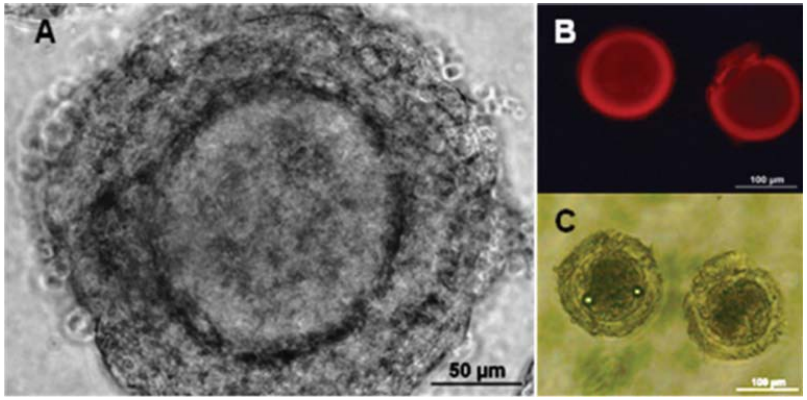
Propagation in culture by enzymatic disaggregation (repeated passaging)

Derivation of postmeiotic germ cells from hESC

Prof. Harry Moore, University of Sheffield, 2009

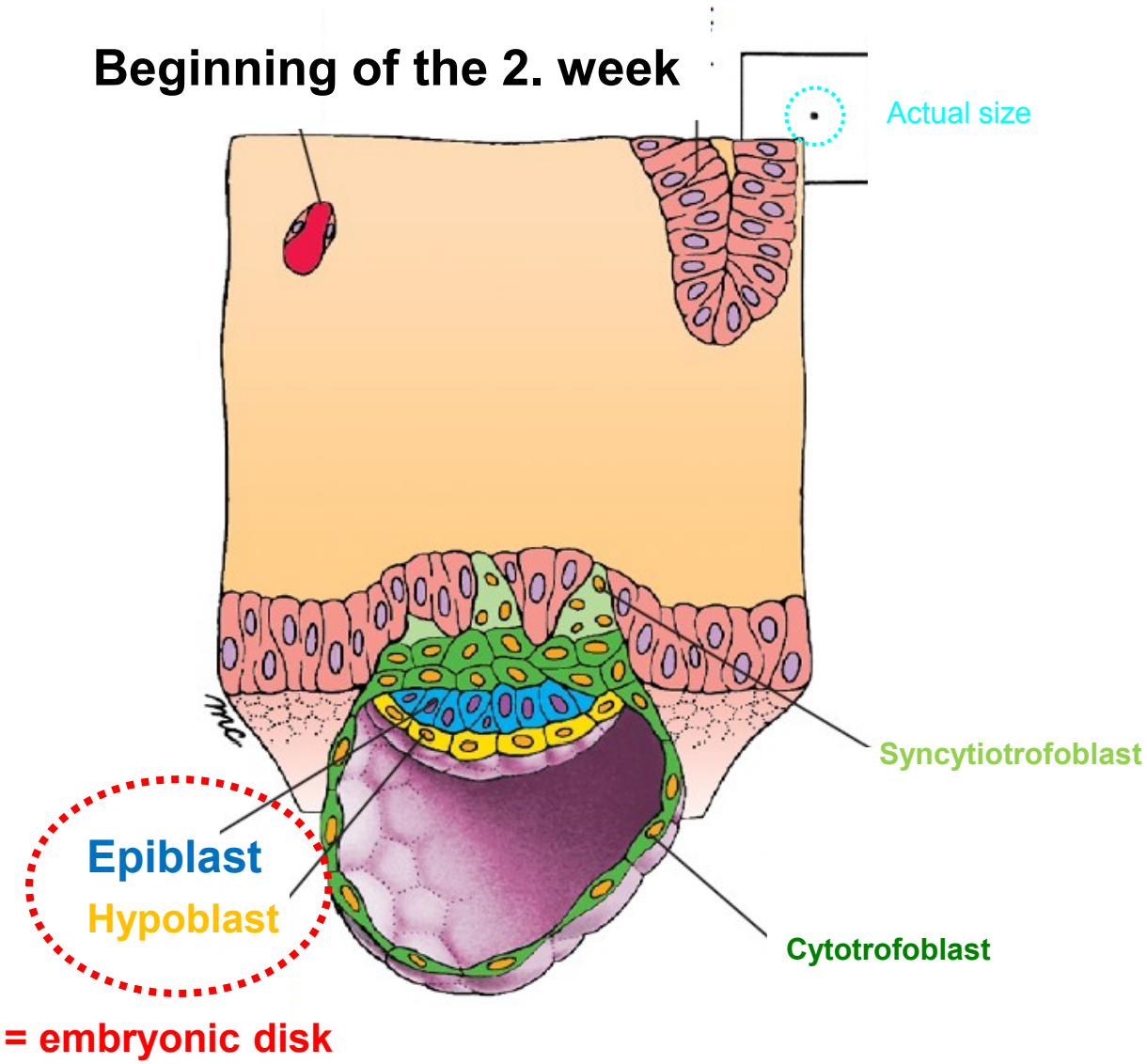


B) C-KIT
C) I-97 antigen
D) Cells with condensed chromatin and signs of flagellum



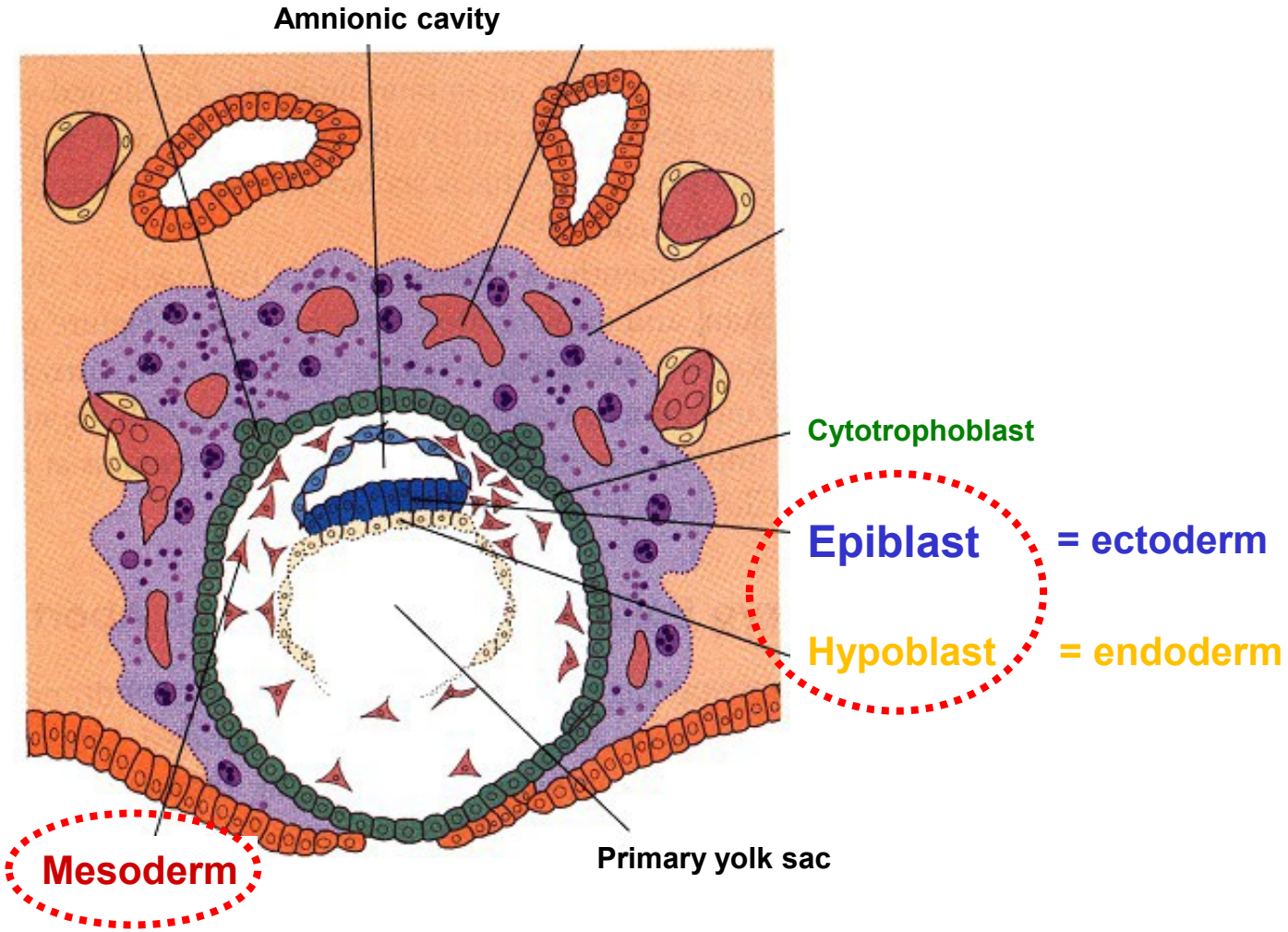
Structures that are highly reminiscent to oocyte-granulosa complexes (zona pellucida is not developed)

Gastrulation – establishment of three germ layers



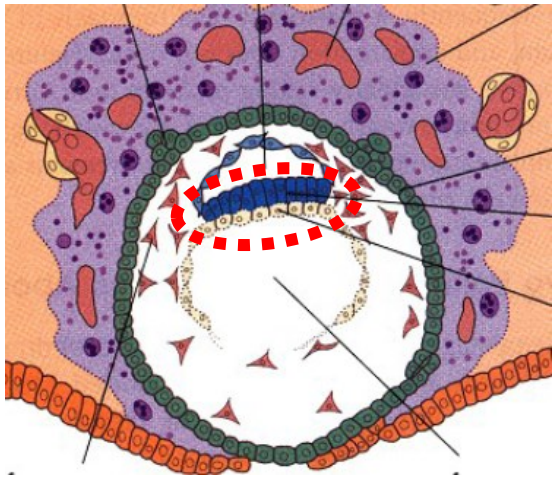
Gastrulation – establishment of three germ layers

Day 8 to 9

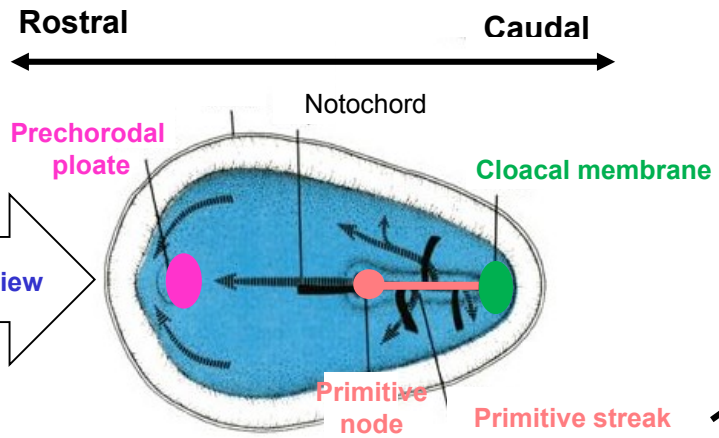


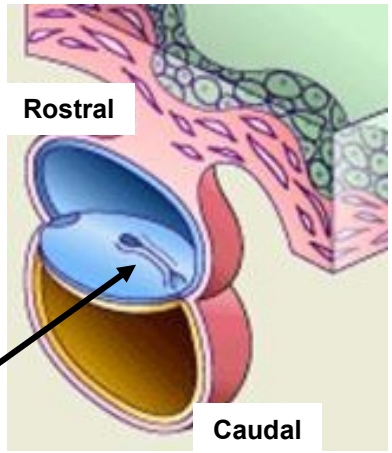
Gastrulation – establishment of three germ layers

Embronic disk – first at day 6 to 7

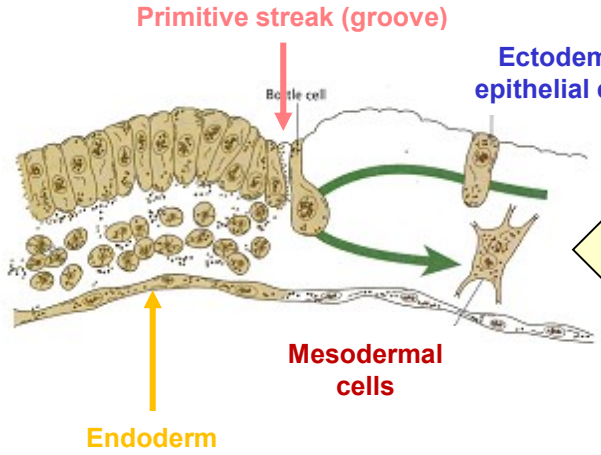


Dorsal view

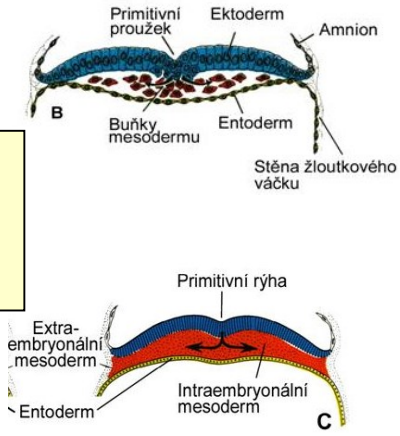




Transversal section



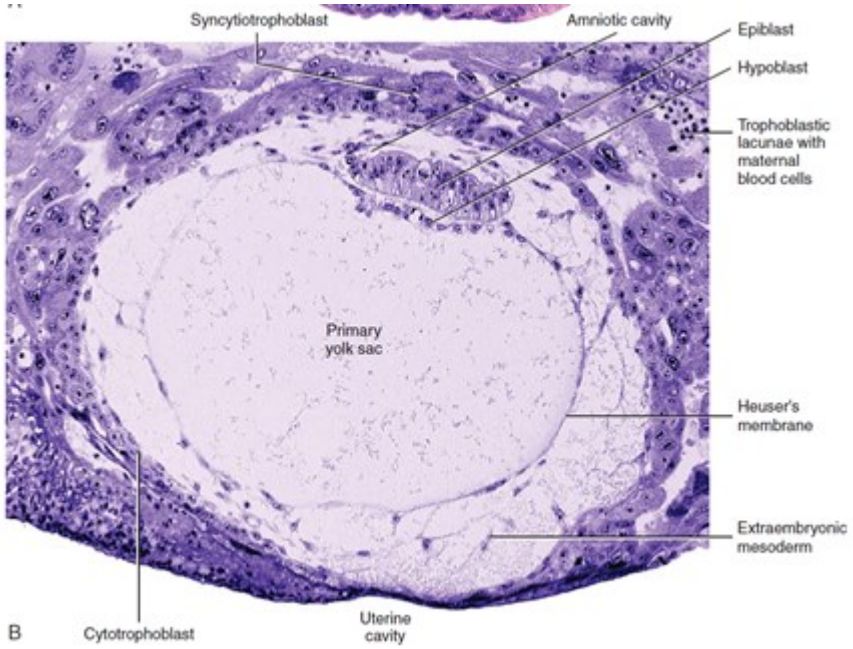
Mesodem specification
(expanded view)



