

# Reproductive biology and Embryology

## **Gametes**

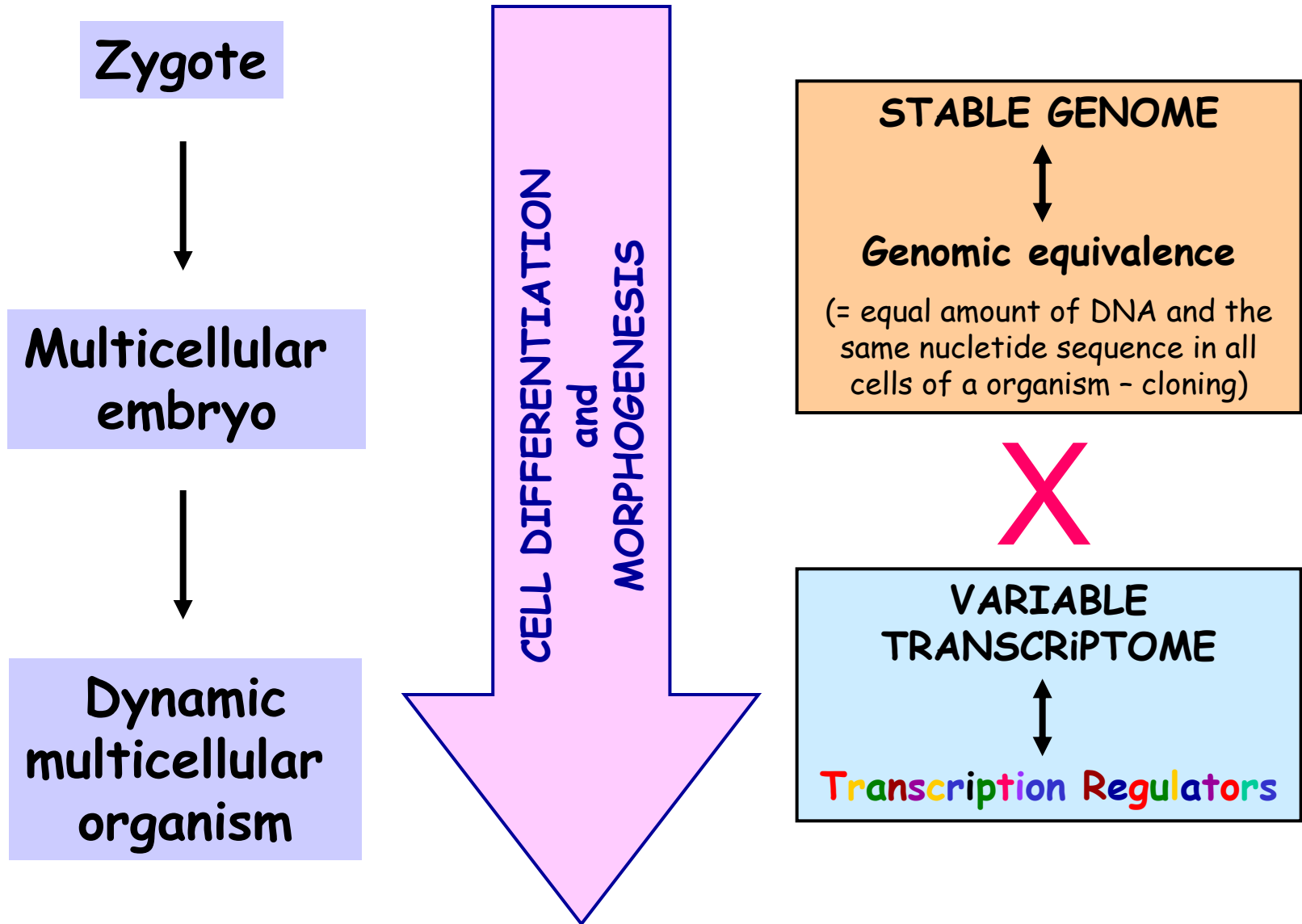
- Meiosis
- Structure and development
- Differences between oogenesis and spermatogenesis
- Regulation of gametogenesis
- Ovarian and menstrual cycles
- Ovulation
- Transport of gametes, sperm capacitation, acrosome reaction

## **Fertilization and Early Embryogenesis**

- Cortical reaction
- Cleavage, morula, blastocyst
- Activation of embryonal genome
- Embryonic stem cells, nuclear transfer (cloning)

**Brno, April 2013**

# Embryology: what does it cover?



# Embryonal x Fetal Development

Early embryo  
before implantation

All the organs  
are established

Parturition

Embryo

Fetus

Fertilization

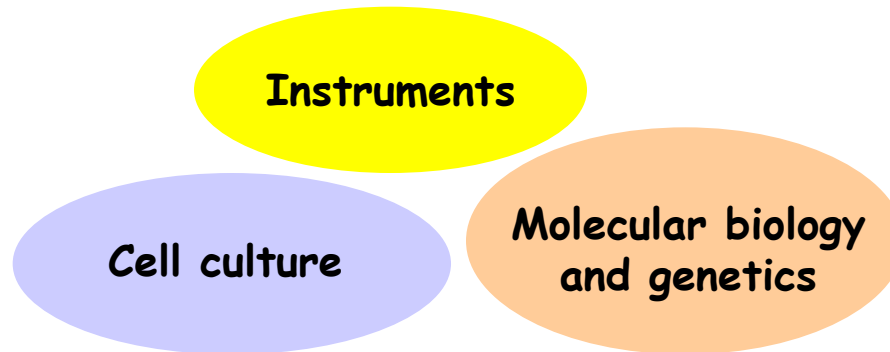
Week 8

Week  
39-40

The  
primitive  
heart  
starts  
beating at  
4 weeks.



# Any use of understanding principles of reproduction and embryonal/fetal development?



- **Infertility treatments**
- **Contraception**
- **Avoidance of developmental abnormalities**
  - Genetic basis of gamete development
  - Examination of genetic status (amniotic fluid)
  - Understanding the effects of teratogenic compounds
  - Intrauterine examination - sonography
  - Intrauterine surgeries
  - Others to come

# Reproduction

- allows for continuity of a given species via propagation of its individuals
- key element in reproduction is the transfer of DNA duplicate from parents onto progeny

Individuals of **different sex** produce **different gametes**



Key element in **sexual reproduction**

# Sexual reproduction mediated by gametes

may seem to be too complicated and much less effective than asexual but

**serves very significant adaptation role.**

This adaptation role realizes via unique genetic processes, which take place during development of gametes - eggs and sperm.

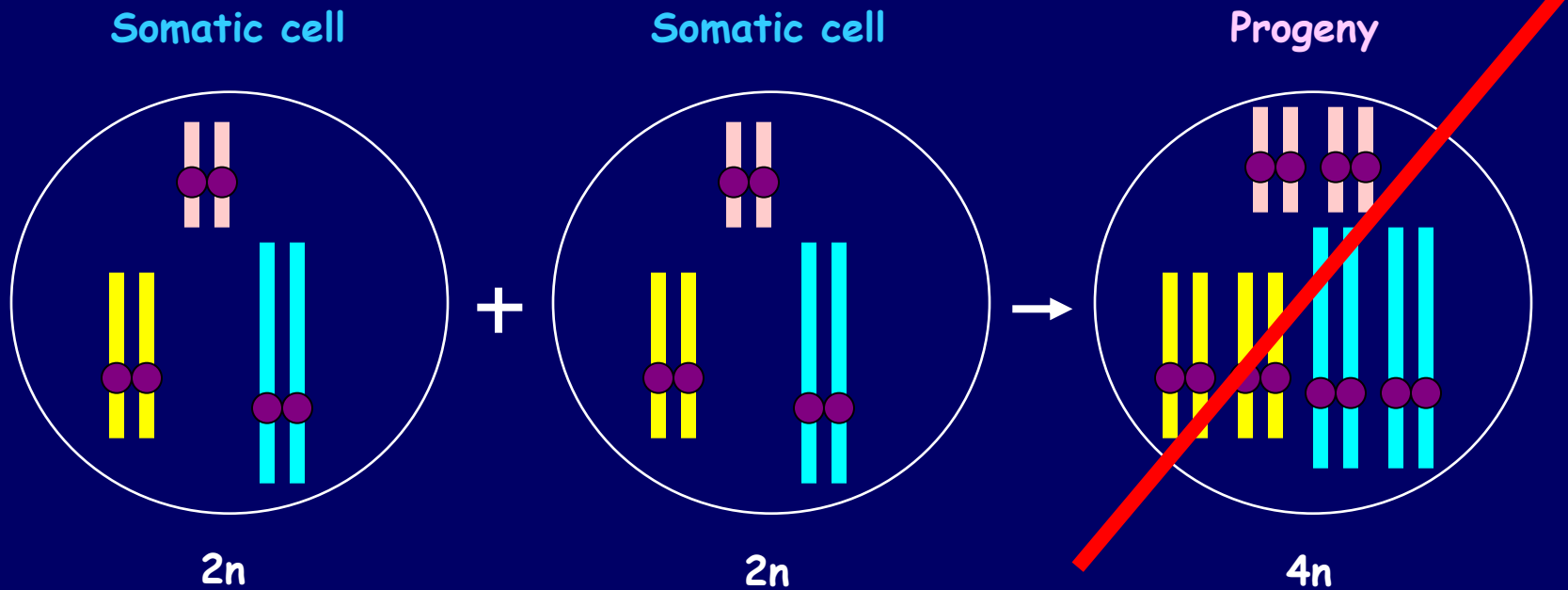
Although development of eggs and sperm differ in many morphogenetic details, key genetic processes taking place in both types of gametes are principally the same.

Genetic processes that are crucial for gametogenesis take place during meiotic cell division - MEIOSIS

These genetic processes include:

- Reduction of the number of chromosomes
- Independent segregation chromosomes
- „Crossing over“