= epithelo-mesenchymal transition

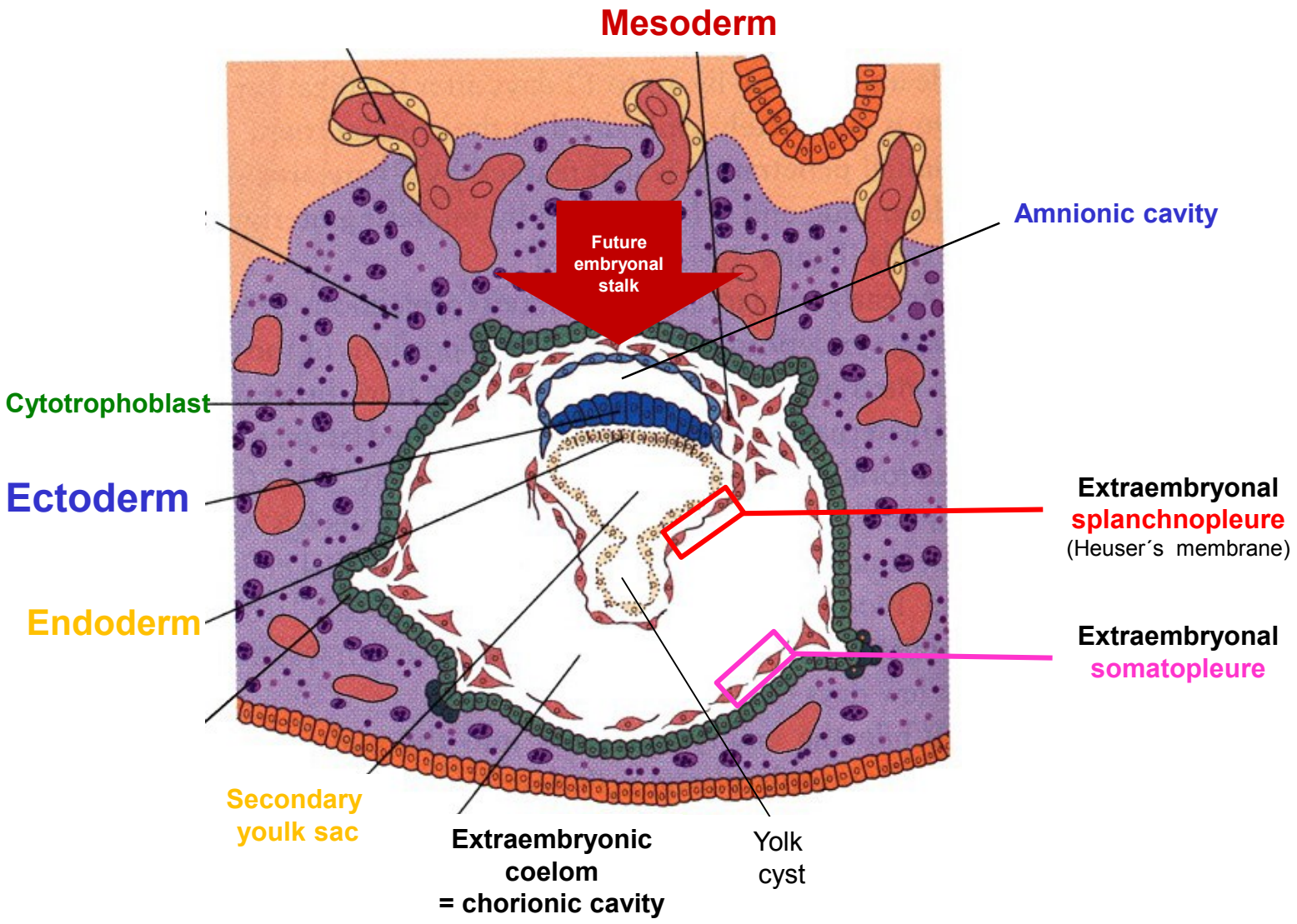
Gastrulation – establishment of three germ layers

Day 9 – primary yolk sac

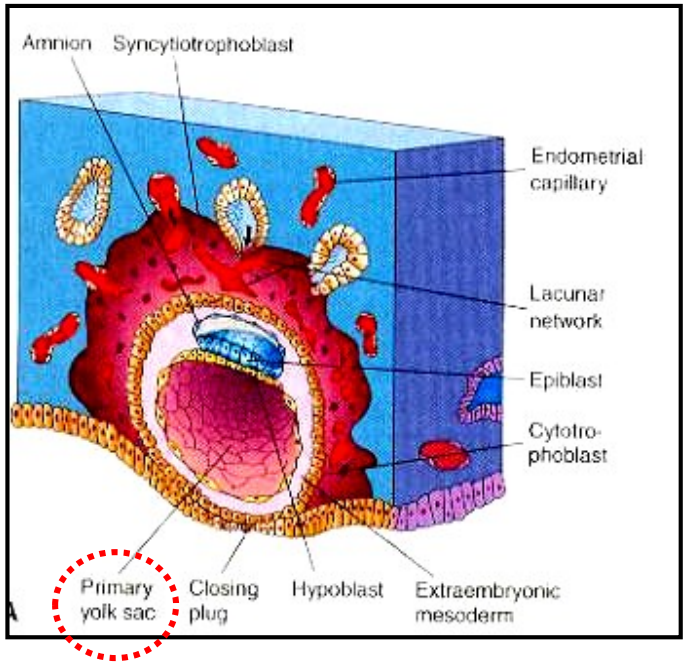


Gastrulation – establishment of three germ layers

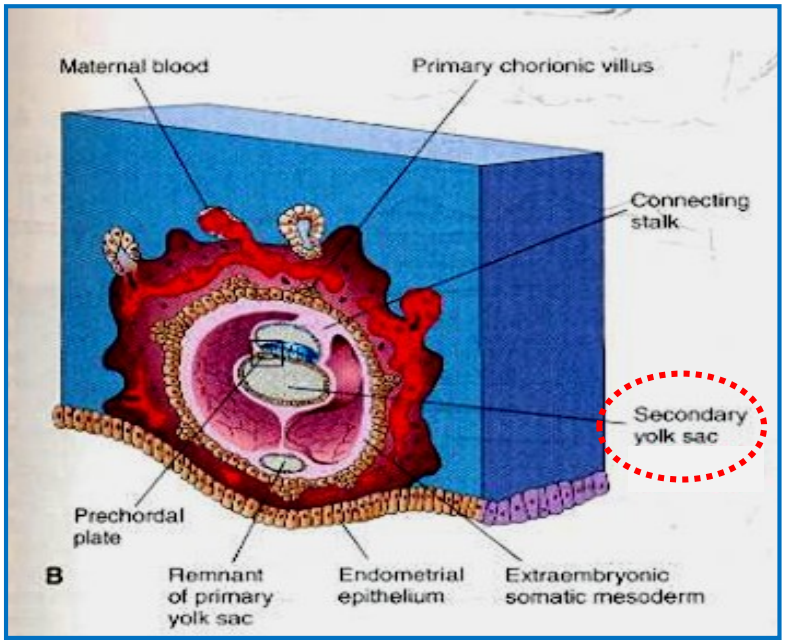
Day 12 - 13



Extraembryonal structures – yolk sac 1



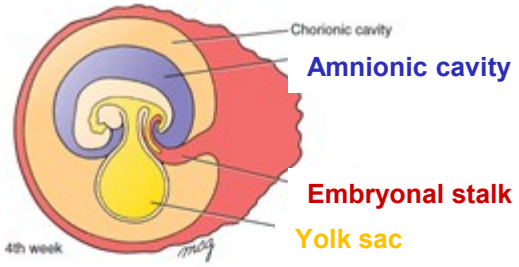
End of the 2. week



Wall of yolk sac = endoderm + mesoderm

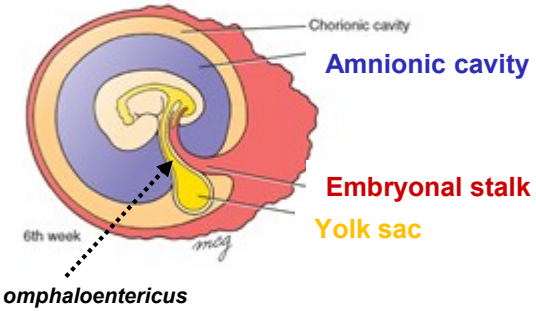
Heuser´s membrane

Extraembryonal structures – yolk sac 2



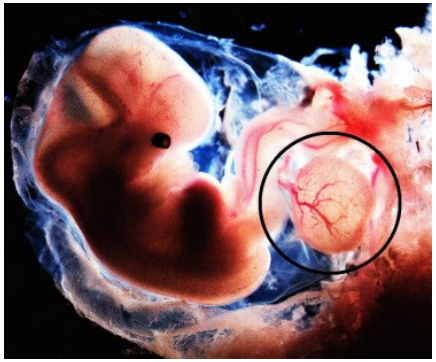
Functions of yolk sac:

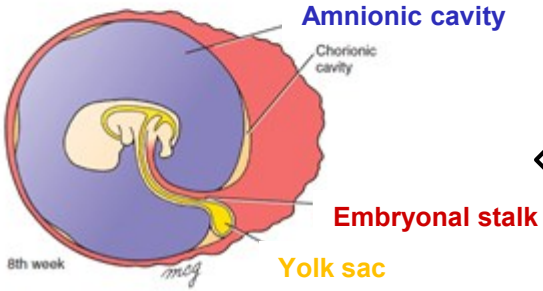
- does not contain yolk (oligolecithal egg)
- 3. week – hematopoesis (since 6. week in liver)
- 3.- 4. week– PGC
- 4. week – incorporation into primitive gut
- since 6. week – loss of link to gut – obliteration
- abnormal persistence - Meckel diverticle



Ductus omphaloentericus

approx 6. week

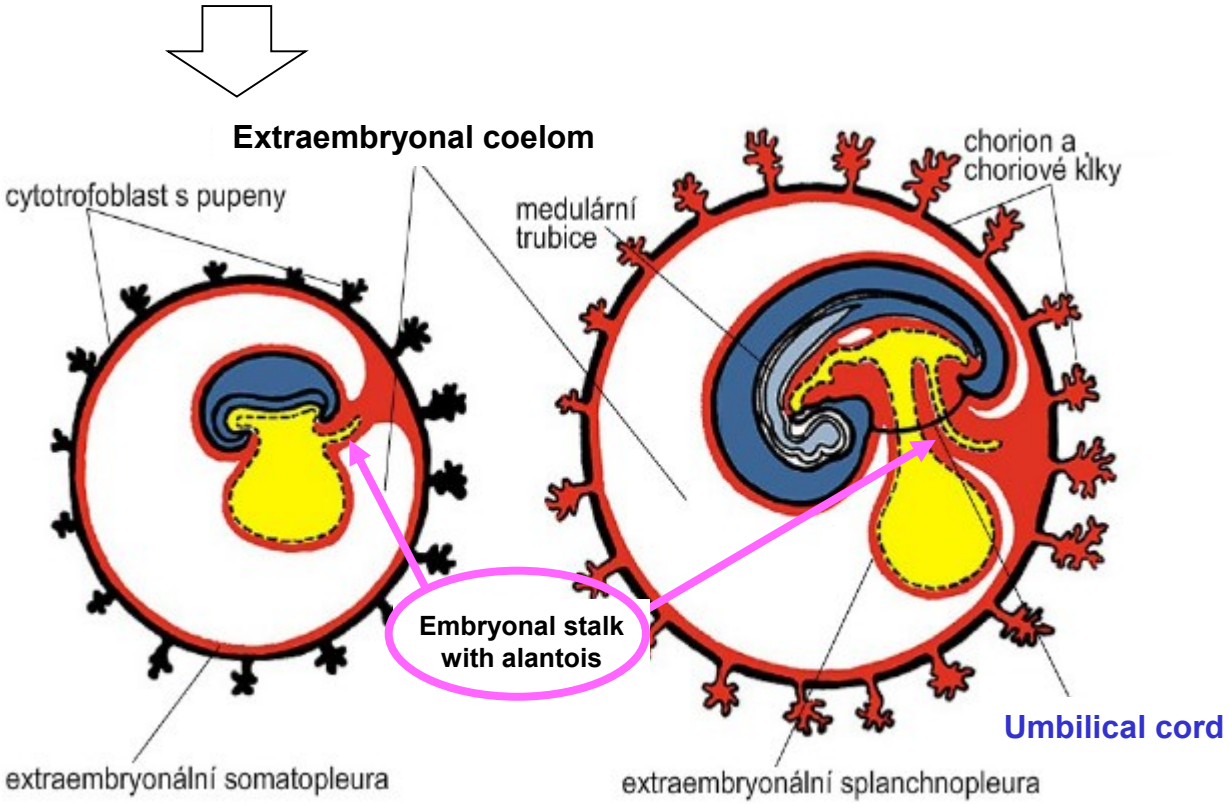




approx 8. week

Extraembryonal structures – amnion (internal fetal membrane)