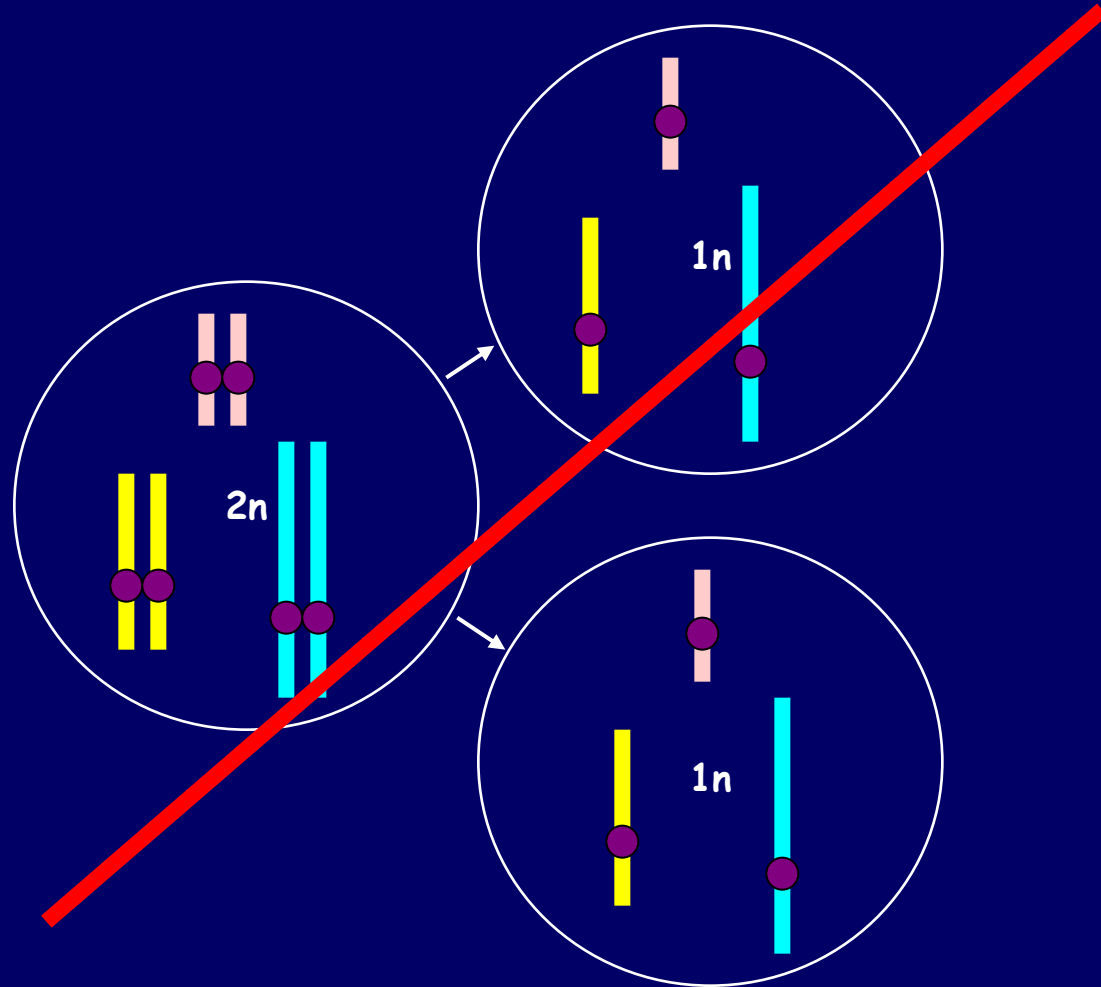
# Reduction of the number of chromosomes Why?

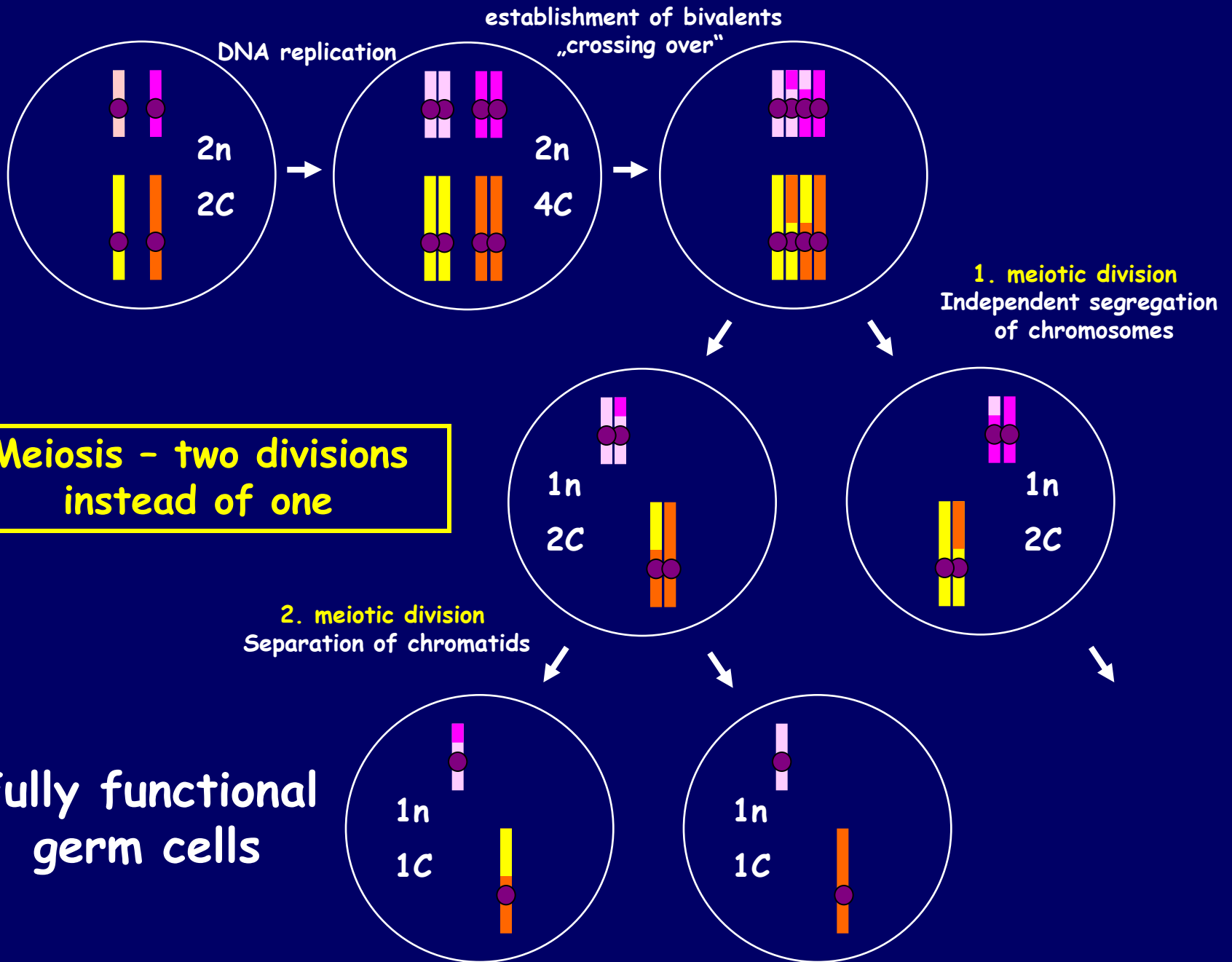


Gametes have to contain haploid number of chromosomes ( $n$ ) in order to prevent multiplication of chromosomes in progeny above a diploid number ( $2n$ )



In principle, the number of chromosomes could be reduced in one step by just separating homologous chromosomes without preceding replication of DNA (DNA synthesis)





- Independent segregation of chromosomes
- „Crossing over“
- Fertilization

are sources of genetical diversity, that underlies adaptation of living organisms.

S  
A  
M  
E

Genetic function

♂ Sperm

×

♀ Eggs

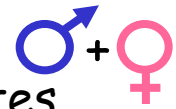
Significance for  
embryogenesis  
(reproduction)

Morphological  
and physiological  
properties

Development and  
underlying regulatory  
mechanisms


D  
I  
F  
F  
E  
R  
E  
N  
T


## Primordial germ cells - PGC



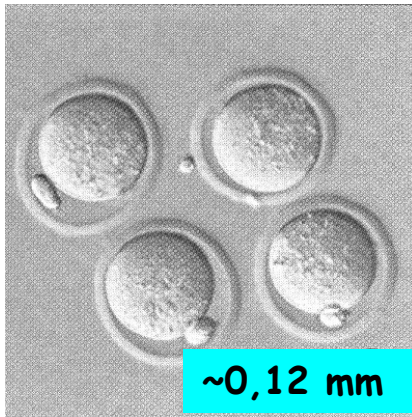
- stem cells, which are common to both sperm cells and oocytes
- originate in yolk sac (extraembryonally)
- divide mitotically while migrating into gonad anlagen (genital ridges) (due to signals from surrounding environment - laminin, kit-ligand, TGF-beta1, ...)
- in man PGCs are sexually indifferent until the 6th week of embryonic development

### DEVELOPMENTAL PROCESSES

- after reaching puberty, sperm cells are produced in testes continuously until high age (two testes of man produce about 1000 spermatozoa every second) 

- numbers of oocytes (follicles) in ovary is given at the time of birth and do not increase (in woman ~500 000) 
- only small number of oocytes develop into fertilizable eggs (in woman ~400)
- at the time of menopause, ovary contains only small number of remaining oocytes (in woman ~100-1000)

# Oocyte



One of the biggest and most „precious“  
(by both number and significance) cells.

Paradoxical cell

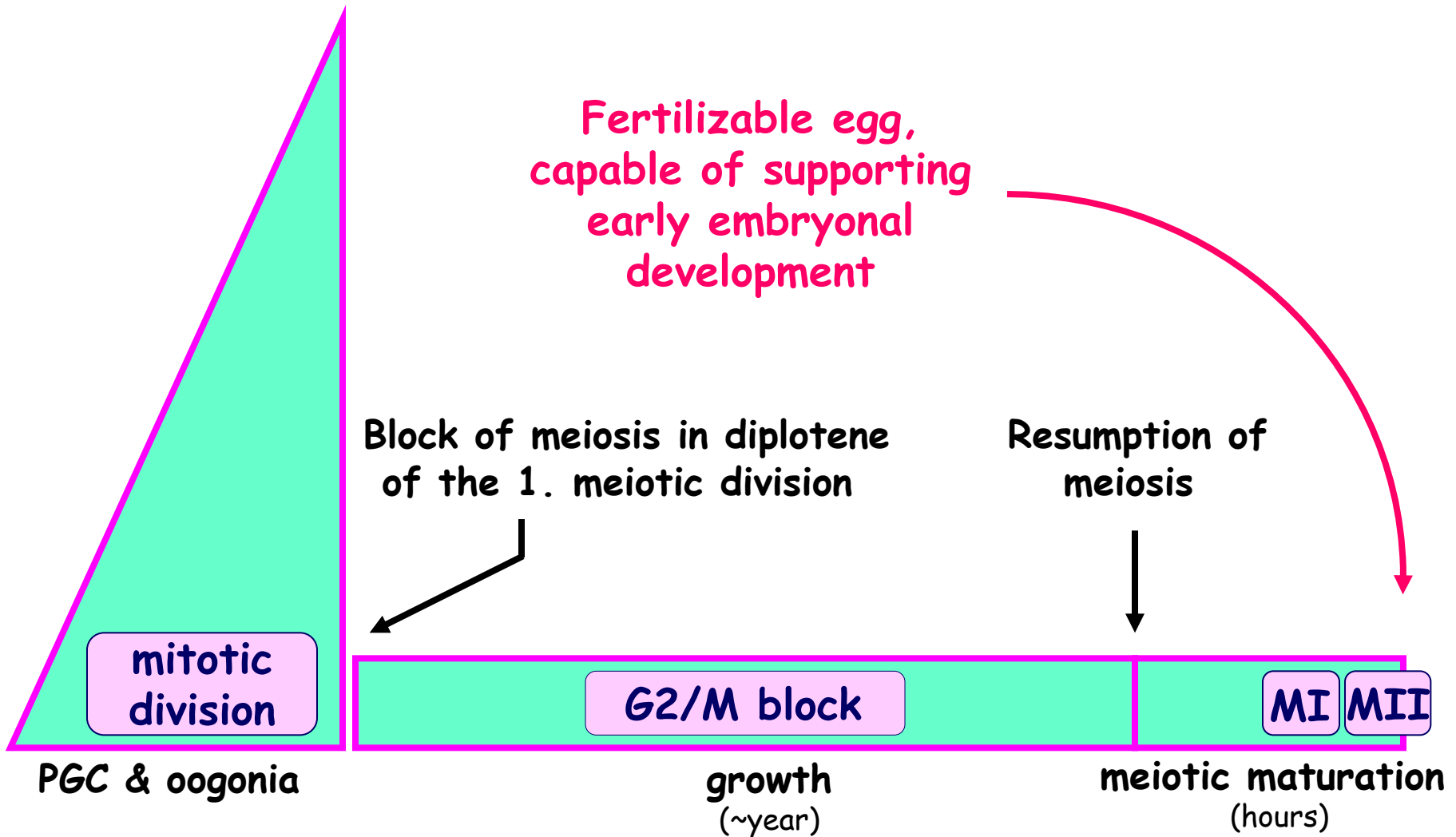
**Highly specialized cell.**  
The only cell in the female body that can undergo meiosis and fertilization, and thus give rise to a new individual.

&

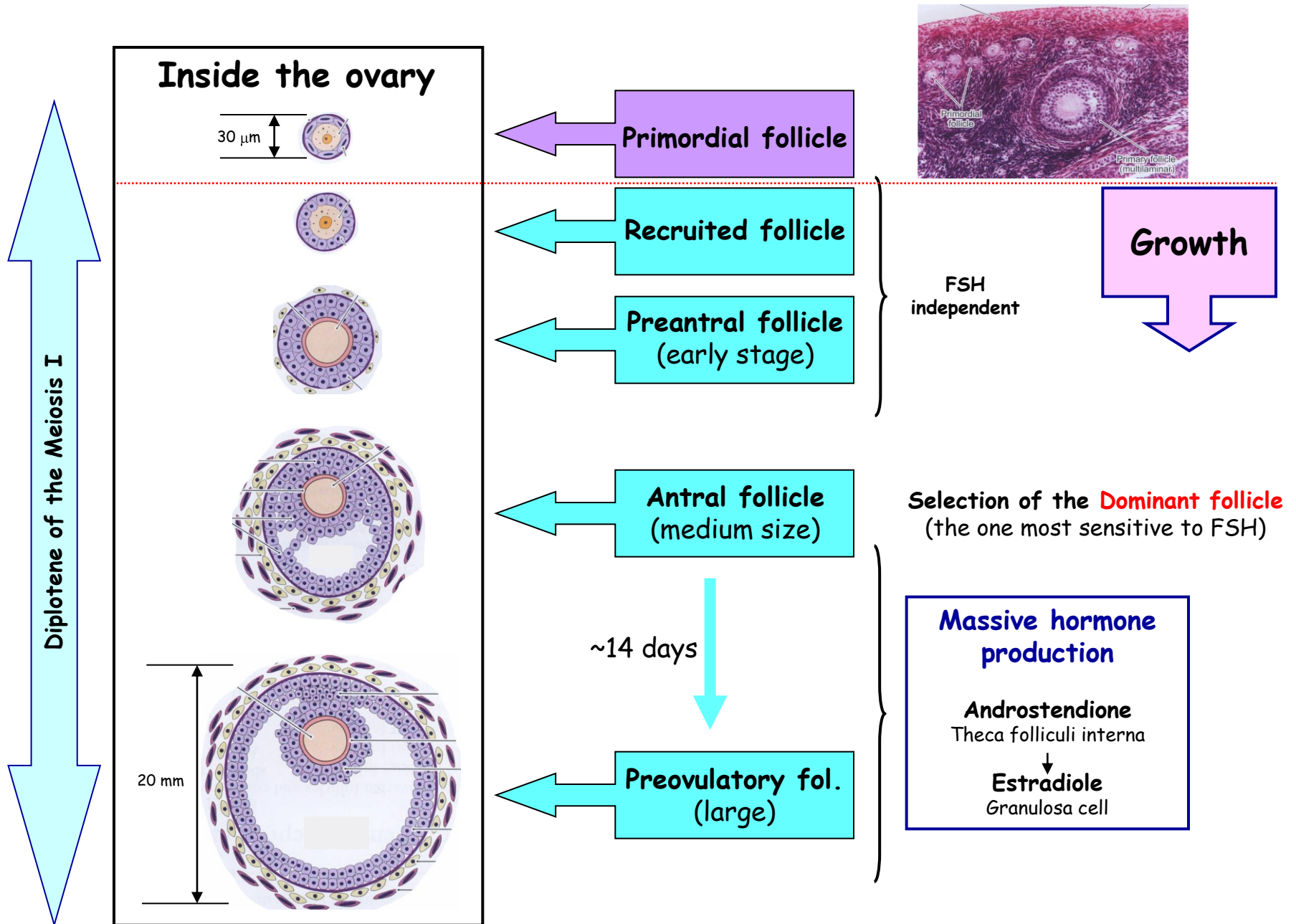
**„Totipotent“ cell**  
It can generate all the cellular diversity that is typical for multicellular organism.

**Even the era of cloning did not replace the functions of egg !**

# Key periods of oocyte development



# Where and how the oocyte development is achieved ? (1)





# Where and how the oocyte development is achieved ? (2)

## Oocyte growth

Takes place in **ovary** (along with the growth of follicle)

&

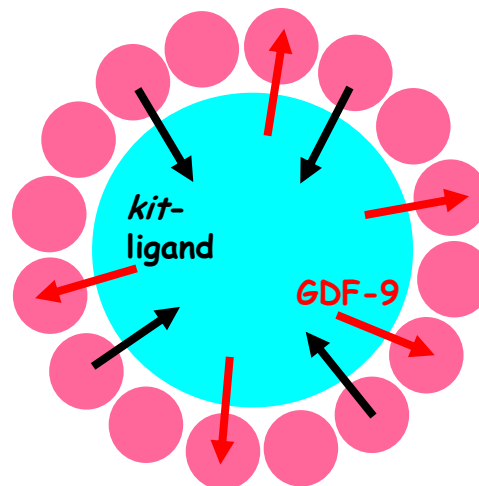
**Signal** that initiates growth is **not known**  
(it is not FSH - hypophysectomy does not prevent growth)

&

It is fully dependent on the **contact of oocyte with granulosa cells** of the follicle (mediated for example by the gap junction protein connexin-37)

&

**Communication** between oocyte and granulosa cells is **bidirectional**



# Where and how the oocyte development is achieved ? (3)

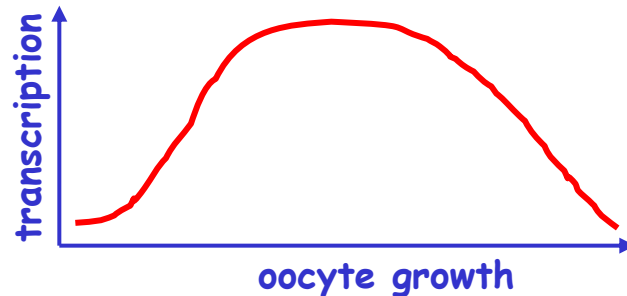
## Oocyte growth

Slow process (several months in woman)

**100x increase in volume** - **accumulation of organelles a molecules** providing egg with the ability to support early embryogenesis until reaching autonomy (about  $10^5$  mitochondria accumulated in oocyte supports embryogenesis until blastocyst stage)

**Intensive transcription** - accumulation of mRNA in dormant state (regulated by polyadenylation and ?)

Fully grown oocyte - ~2,5 ng total mRNA



Transkriptome and proteome - underlie unique properties of oocyte

**Intensive translation** - many proteins (very limited knowledge)

Example: ZP1, ZP2, ZP3 - proteins of *zona pellucida*

Fully grown oocyte - ~120 ng total protein

# Where and how the oocyte development is achieved ? (4)

## Epigenetic changes occurring during oocyte growth

### Reactivation of X chromosome

- **somatic cells** - one X chromosome is inactivated by hypermethylation of cytosine residues in molecule of DNA
- **growing oocyte** - both X chromosomes are active (crucial for oocyte development - karyotype 45, XO results in an abnormal development of ovaries)

&

### Genomic imprinting

- epigenetic modification of autosomal chromosomes that leads to monoallelic expression of genes - due to activity of enzyme DNA methyltransferase
- PGCs are globally demethylated
- imprinting is newly established during oocyte growth (about 70-80 genes)

**Abnormalities in imprinting may result in spontaneous abortions in assisted reproduction !!!**  
(*in vitro* manipulation with gametes and embryos may produce abnormalities in imprinting)

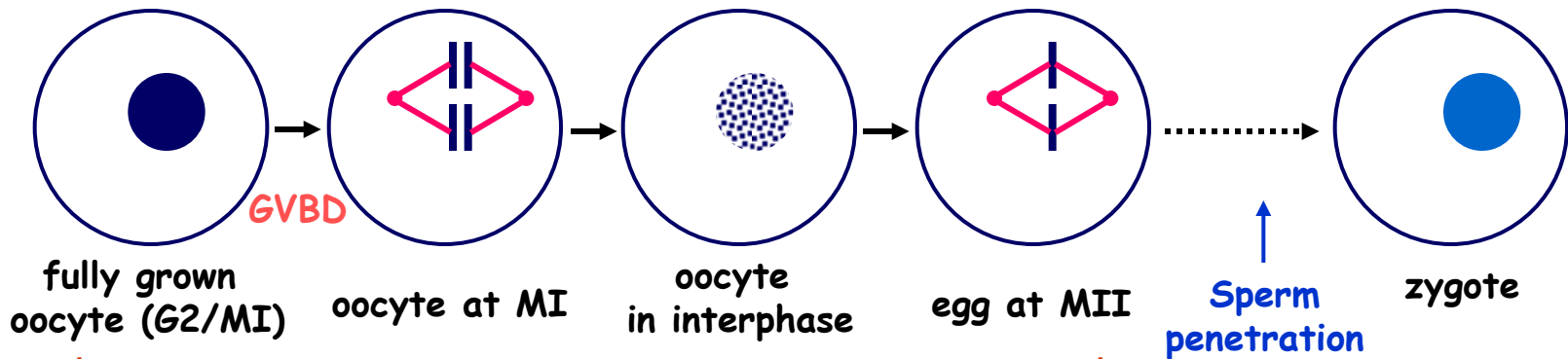
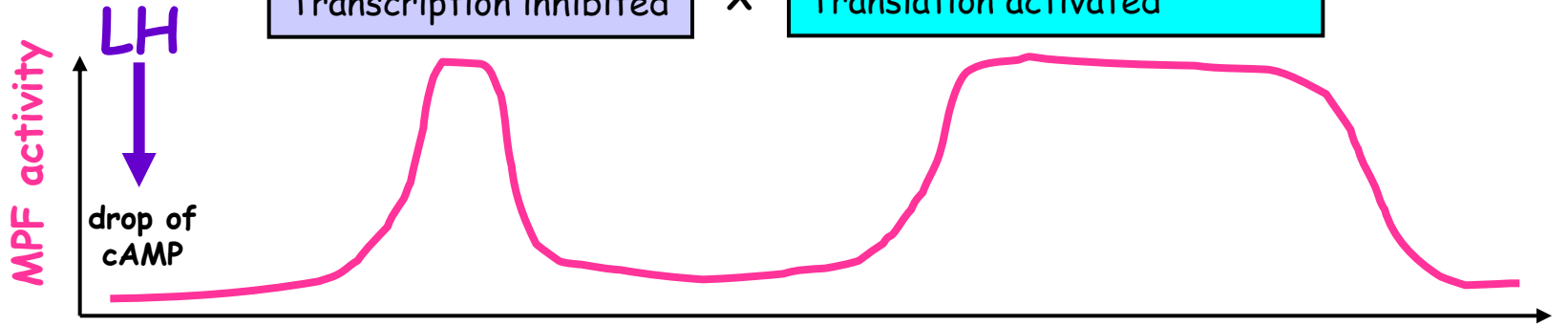
# Where and how the oocyte development is achieved ? (5)