Beginning of the 3. week – diverticle of caudal wall of yolk sac

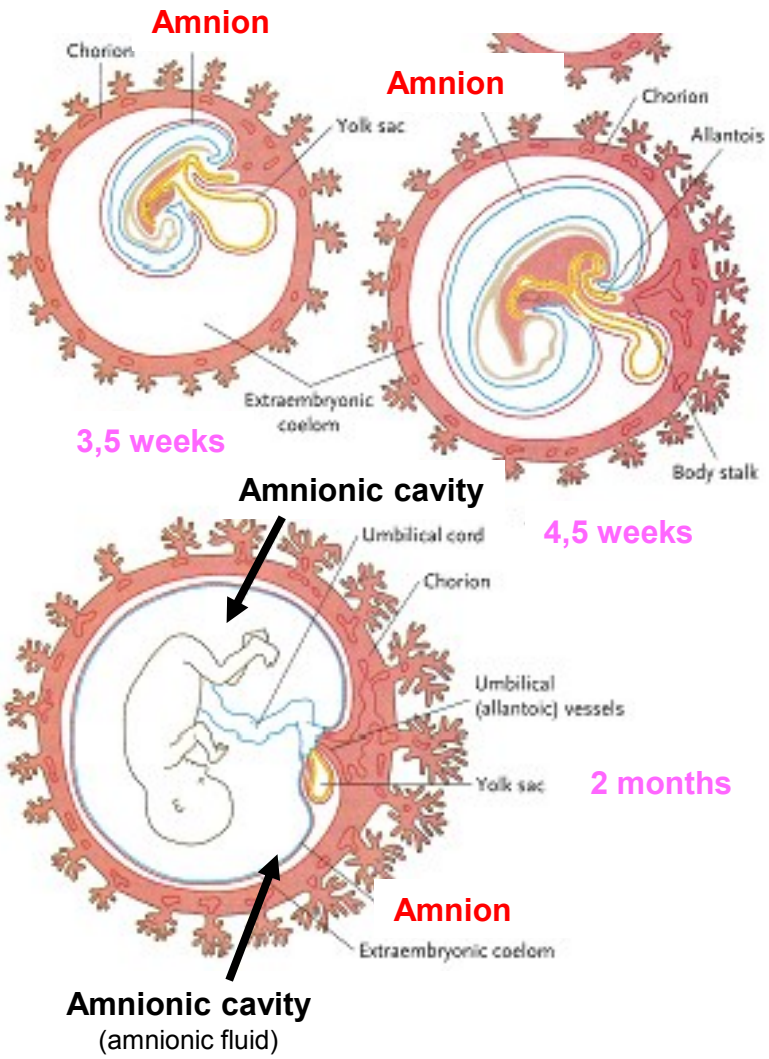


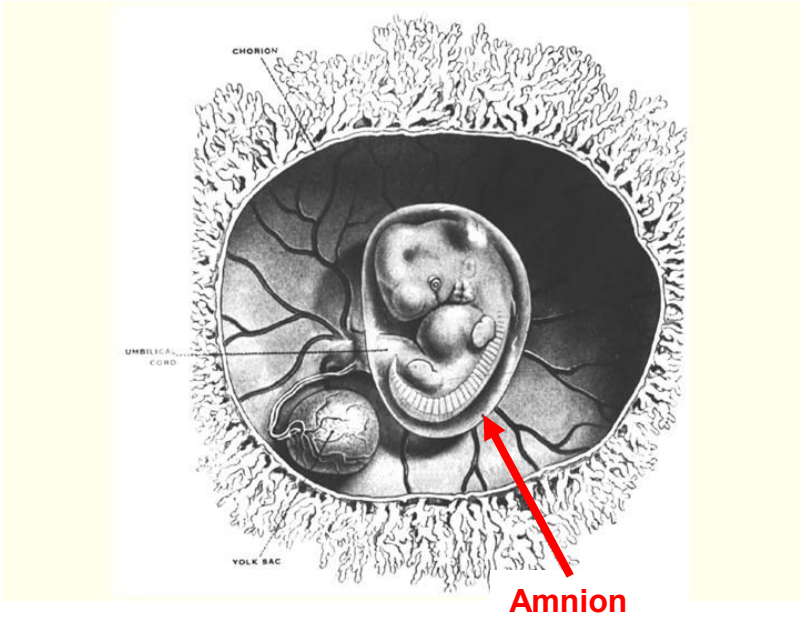
Functions:

- In humans rudimentar structure
- **vessels of alantois – umbilical vein and arteries**
- 2. month – extraembryonal part degenerates
- 3. month – intraembryonal part – urachus (link to urinary bladder)

Extraembryonal structures – amnion (internal fetal membrane)

thin, transparent = flat simple ectodermal epithelium + extraembryonal mesoderm (somatopleure)

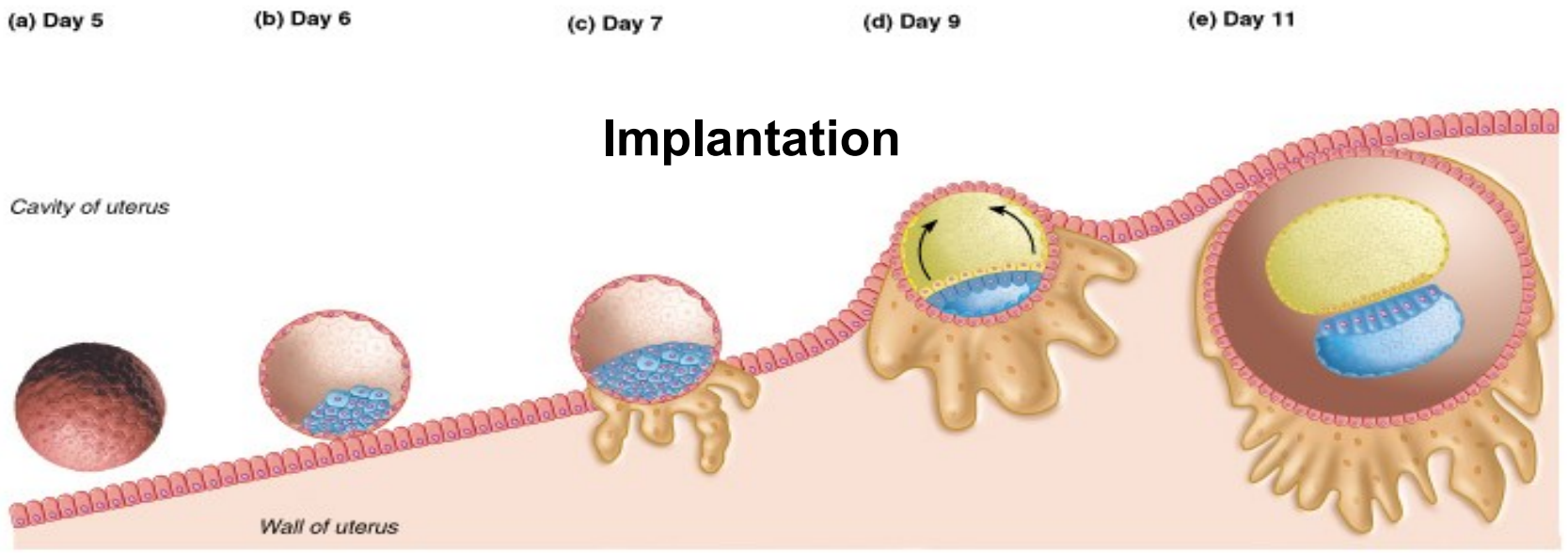




Amnionic fluid:

- 99% water; 800 až 1000 ml in the last month of pregnancy
- source = diffusion from endometrium through amniochorion
- source = transfer through placenta
- source = transfer through skin of fetus
- source j = excretion by fetal kidneys (since 11. week)
- very fast turnover – passage via fetus (digestive + respiratory tracts)
- function – free movement without adhesion (space for symmetrical growth)
- function – barrier (temperature, mechanical damage, infection)
- function – chemical homeostasis

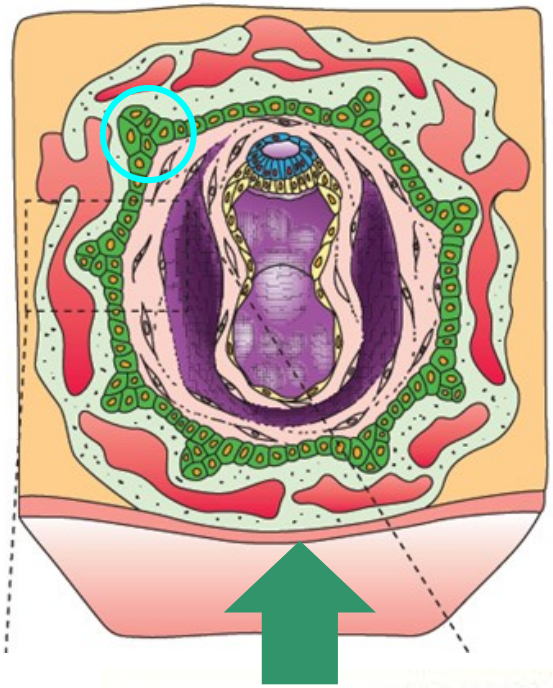
Extraembryonal structures – chorion (external fetal membrane)



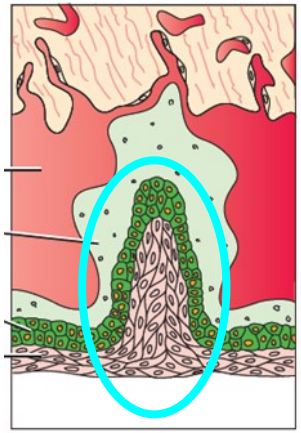
Syncytiotrophoblast invades into surrounding stroma