The last hours before ovulation - meiotic maturation

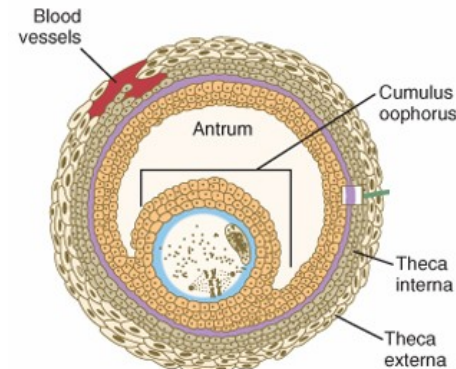
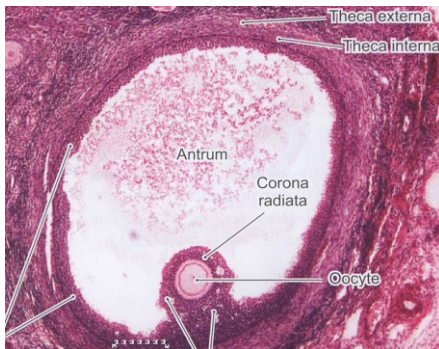
Transcription inhibited

X

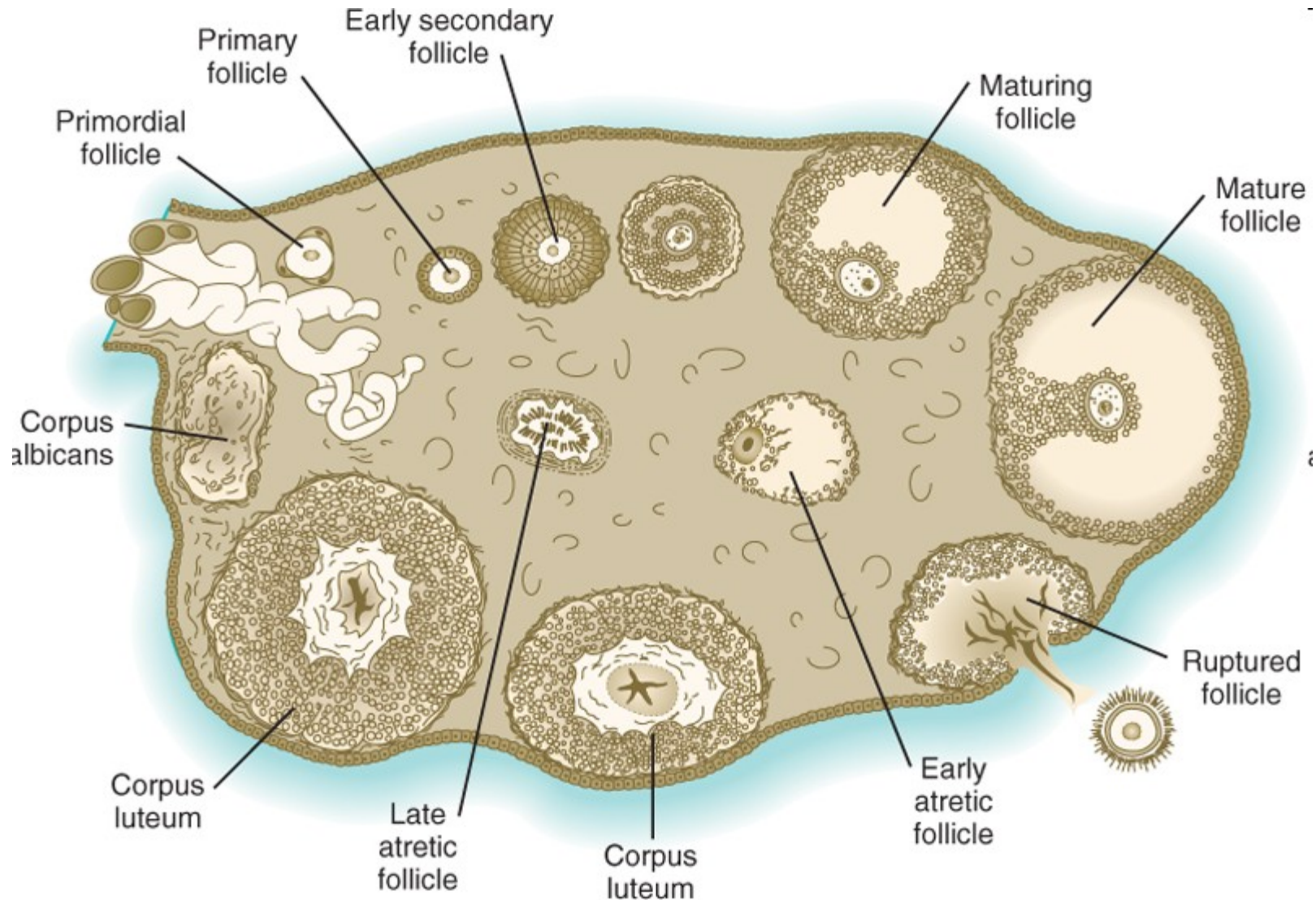
Translation activated



25 hours

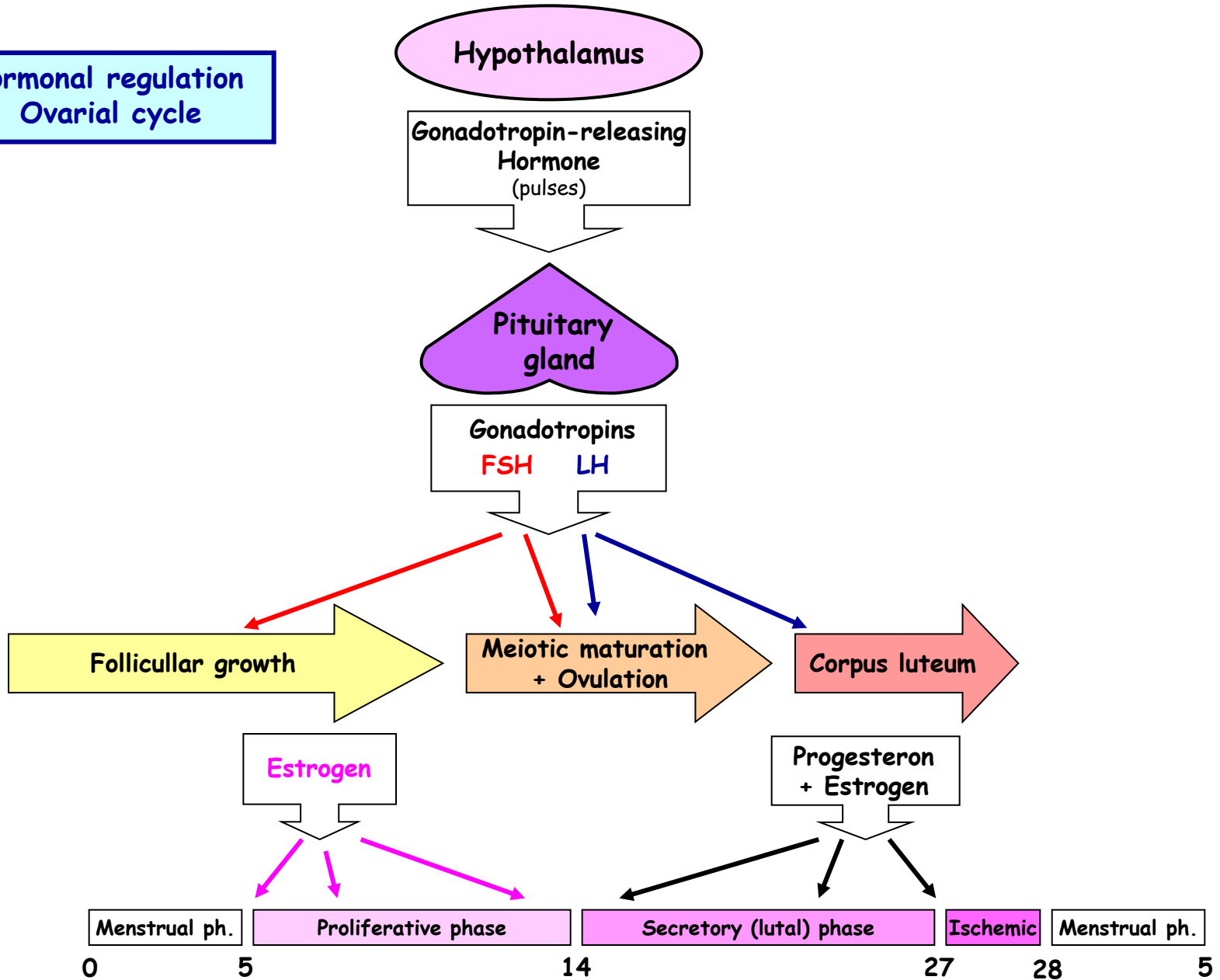


# The final look into the ovary

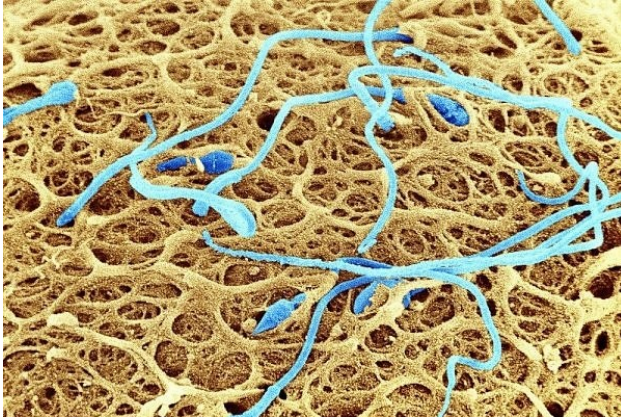


# Where and how the oocyte development is achieved ? (6)

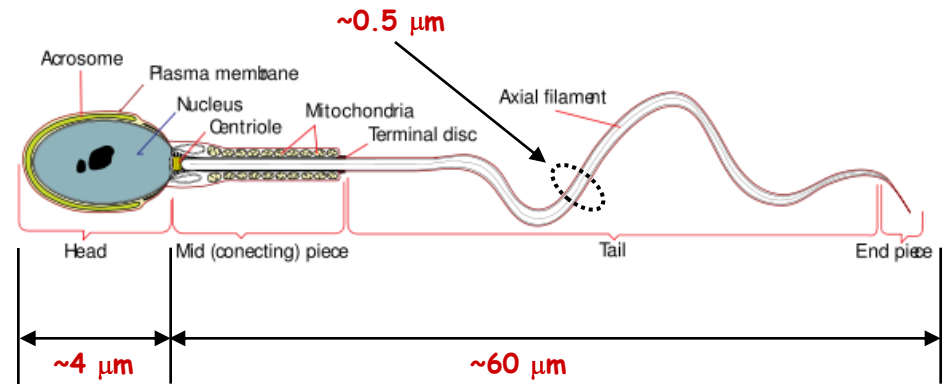
Hormonal regulation  
Ovarial cycle



# Sperm cell development - Spermatogenesis (1)



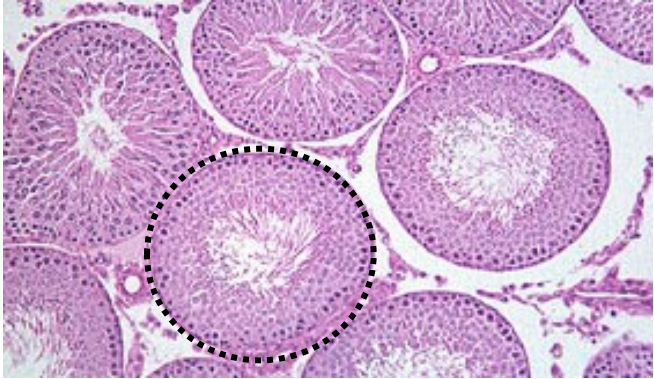
Sperms on the oocyte



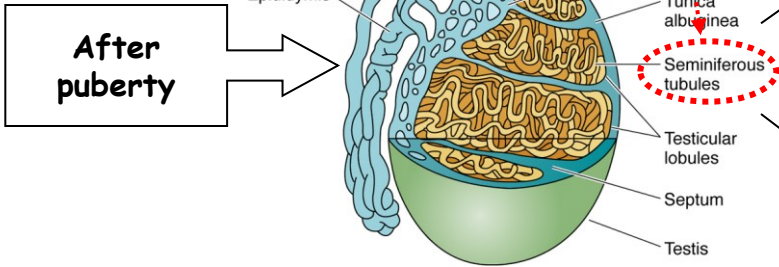
## Minimal ejaculate (WHO)

- Volume - 1.5 ml
- Sperm number - 15.1 millions/ml
- Motility - 40%

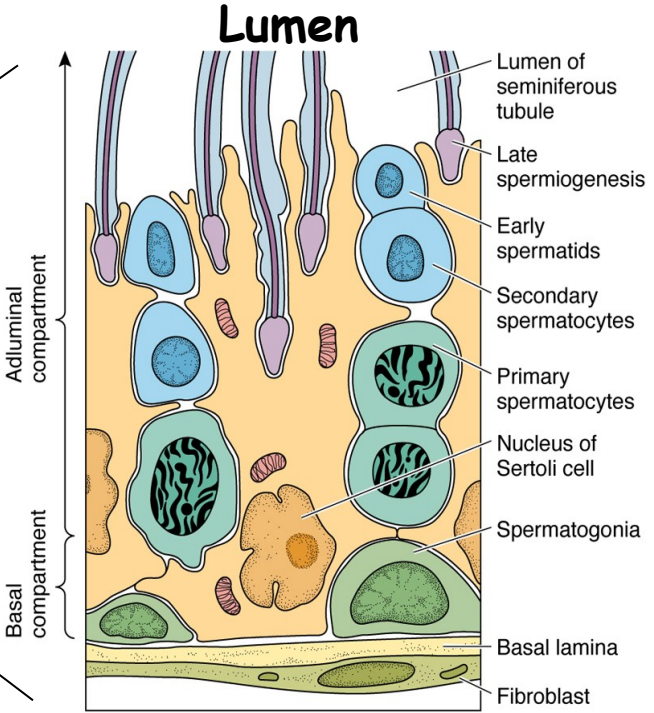
# Sperm cell development (2)