Extraembryonal structures – chorion – chorionic villi

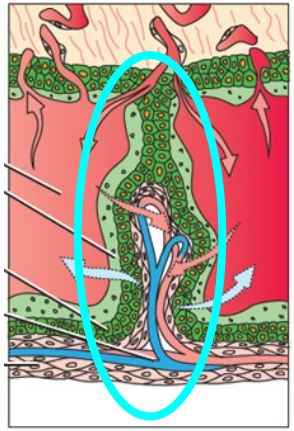
Day 11 až 13

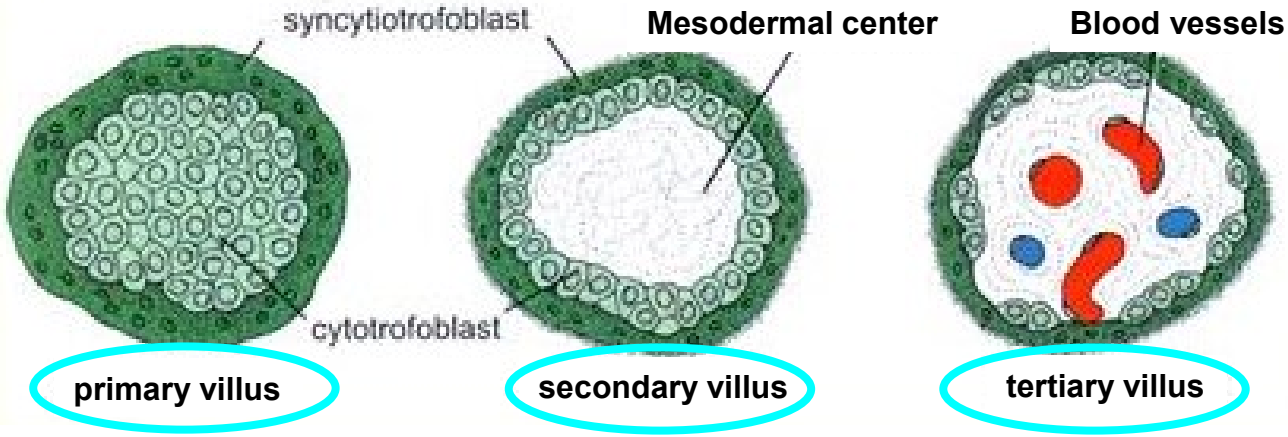


Day 16

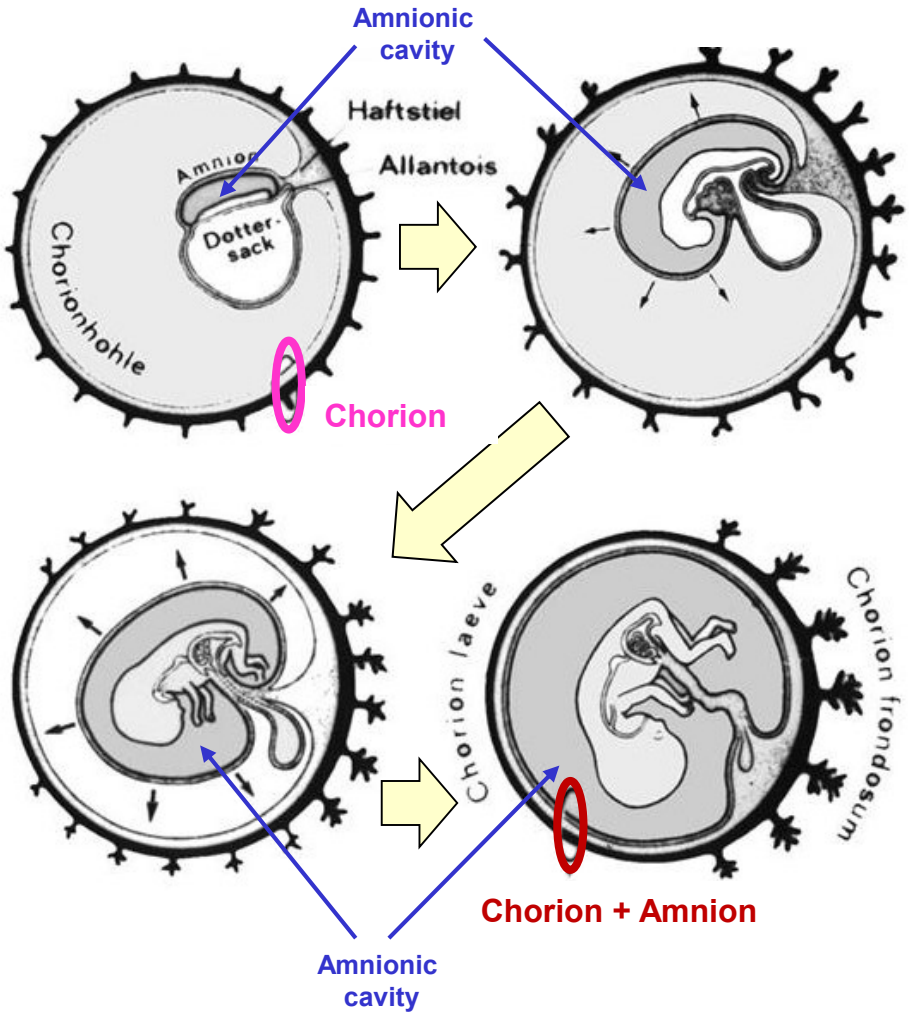


End of the 3. week





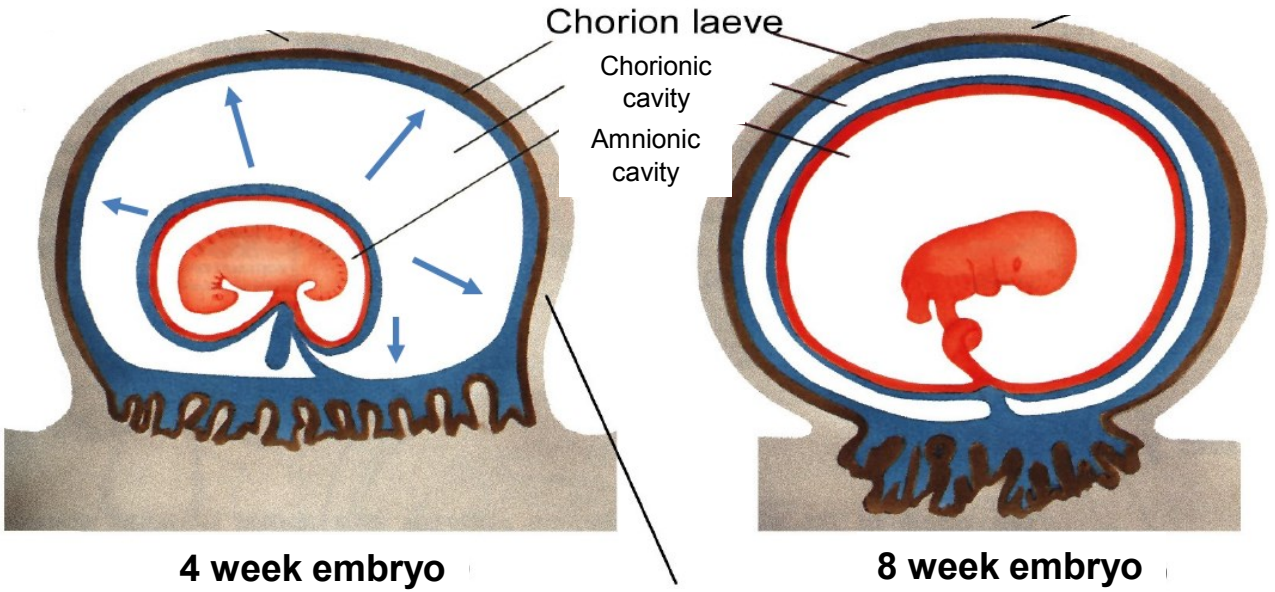
Extraembryonal structures – chorion – expansion of amnion



Extraembryonal structures – growth of amnion and chorion

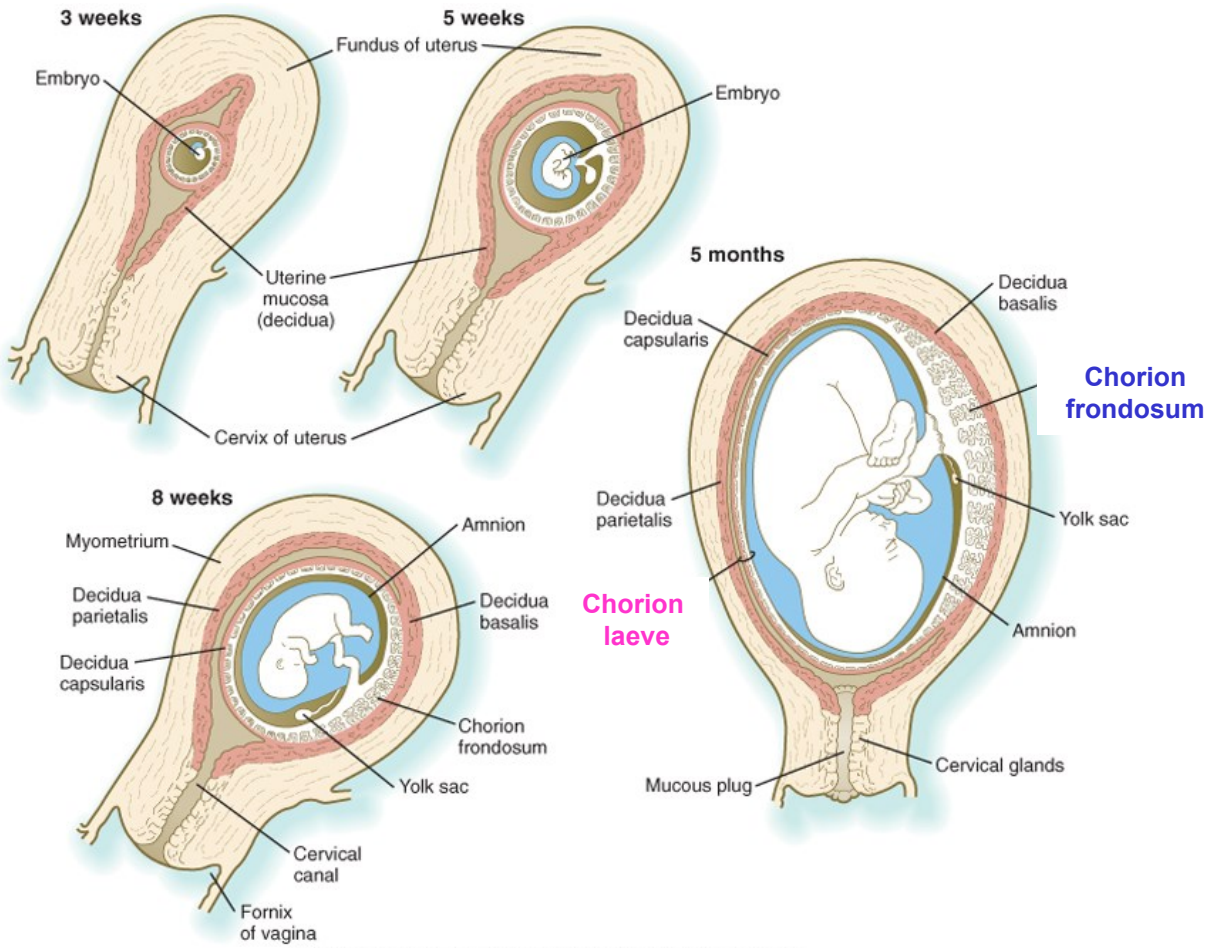
CHORION = cytotrofoblast + mezoderm (ex.)
AMNION = mezoderm (ex.) + ektoderm

Growth of amnionic and chorionic cavities



Extraembryonal structures – chorion – *frondosum x laeve*

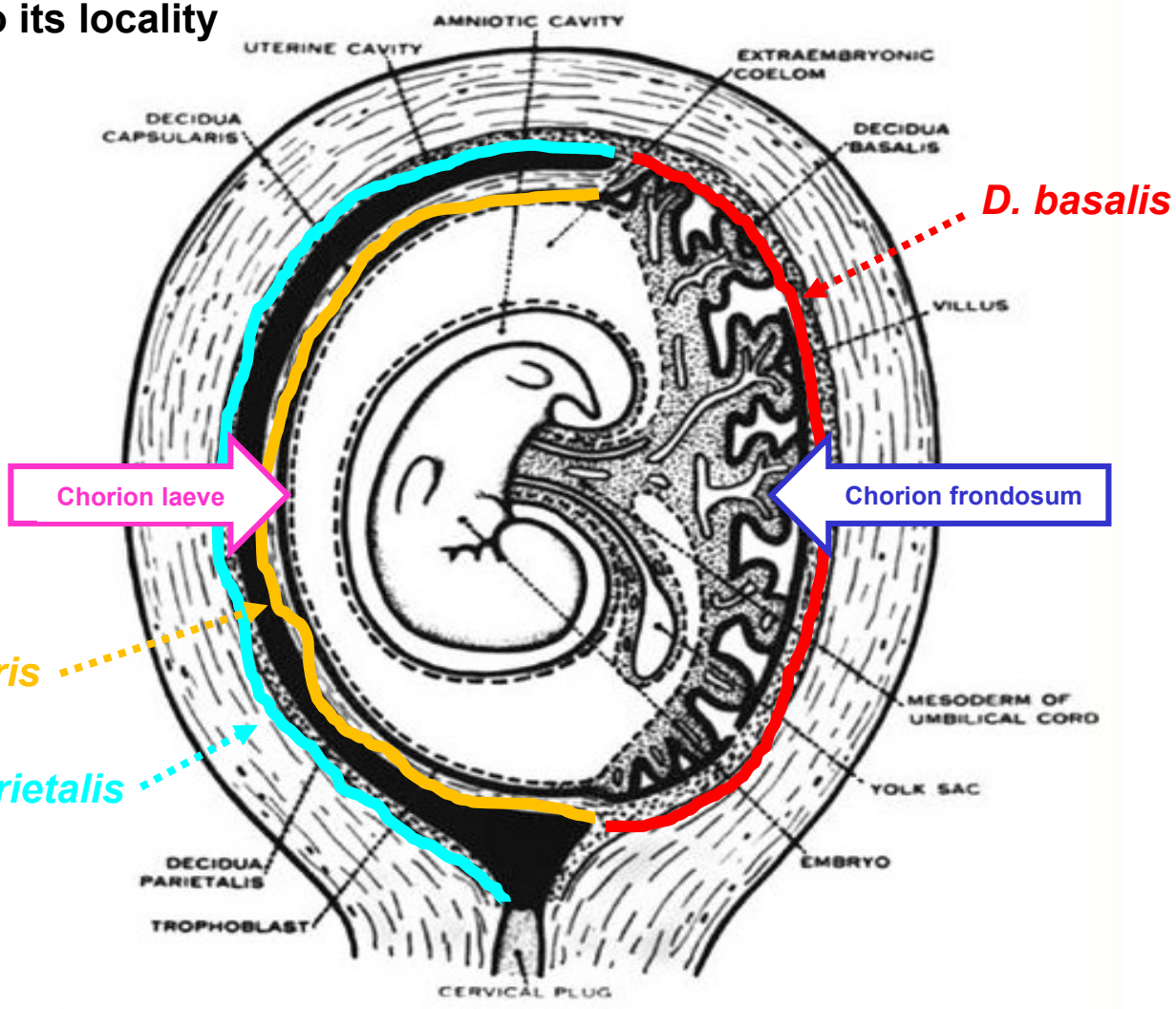
Ch. frondosum – vilous
Ch. laeve - smooth