**Before puberty** → Slowly mitotically dividing spermatogonia in **genital ridges**



~0.25 mm  
~0.5 km



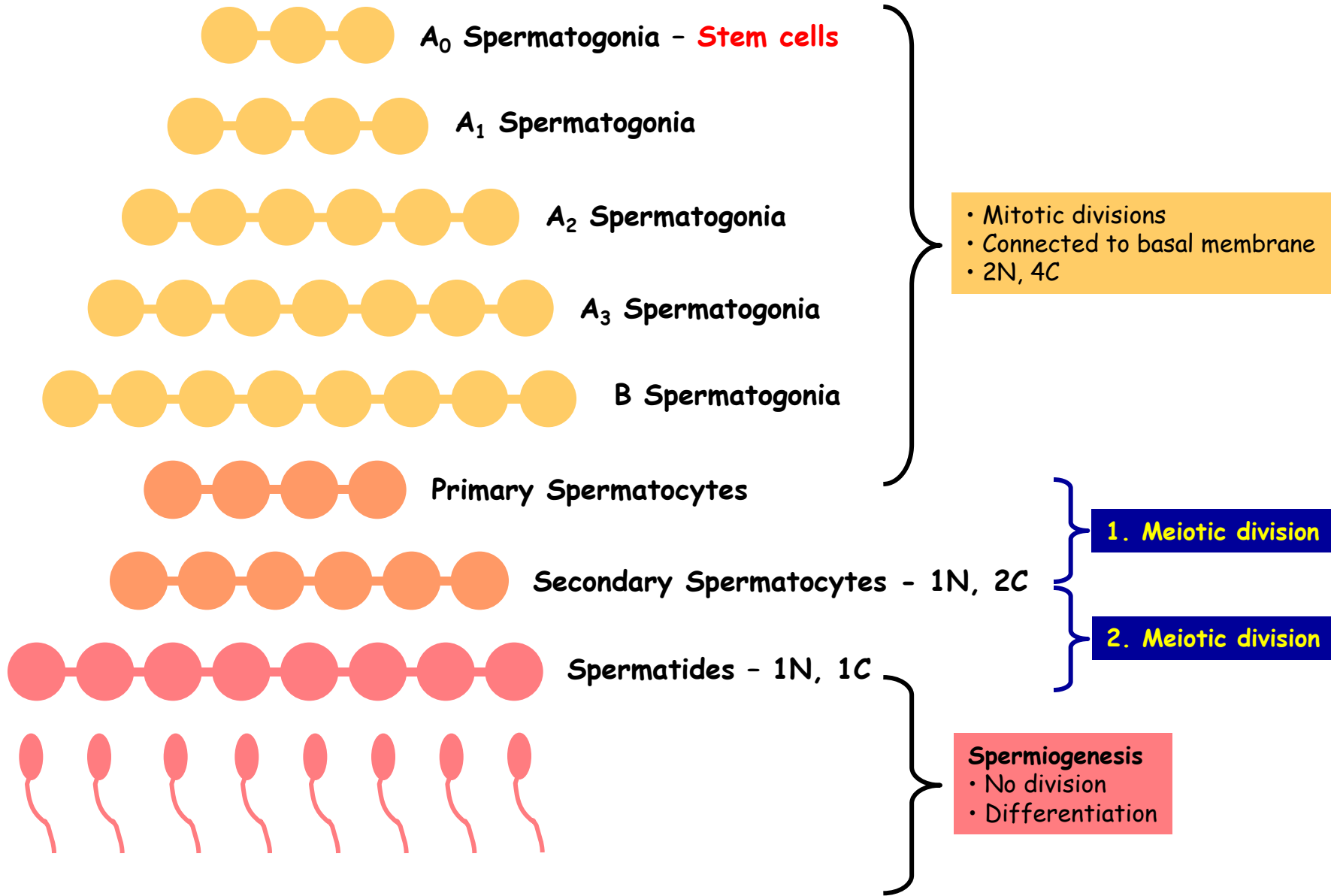
**Spermatocytogenesis (mitotic)**

**Meiotic phase**

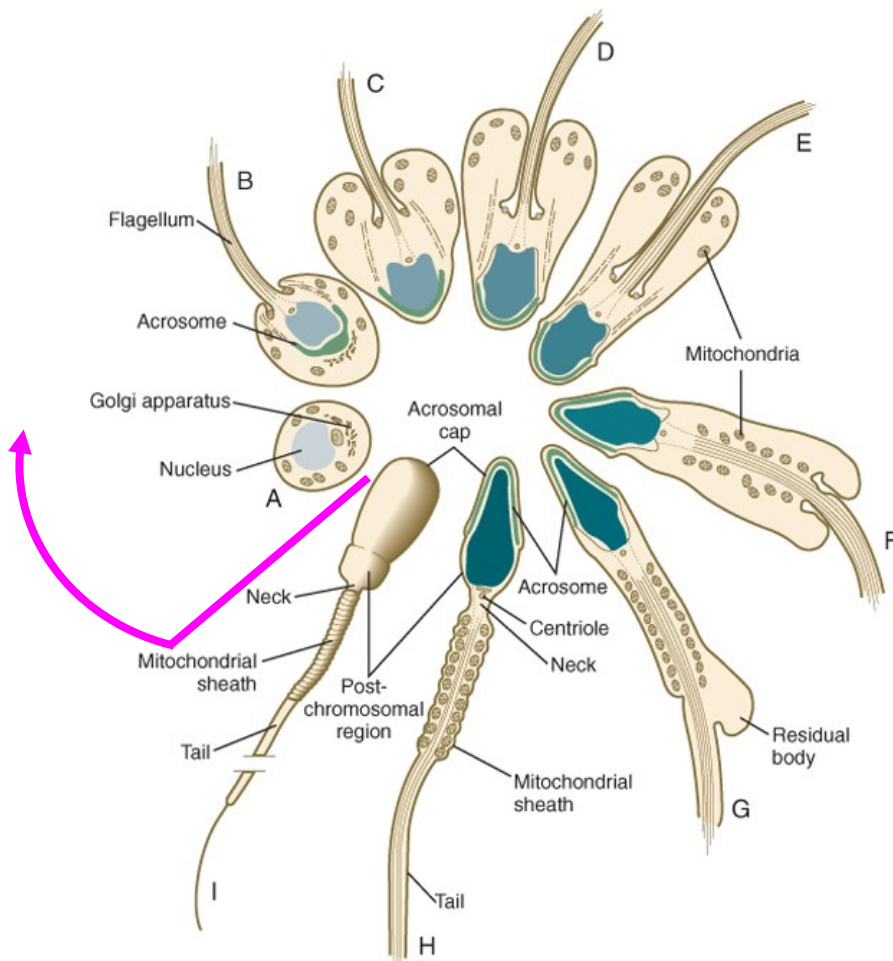
**Spermiogenesis**



# Sperm cell development (3)



# Sperm cell development (4) - Spermiogenesis

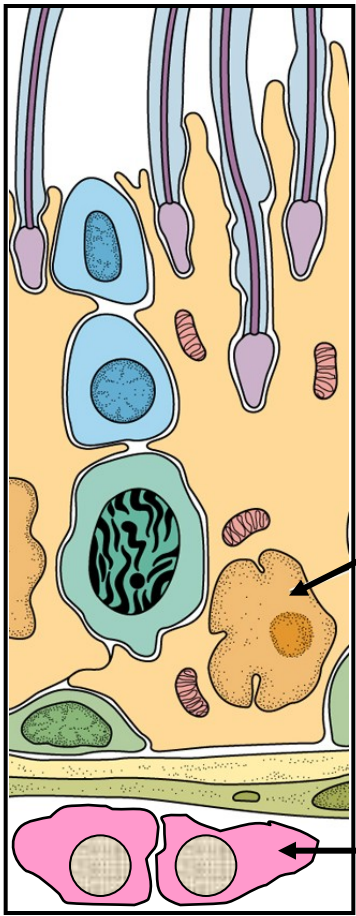


**Histones to Protamines**  
**Genome inactivation**  
**Loss of cytoplasm**

## Sperm production

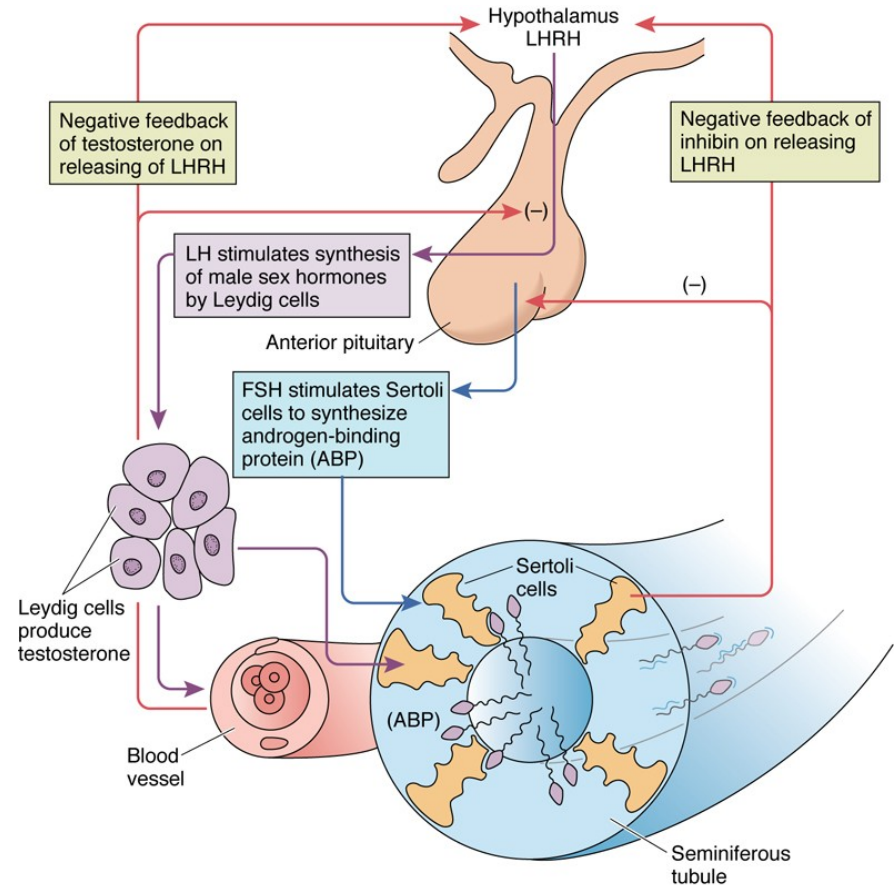
- 1 million sperms every hour
- Spermatogenesis takes ~70 days
- Transport through epididimis ~8-17 days
- Cyclic character  
(Cycle of the seminiferous epithelium - 16 days  
- the same developmental stage at the same place)

# Sperm cell development (5) - Regulation



- Sertoli cells**
- Support , protect, and nourish
  - Phagocyte
  - Blood-testis barrier (zon. occlud.)
  - Produce anti-mullerian hormone
  - Produce fructose
  - Produce inhibin (inh. FSH prod.)

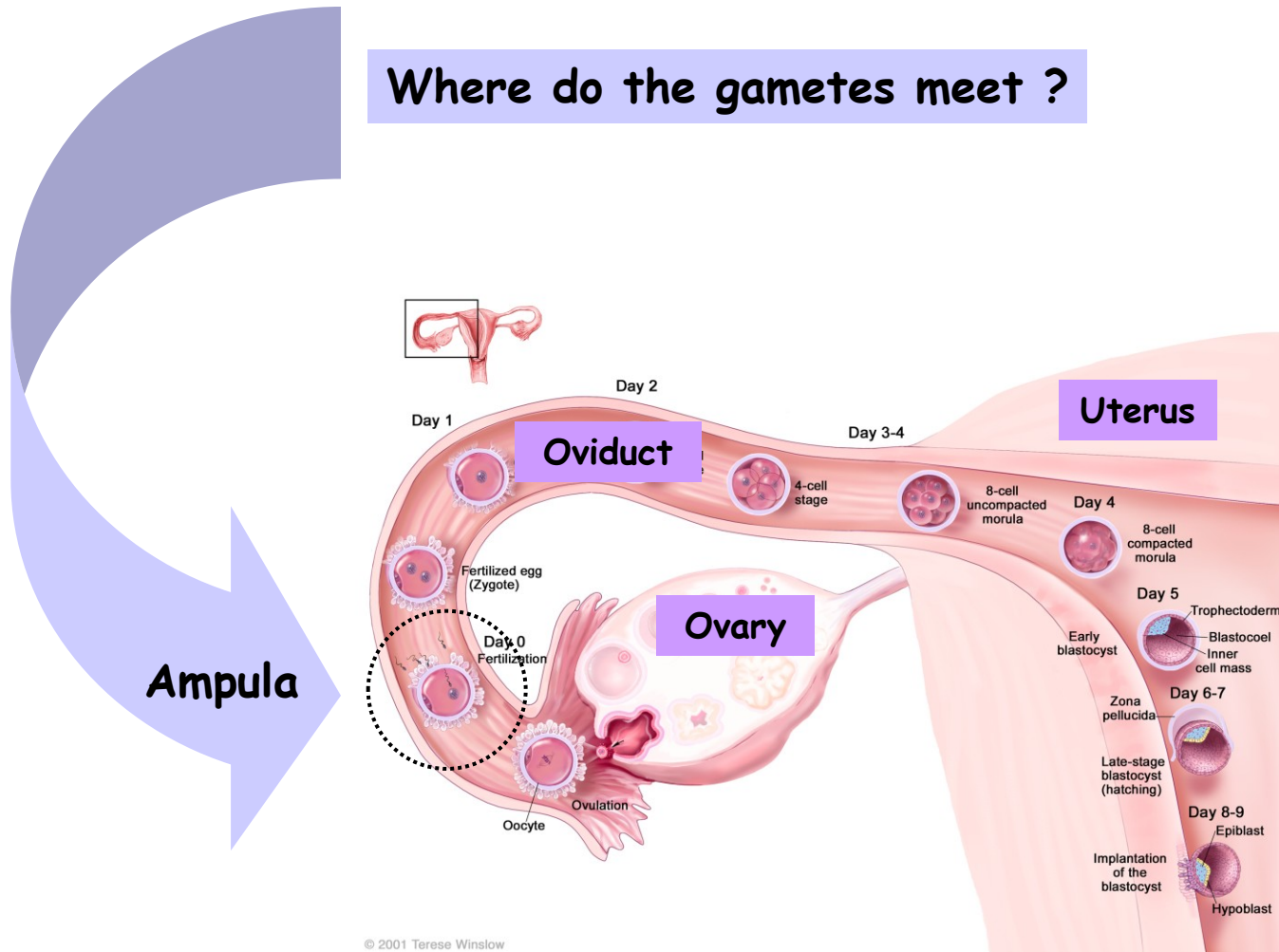
- Leydig cells**
- In interstitium
  - 10 % of testis
  - Produce testosterone



# Fertilization (1)

= the process that culminates in the union of one sperm nucleus with the egg nucleus within the activated egg cytoplasm

Where do the gametes meet ?



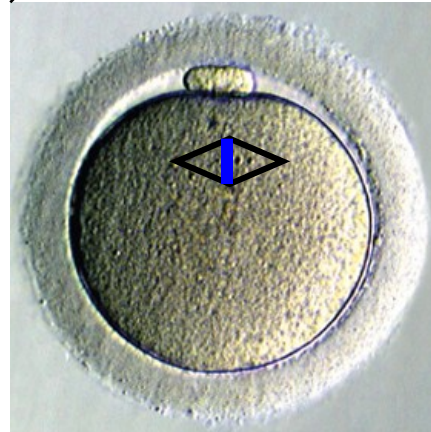
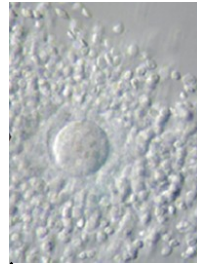
# Fertilization (2)