Extraembryonal structures – chorion – decidua

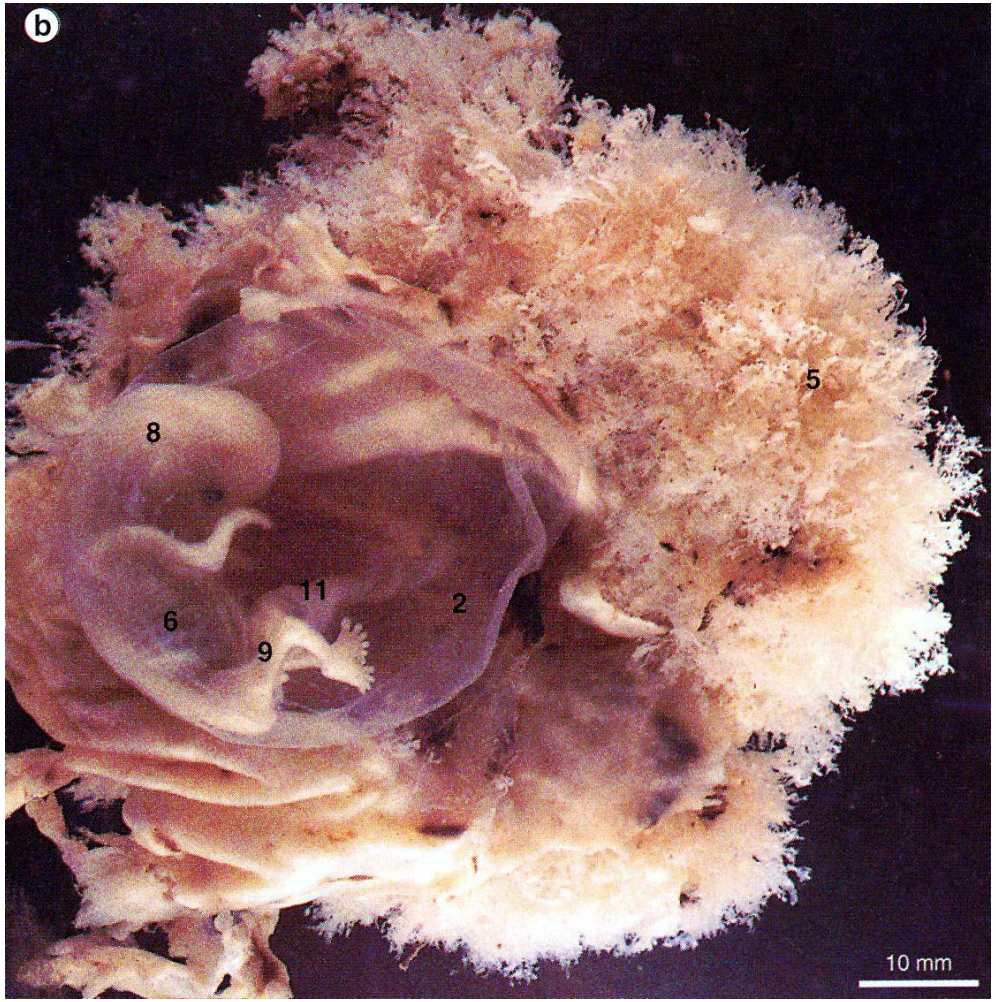
Decidua – according to its locality

- ***basalis***
- ***capsularis***
- ***parietalis***

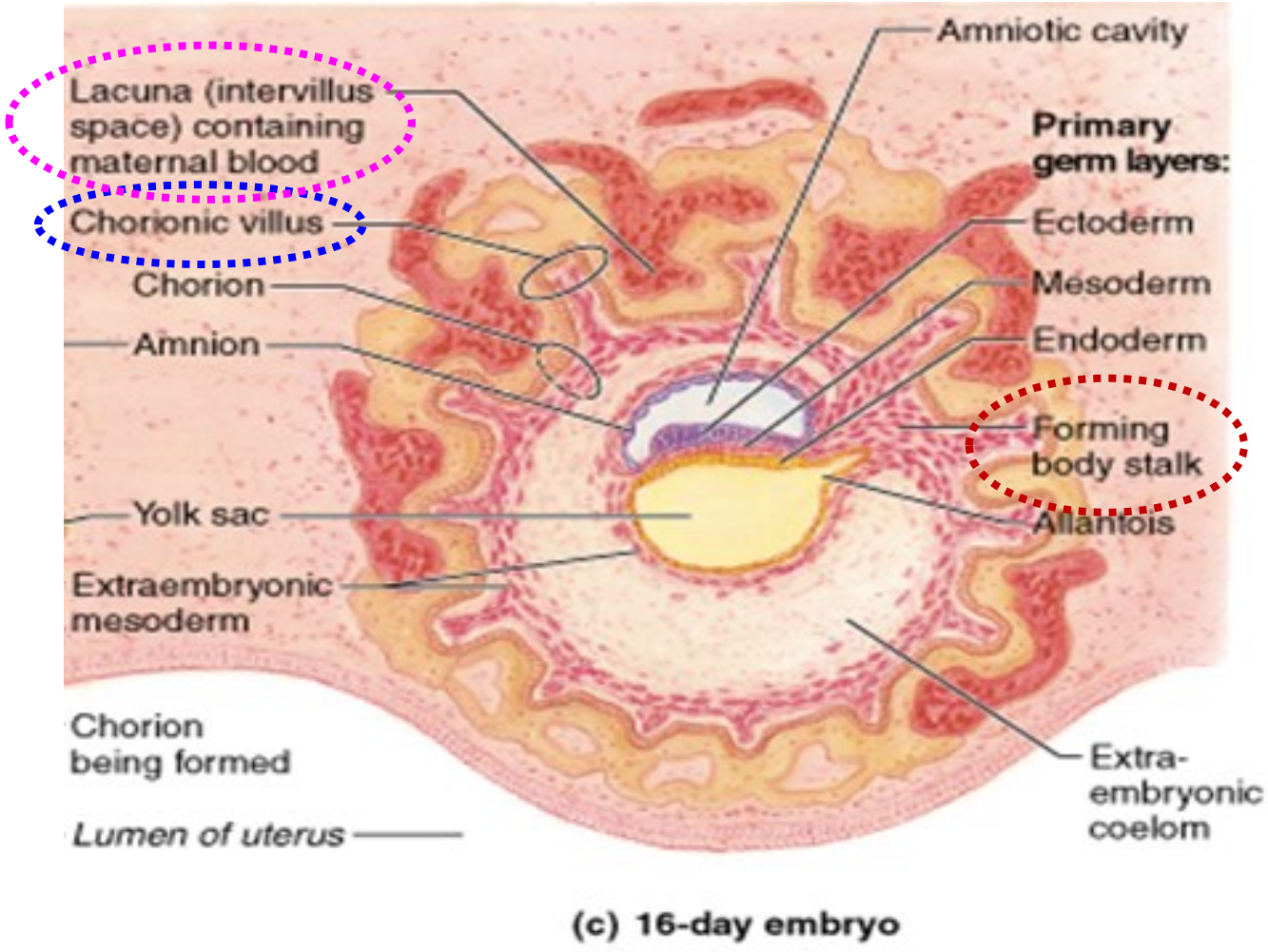


Decidua basalis – between embryo and myometrium
Decidua capsularis – between embryo and uterine cavity (becomes thinner)
Decidua cparietalis – opposite wall of uterus

Extraembryonal structures – chorion – decidua



Extraembryonal structures – chorion – placenta



(c) 16-day embryo

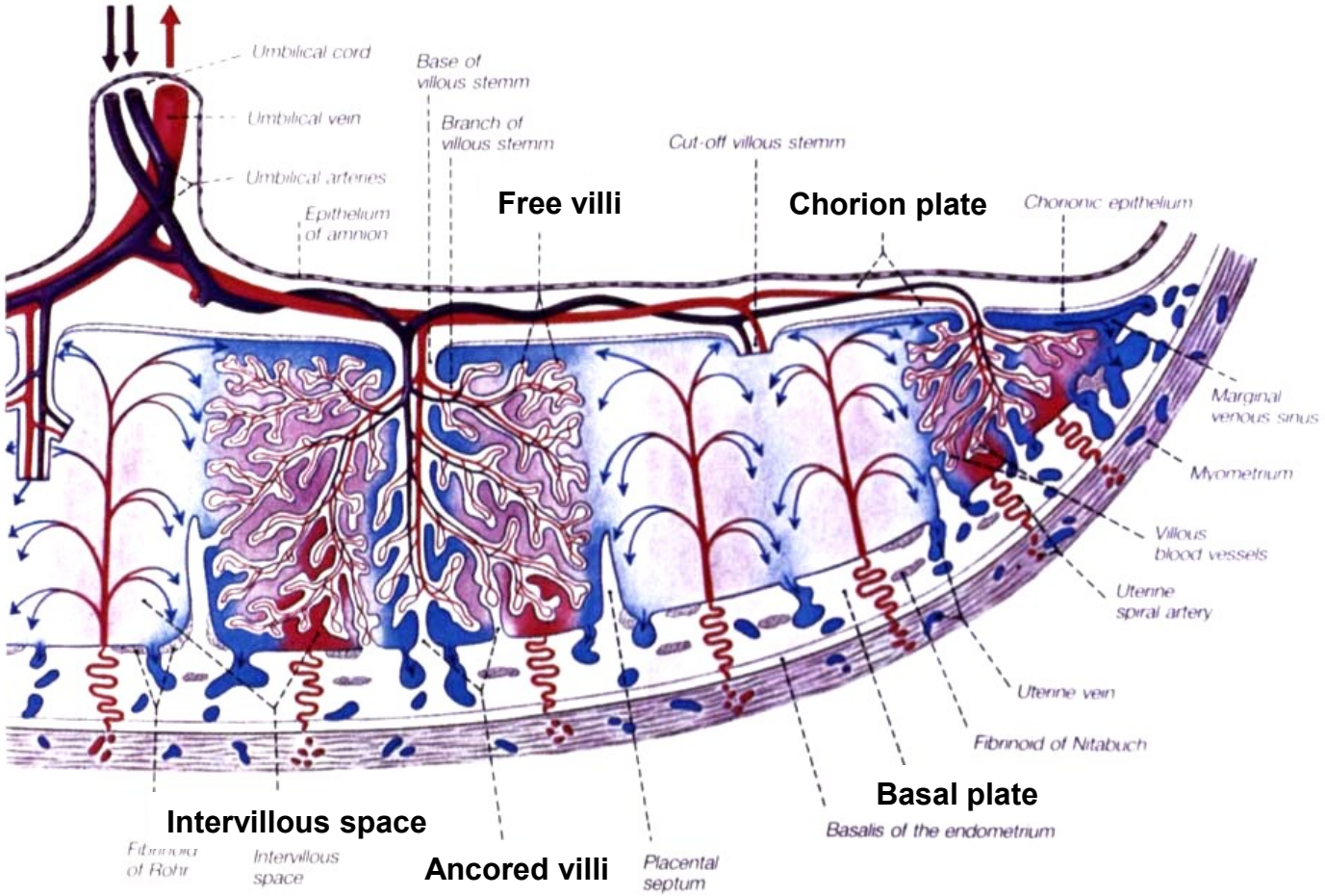
Chorionic villi - finger like projection of embryonic tissue that come in contact with bleeding endometrium
Decidual cells – fibroblast of endometrium (large, cuboidal, very active proteosynthesis)
Placenta – thick disk made by decidua and chorionic villi (formed at the start of month 4)

discoid
15 – 20 cm
400 – 600 g

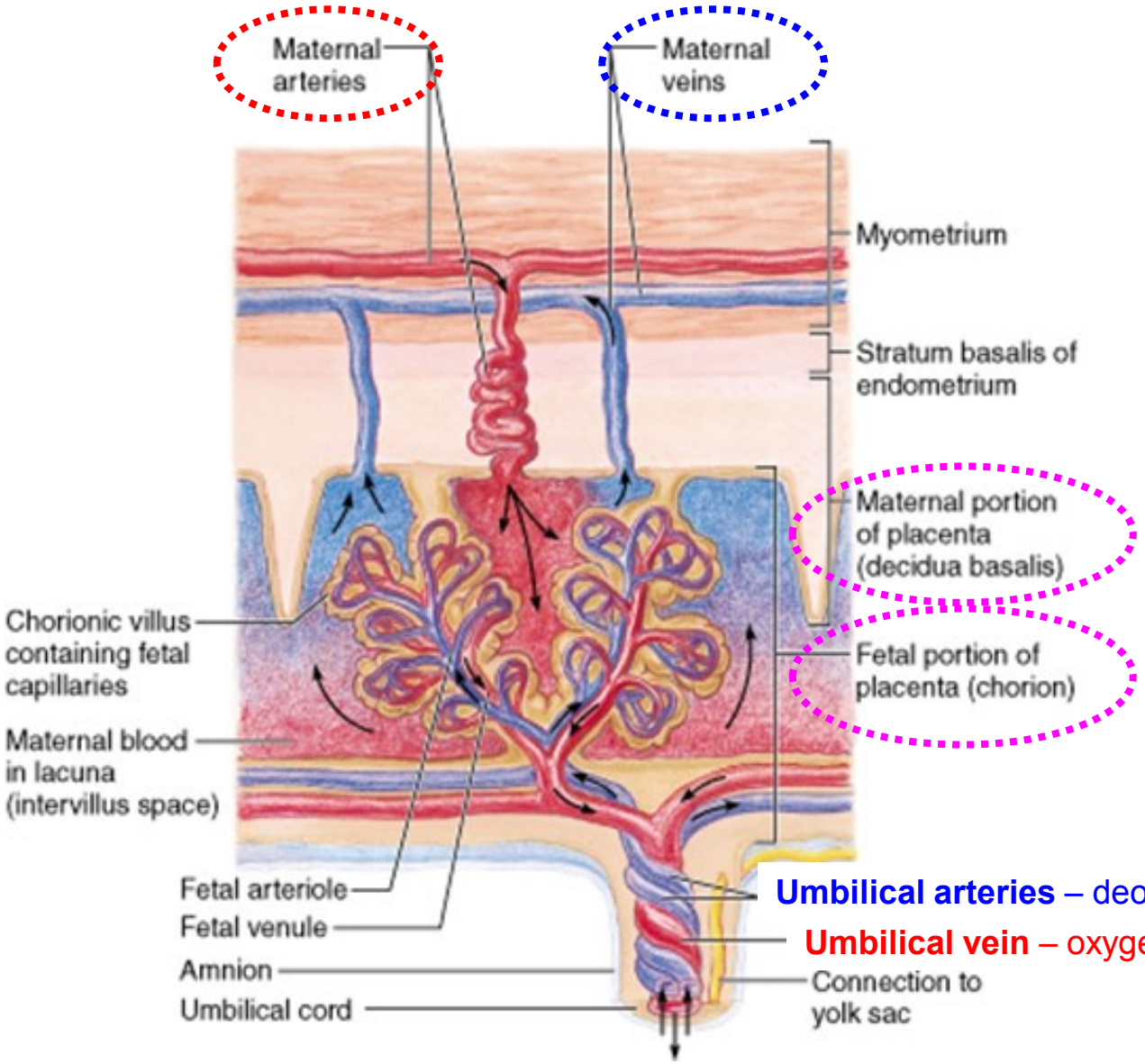
Placenta

Discoidalis + Hemochorialis

- **pars fetalis** – chorion plate, chorion villi (anchored, free = terminal)
- **pars materna** – decidua basalis
- **intervilous spaces** – develop from lacunes



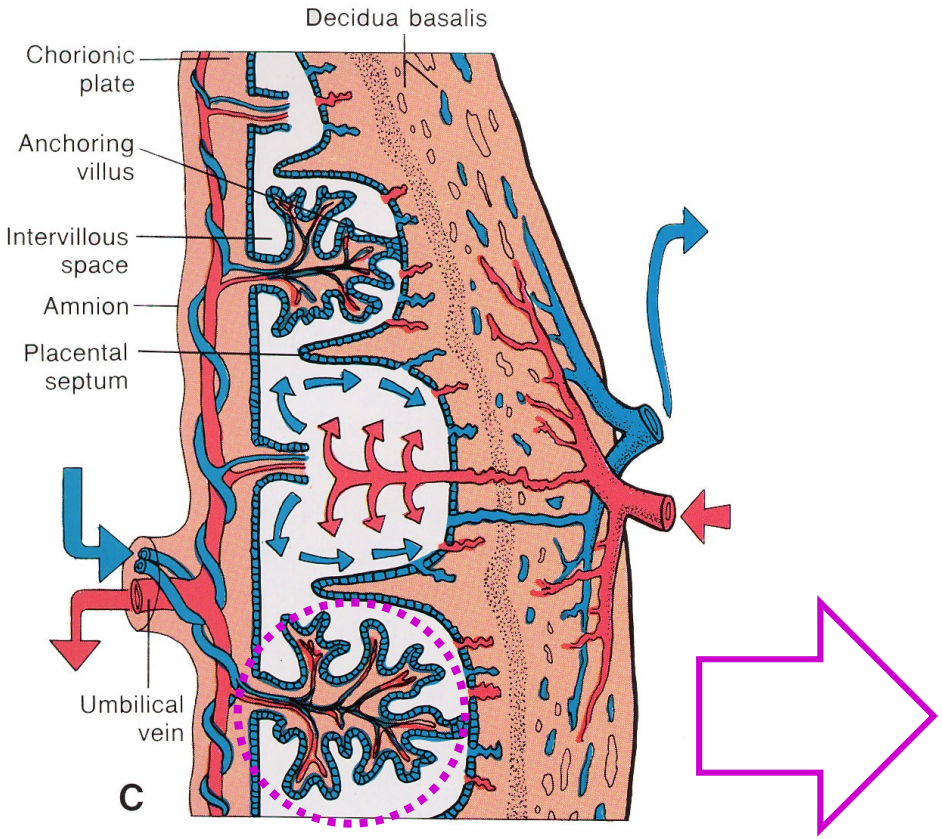
Placenta – blood circulation



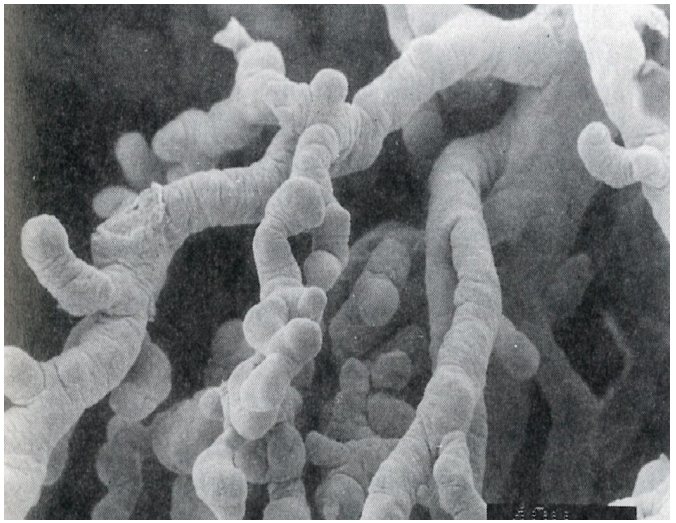
Umbilical arteries – deoxygenated blood

Umbilical vein – oxygenated blood

Placenta – terminal villi

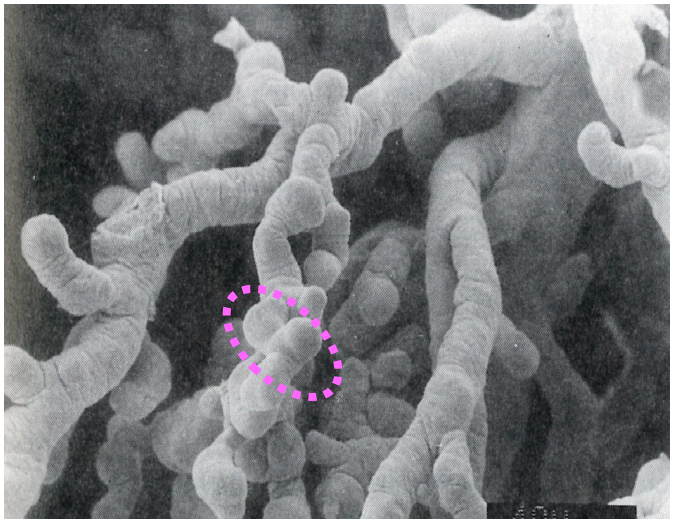


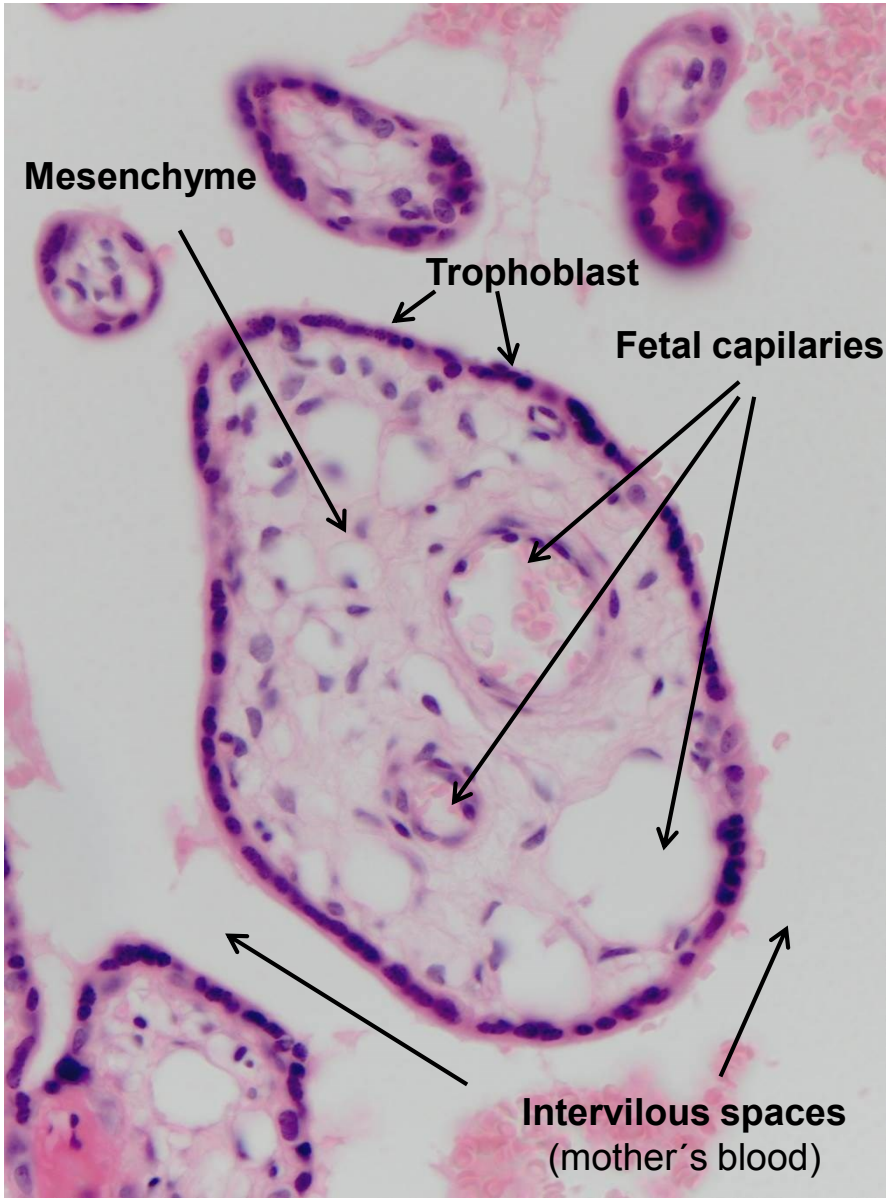
Terminal villi – human – end of pregnancy



Placenta - fetomaternal barrier 1

Terminal villi – human – end of pregnancy



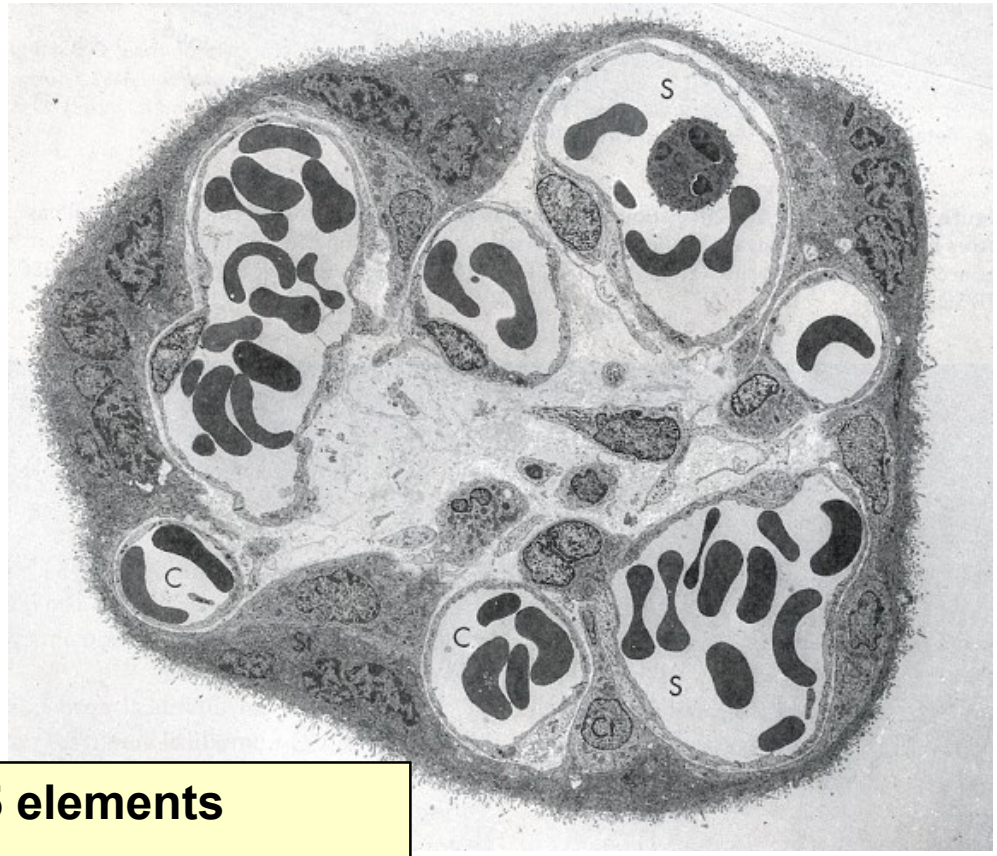


Barrier = 5 elements

- Endothelium of fetal capilaries
- Basal membrane of endothelium
- ***Mesenchyme of villi*** *(extraembr. mesoderm)*
- Basal membrane of trophoblast cells
- Cells of cyto- a syncytio-trophoblast

since month 5 cytotrophoblast looses its continuity

Placenta - fetomaternal barrier 2



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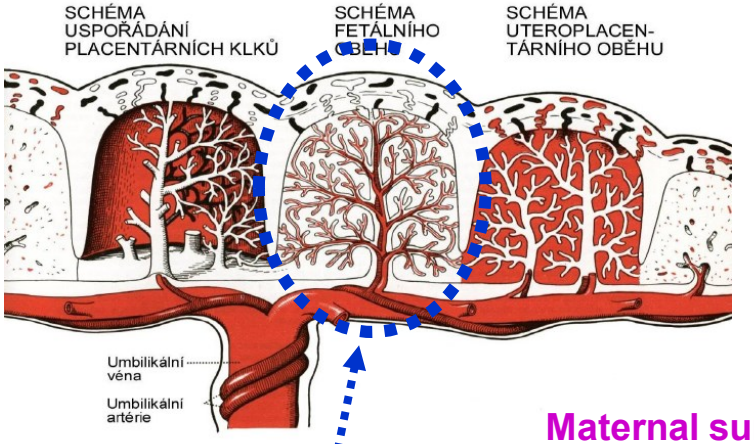
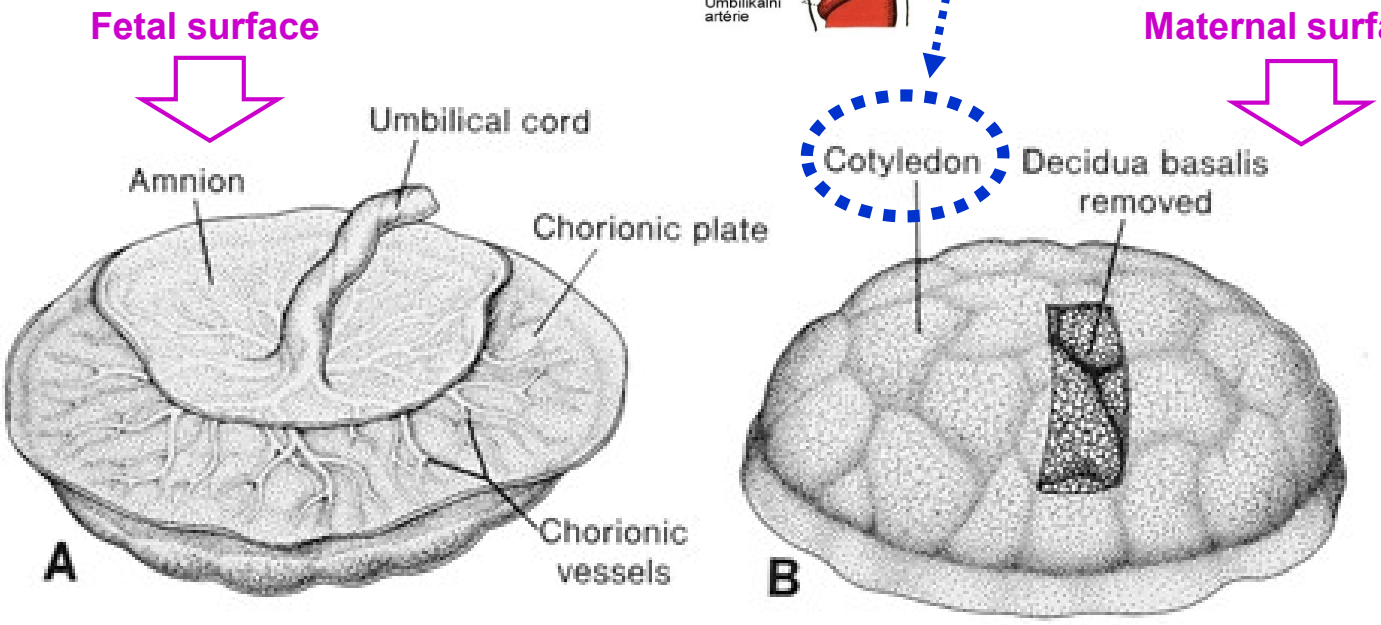
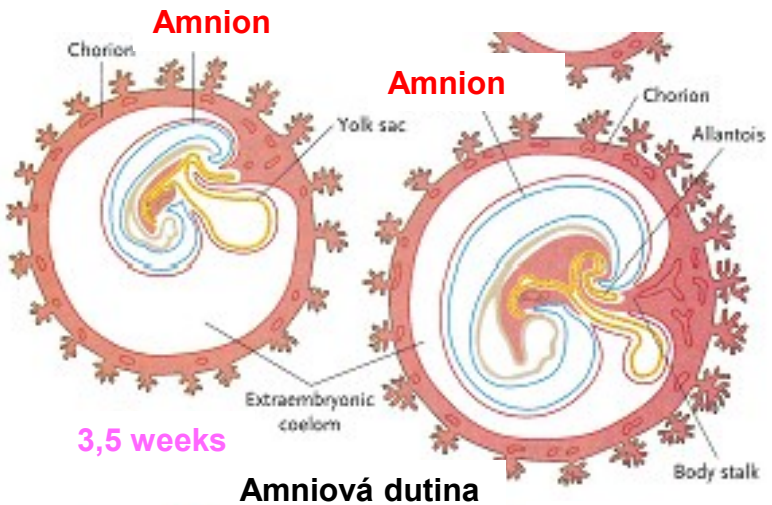
since month 5 cytotrophoblast looses its continuity