Oocyte makes itself ready for being penetrated

LH surge

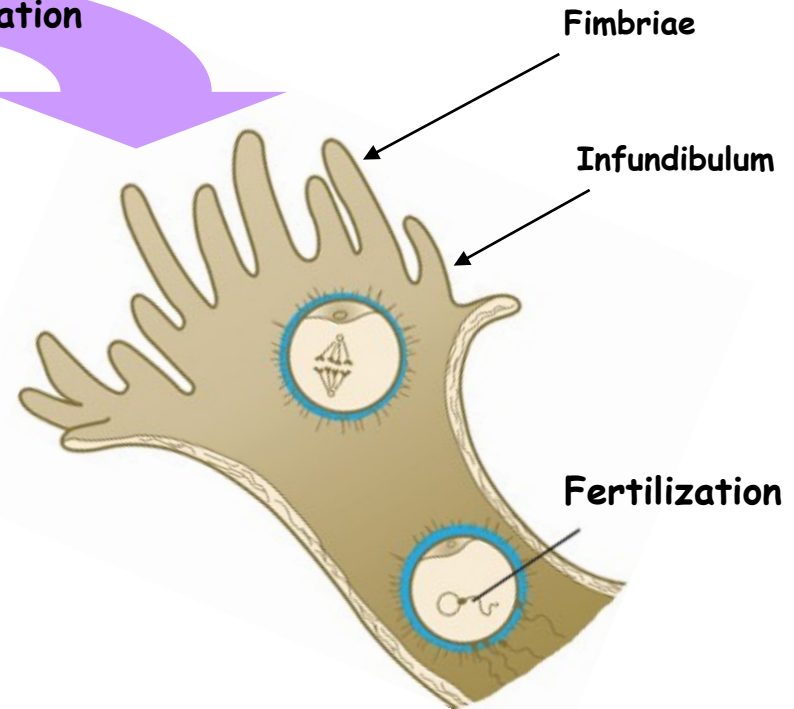


35-40 hrs



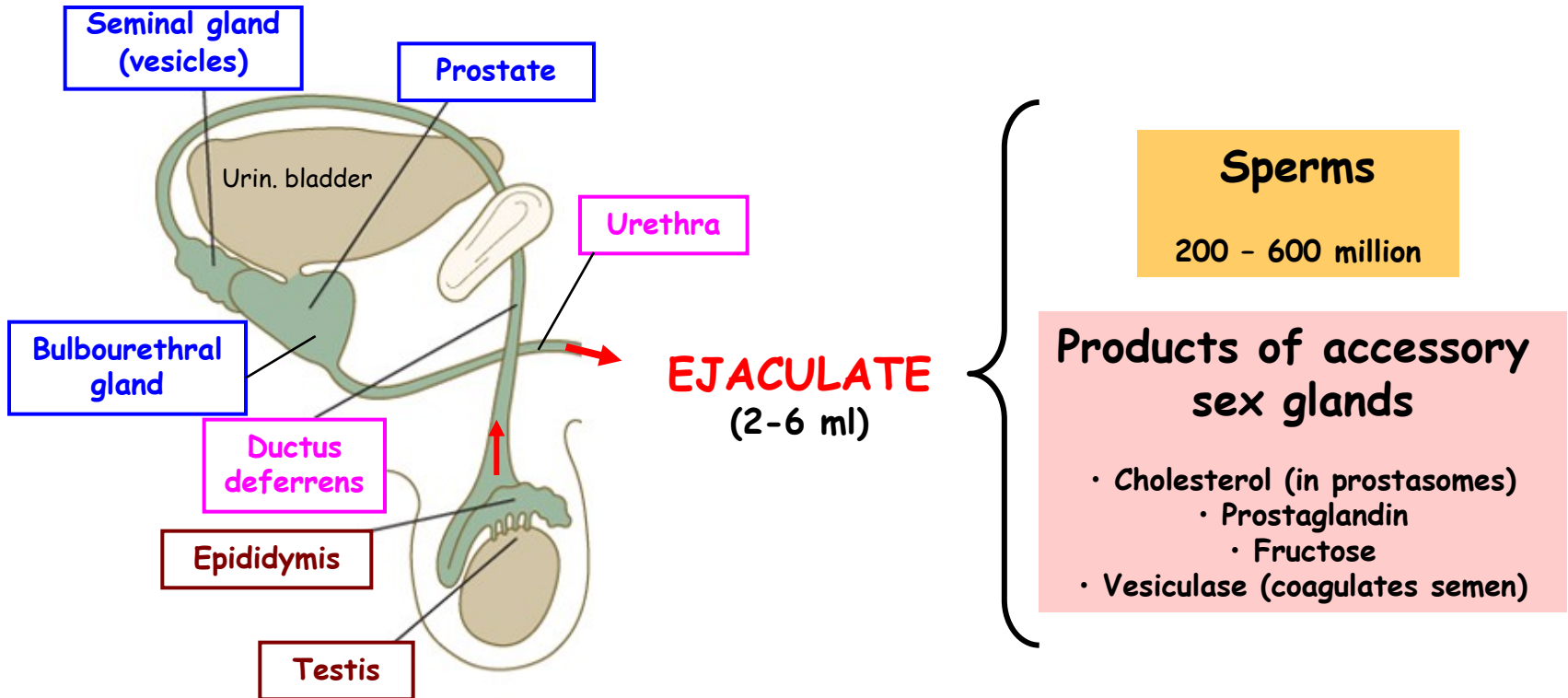
Oocyte is fertilizable for only 12 to 16 hours

Ovulation



# Fertilization (3)

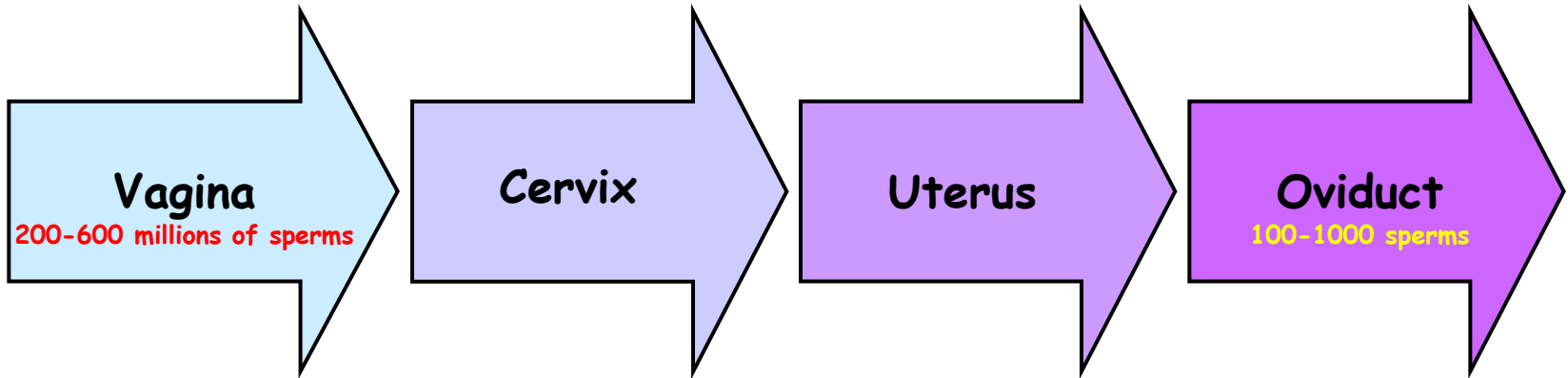
## Travel of sperm to the site of fertilization



# Fertilization (4)

## Travel of sperm to the site of fertilization

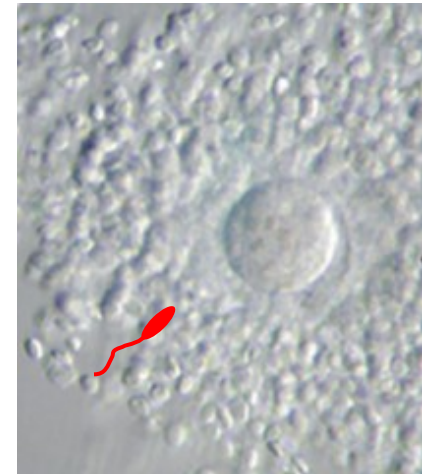
2 - 7 hours



- Acid environment
- Sperms move actively

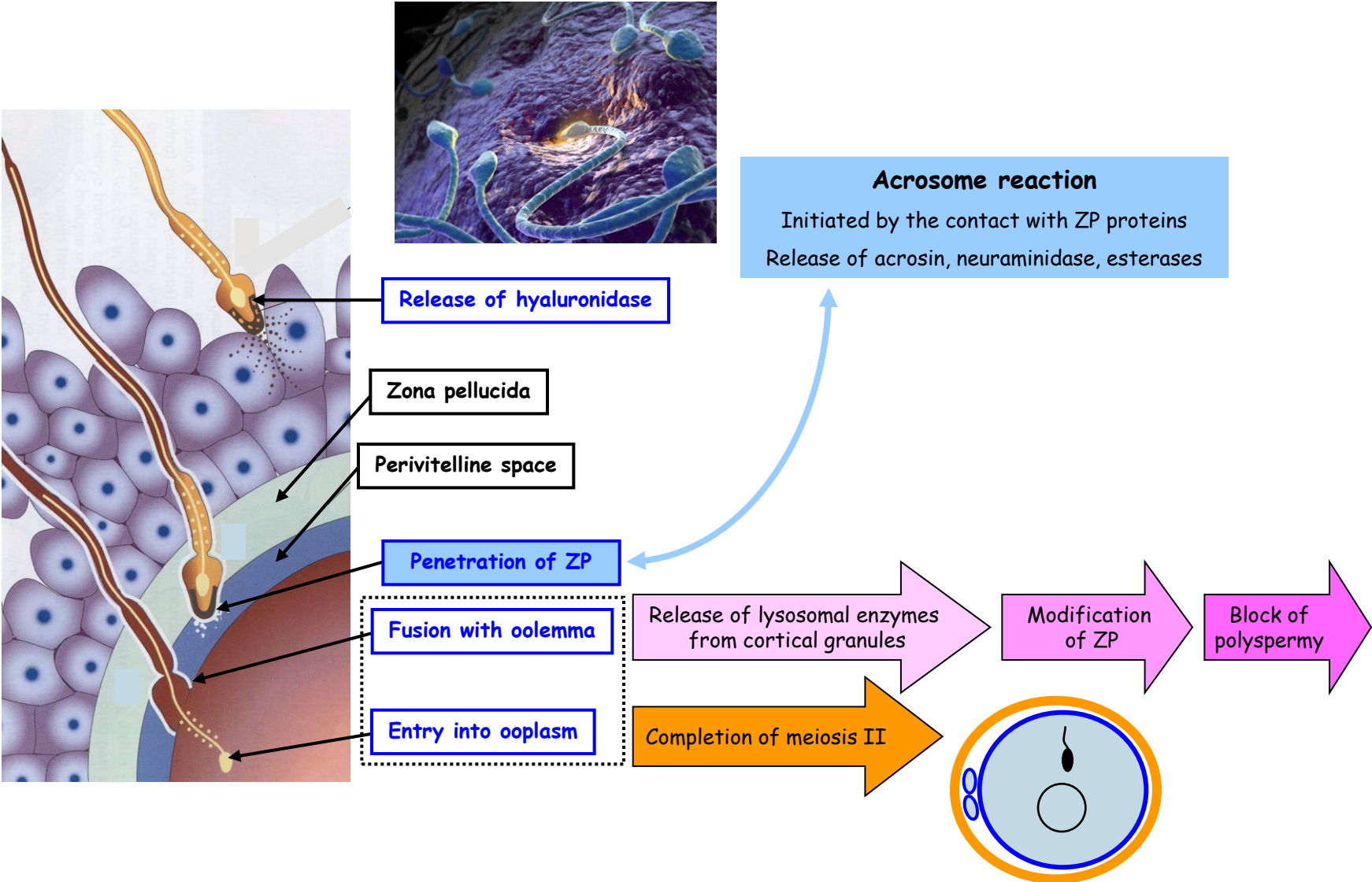
- Cleans sperm
- Cervical mucus stabilizes sperms
- Initiates **capacitation**

- Removal of glycoproteins from the head
- Change to composition of cell membrane
- Increase of motility