Placenta - cotyledons

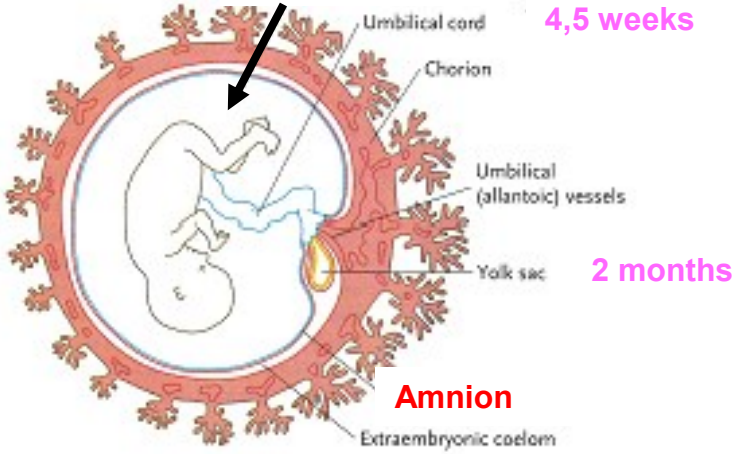




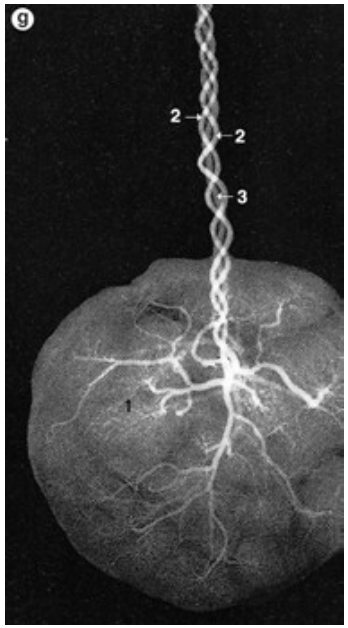
Placenta – umbilical cord 1

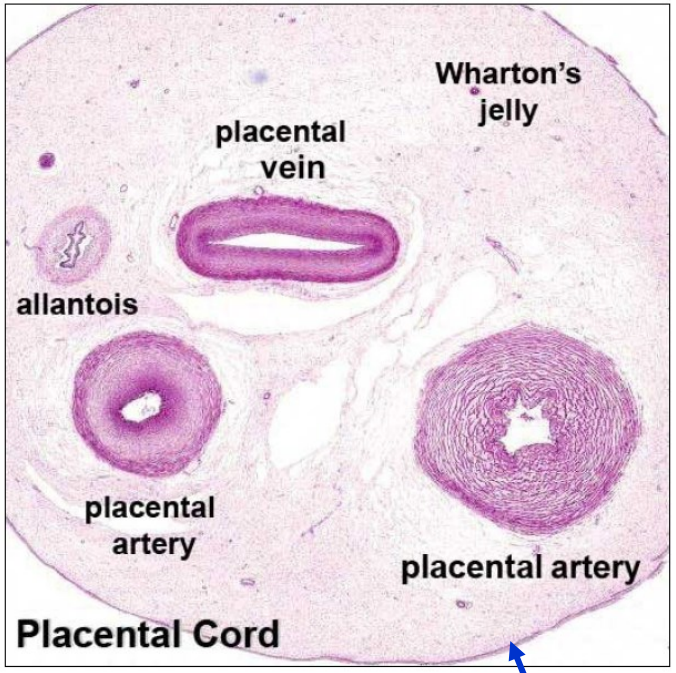


Amniová dutina



Amnionic cavity
(amnionic fluid)

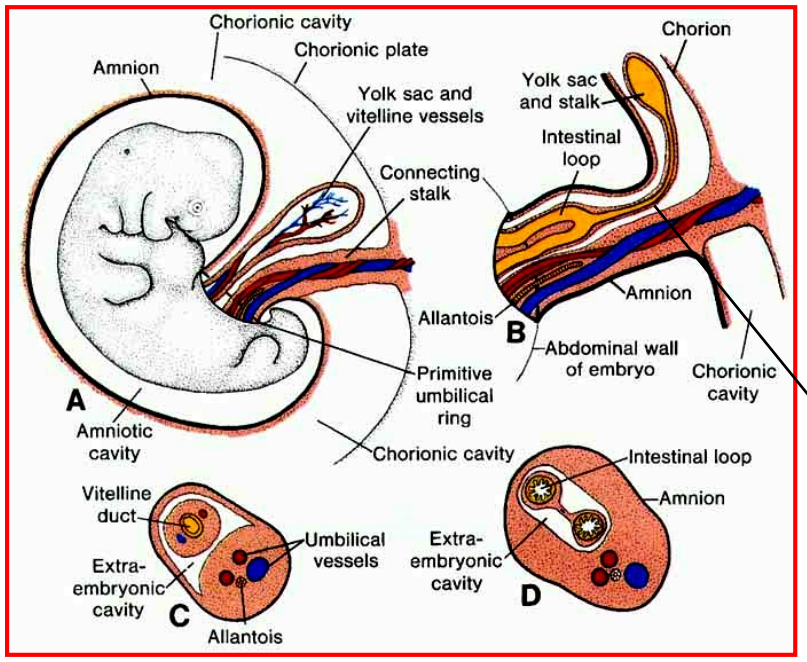


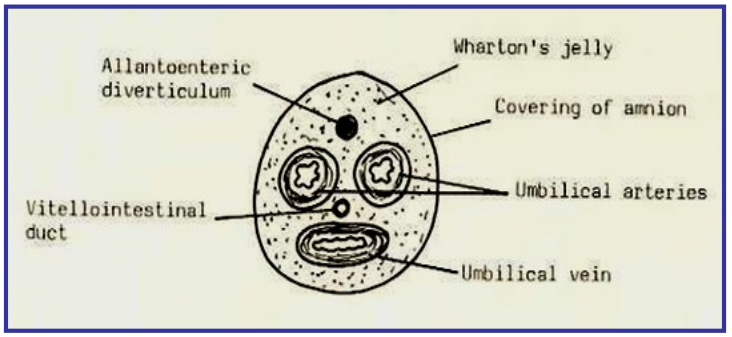


Amnionic ectoderm

- **diameter 1,5 to 2 cm**
- **length 50 to 60 cm**
- **1x vein + 2x artery (**spiral organization)
- **Wharton´s jelly** – loose connective tissue

Placenta – umbilical cord 2





1-Connecting stalk:

Allantois

Umbilical vessels (two arteries & one vein), they all embedded in

Wharton's jelly (extra embryonic mesoderm)

2-Yolk stalk (Vitello-intestinal duct):

(Ductus omphaloentericus)

A narrow, elongated duct which connects gut to yolk sac

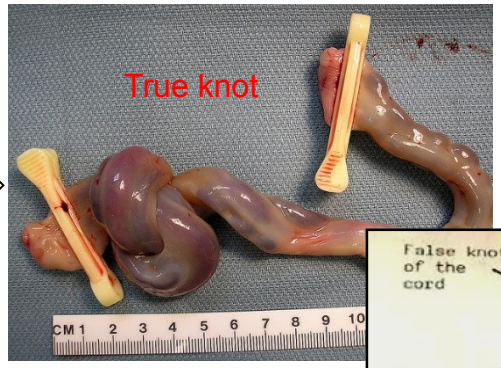
It contains **Vitelline Vessels**

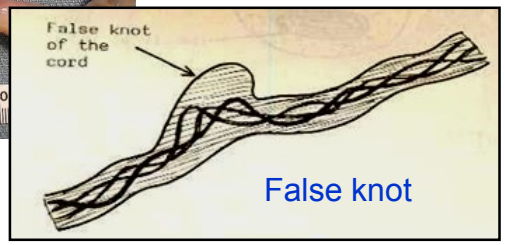
(Later on , it is obliterated and the vitelline vessels disappear).

Umbilical cord - anomalies

- **Short umb. cord < 40 cm**
- **Long umb. cord > 60 cm**
- **Absence of one artery** – fetal hypotrophy

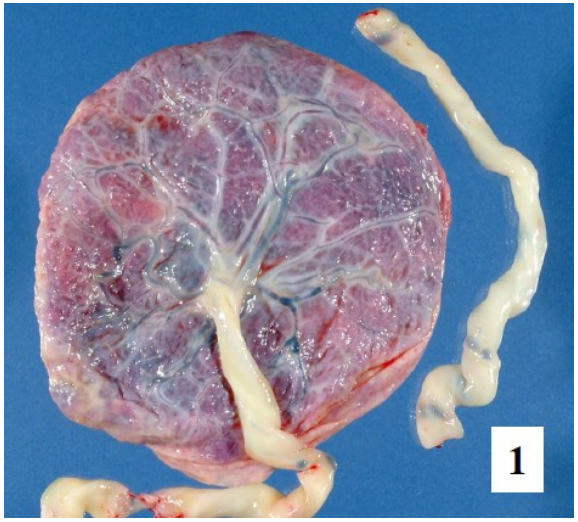
True knot
Fetal strangulation
Umbilical prolapsus



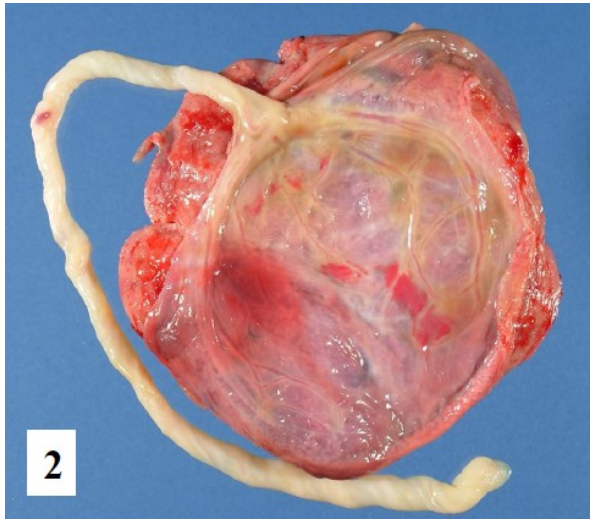


Attachment of umbilical cord to placenta

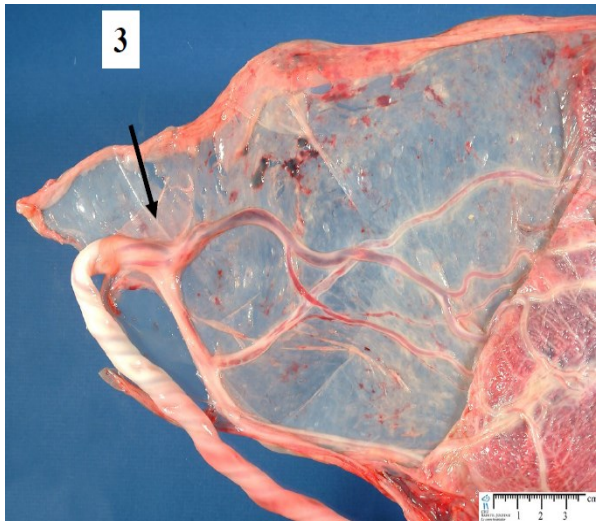
Insertio centralis (norm)



Insertio marginalis



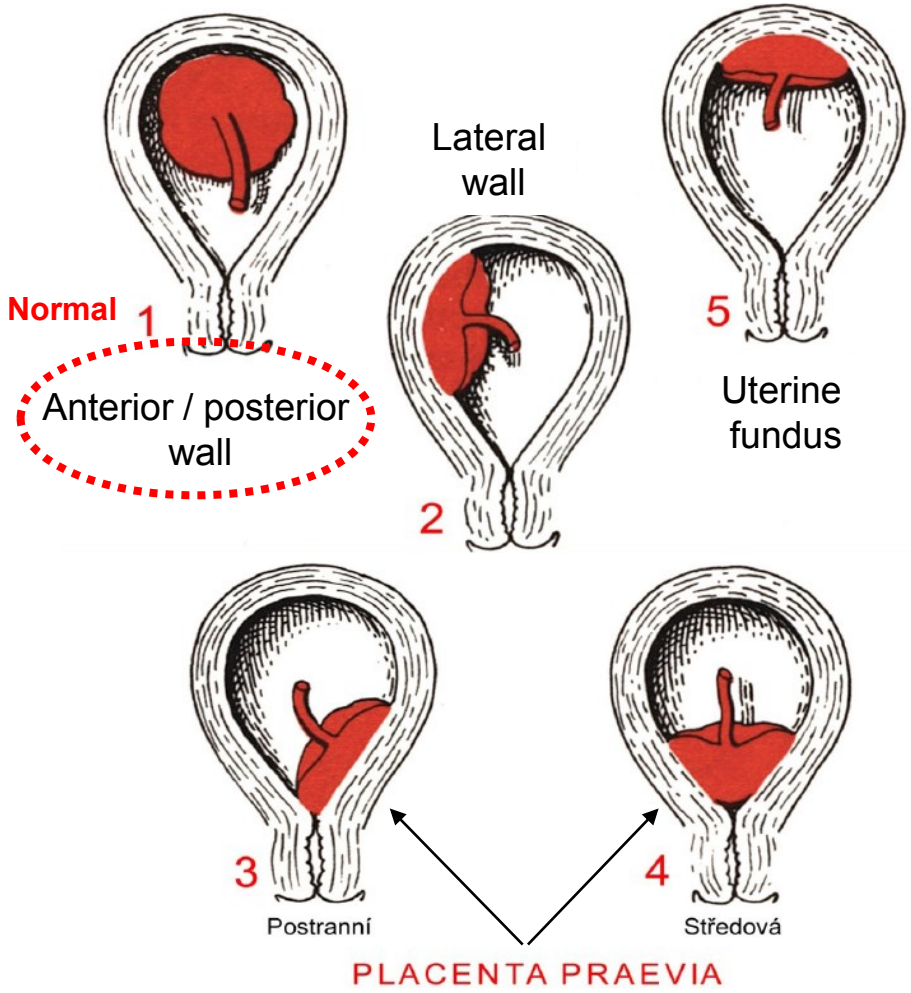
Insertio veluminosa (to chorion laeve)



Placenta – anomalies 1

Location of placenta in uterus

(1 to 5 according to frequency)



Attachment of placenta

(related to myometrium)

- ***Placenta accreta***

  attached to myometrium

- ***Placenta increta***

  grown into myometrium

- ***Placenta percreta***

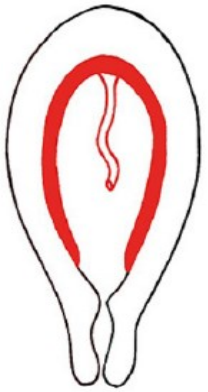
  grown through myometrium

Placenta – anomalies 2

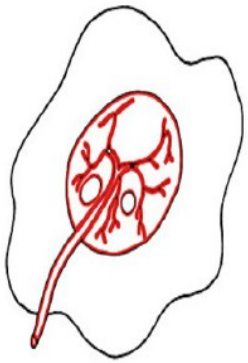
Shape and formation of placenta

Normal placenta

Placenta tripartita

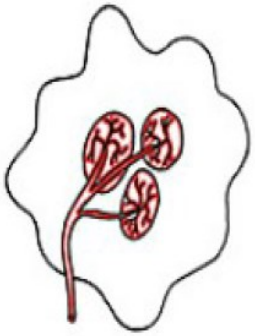


Placenta membranacea

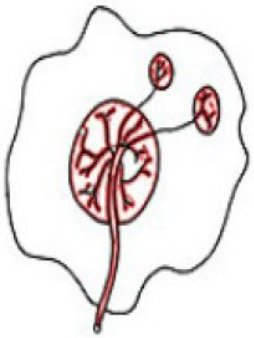


Placenta fenestrata

Placenta duplex



Placenta triplex



Placenta succenturiata

Placenta – multiparous pregnancy 1

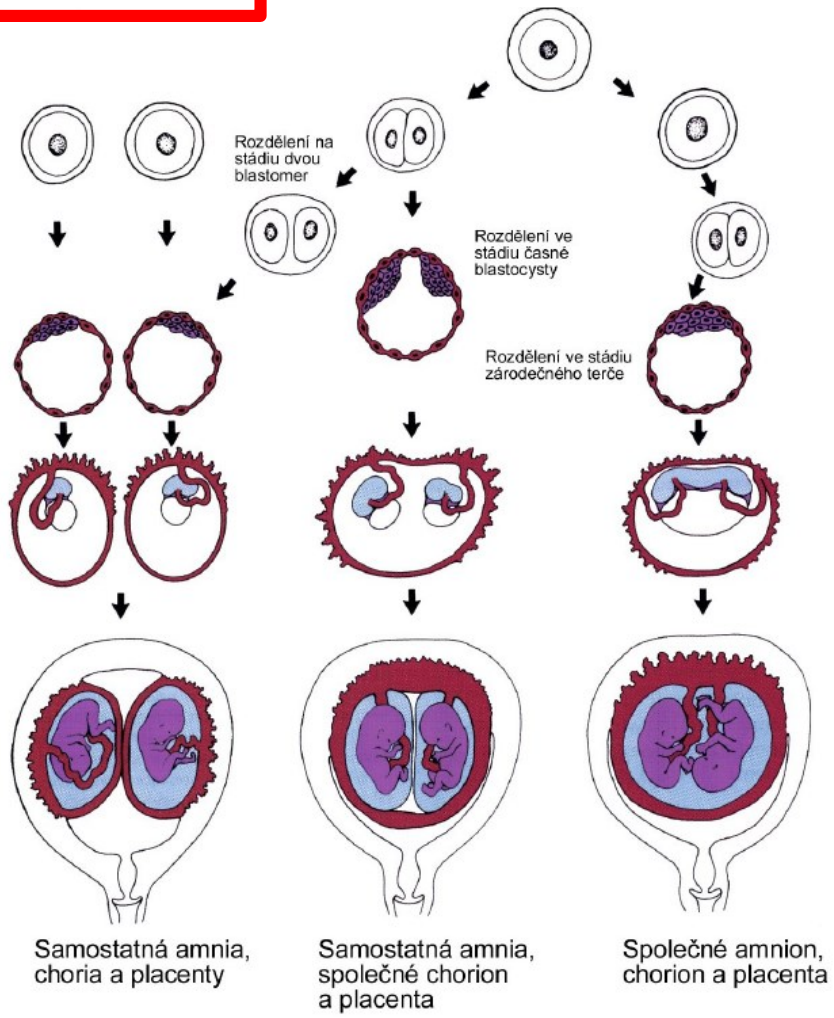
2 oocytes + 2 sperms

2x amnion + 2x chorion + 2x placenta

TWINS

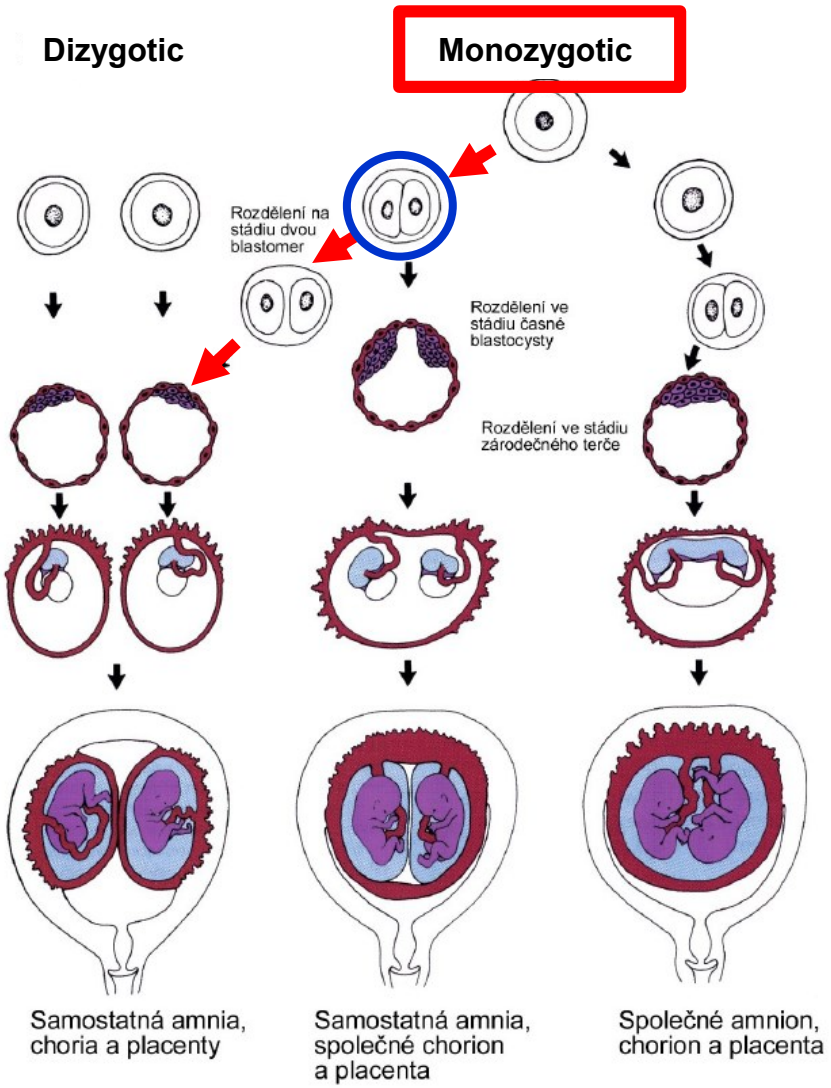
Dizygotic

Monozygotic



Placenta – multiparous pregnancy 2

TWINS



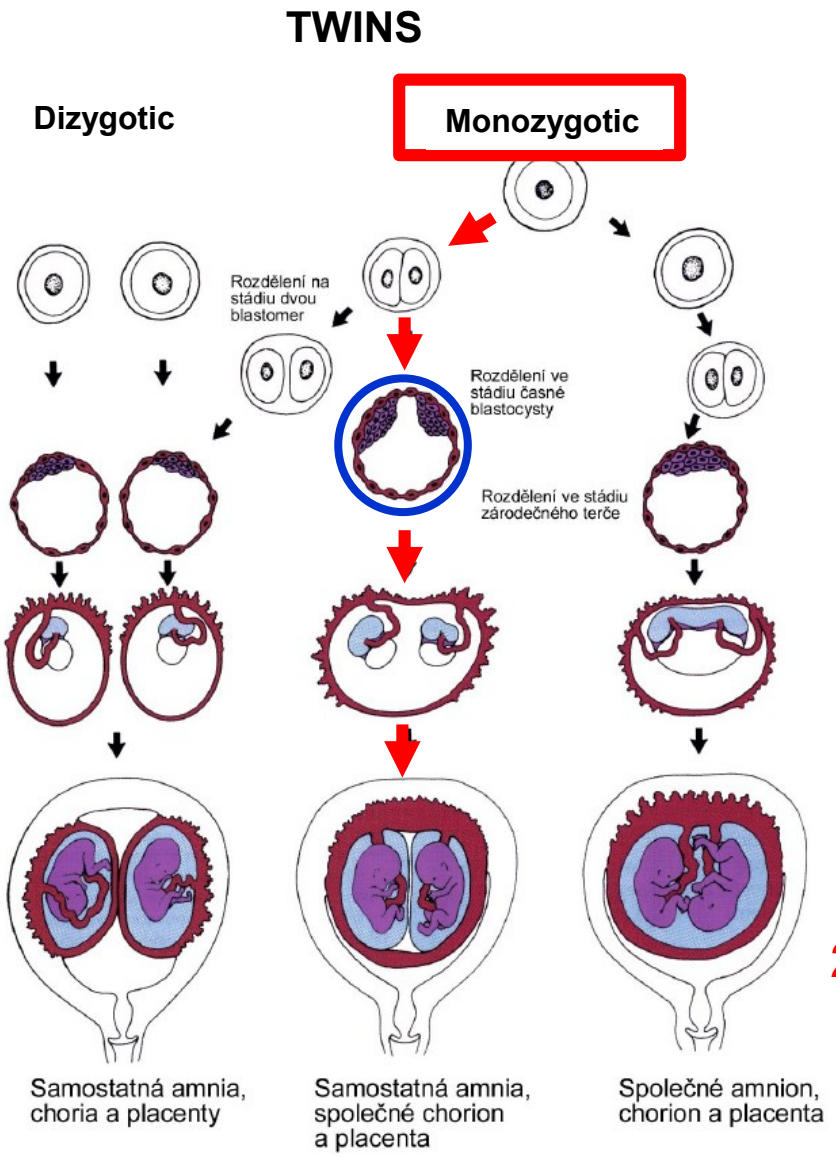
1 oocyte + 1 sperm

Embryo splits at 2-cell stage

2x amnion + 2x chorion + 2x placenta

(as dizygotic twins)

Placenta – multiparous pregnancy 3



Most frequent twins – 65 %

1 oocyte + 1 sperm

Embryo splits at blastocyst stage

Trophoblast common to both embryos

2x amnion + 1x chorion + 1x placenta

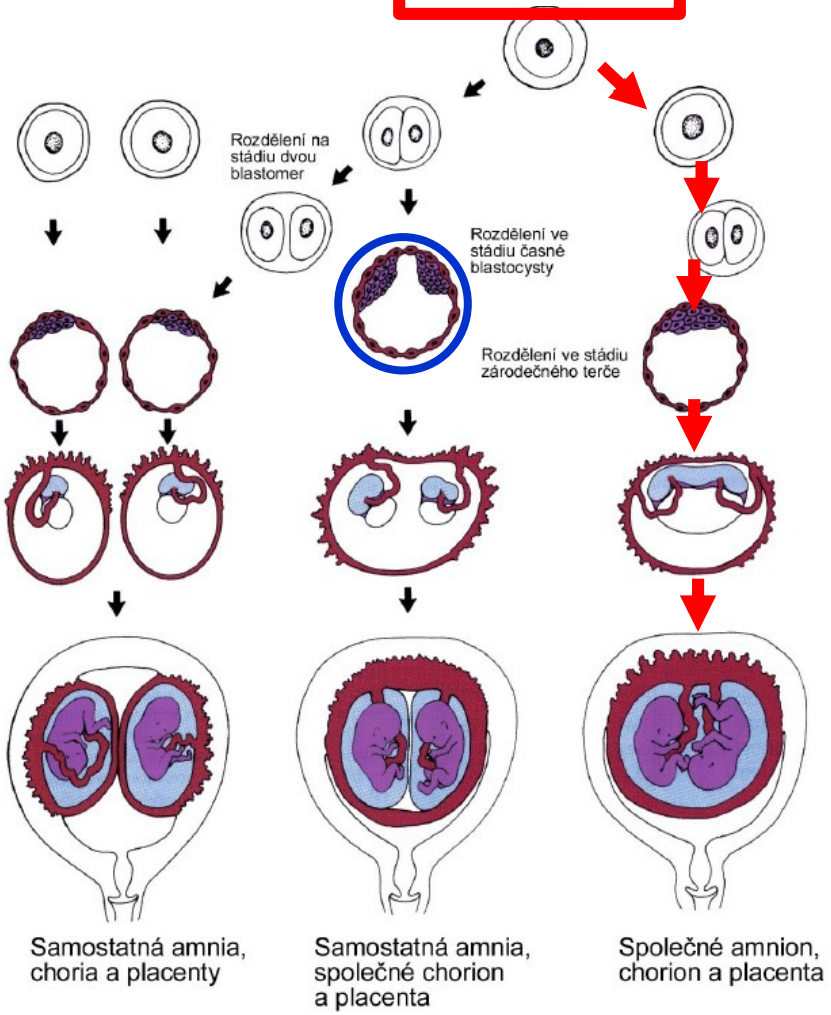
(monochorial, diamniotic)

Placenta – multiparous pregnancy 4

TWINS

Dizygotic

Monozygotic



1 oocyte + 1 sperm

Embryo splits at bilaminr stage

Trophoblast and amnion are common to both embryos

1x amnion + 1x chorion + 1x placenta

(monochorial, monamniotic)

Thank you for your attention !

Questions and comments at:
ahampl@med.muni.cz