# Fertilization (5)

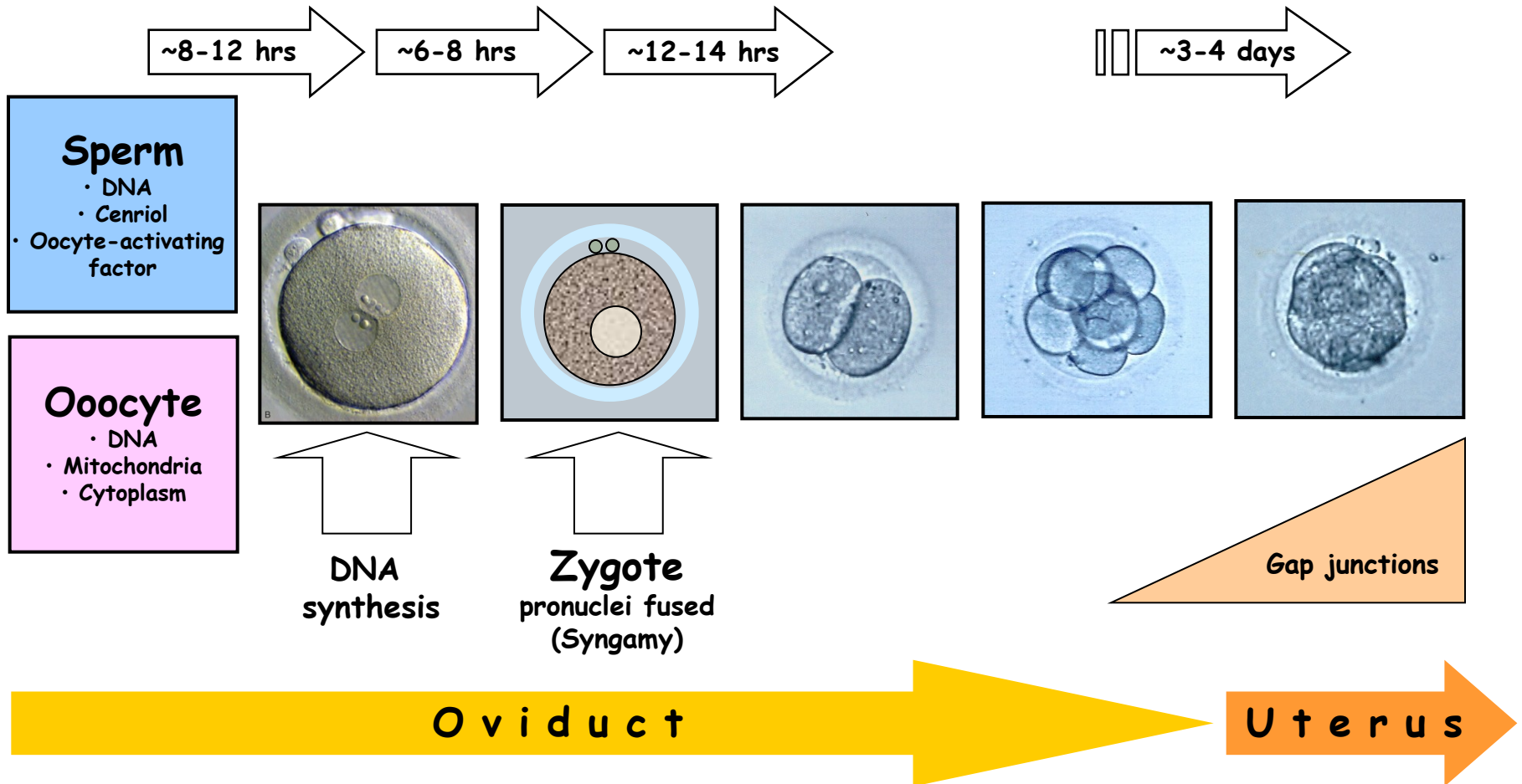
## Entry of sperm into the oocyte



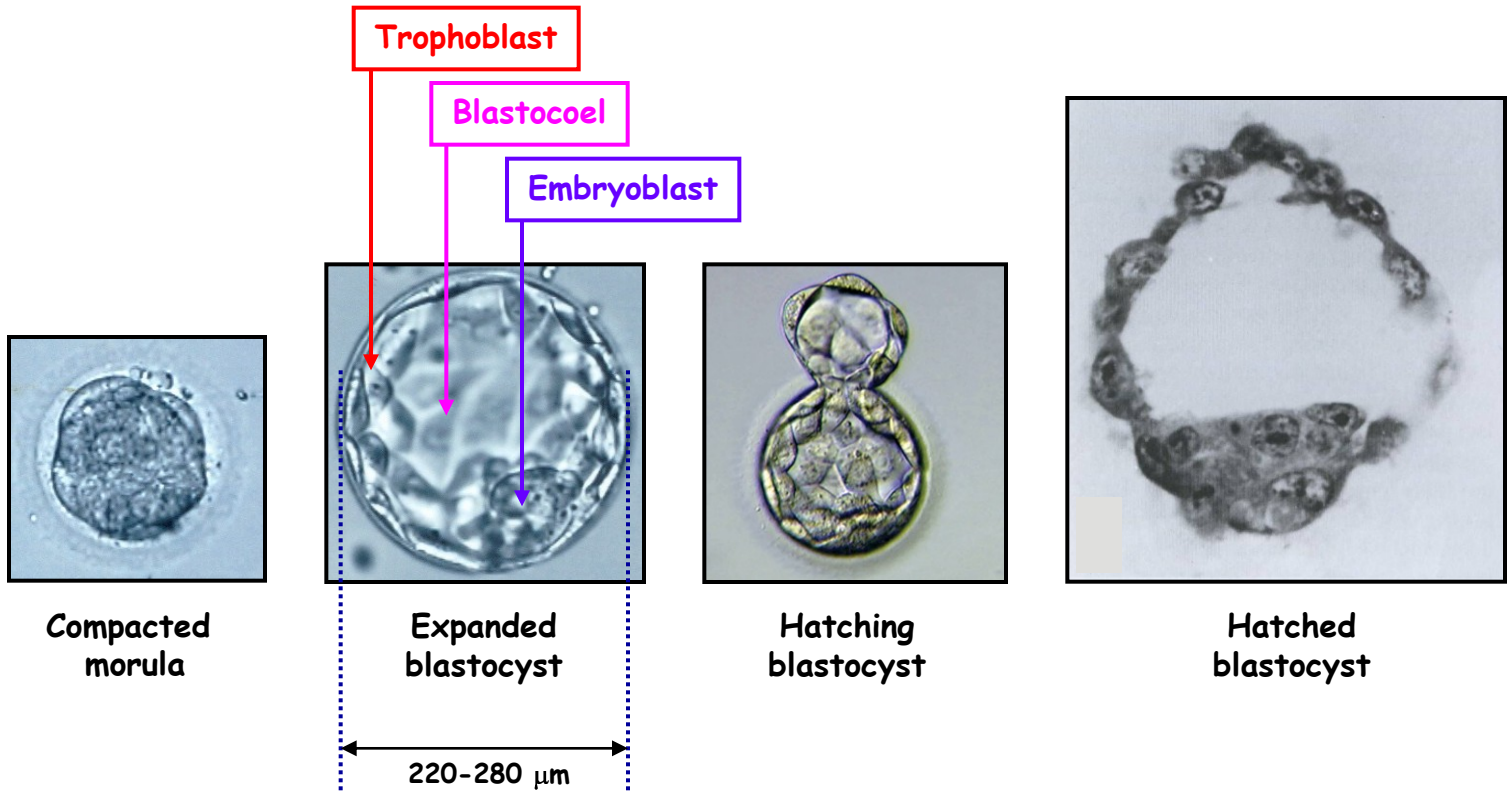


# Fertilization (6)

## Zygote formation and the first cleavages

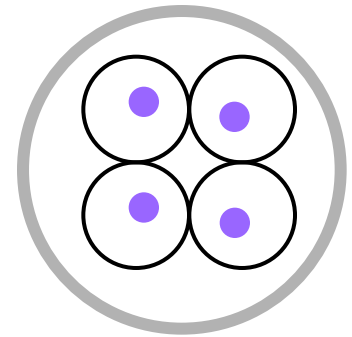
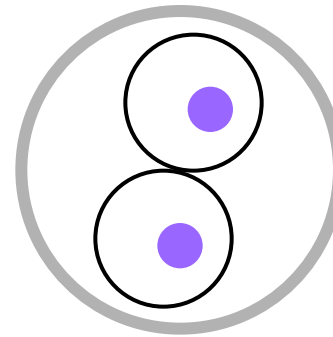
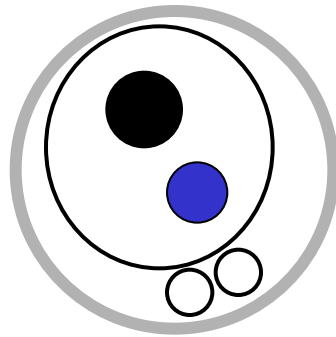
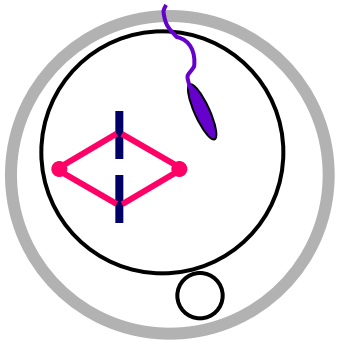
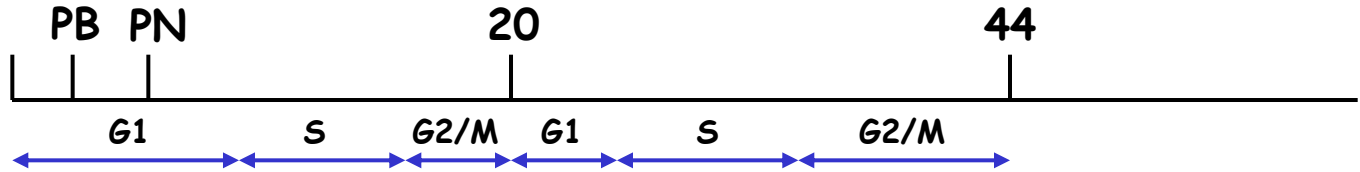


# Blastocyst formation



**Day 5 - 6**  
(60 to 80 cells)

# A potency of oocyte cytoplasm



Translation of maternal mRNA

Translation of zygotic mRNA

Zygotic transcription

Activation of embryonal genome

Repression of transcription

Significance of „enhancers“

# 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

Transcripts that replace  
degraded maternal  
mRNAs

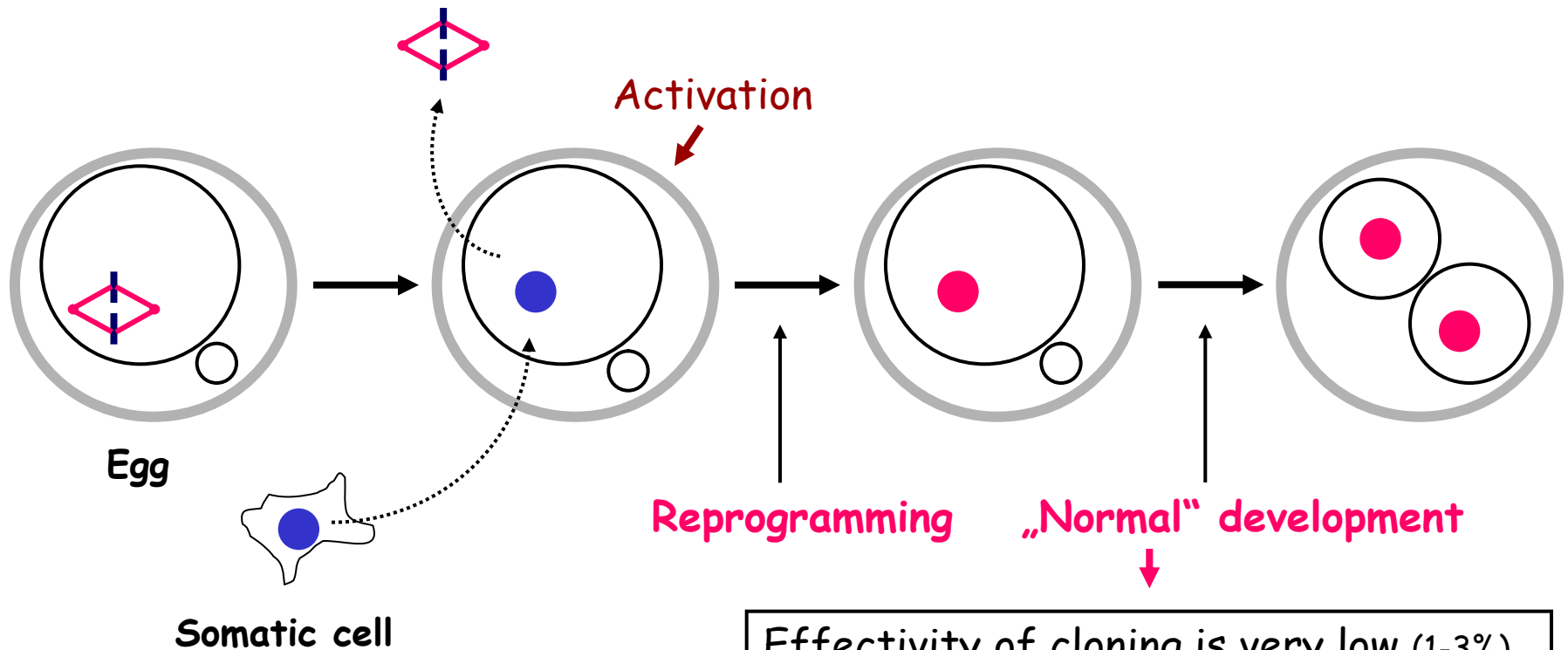
Novel transcripts that  
underlie **new pattern of  
gene expression**

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)

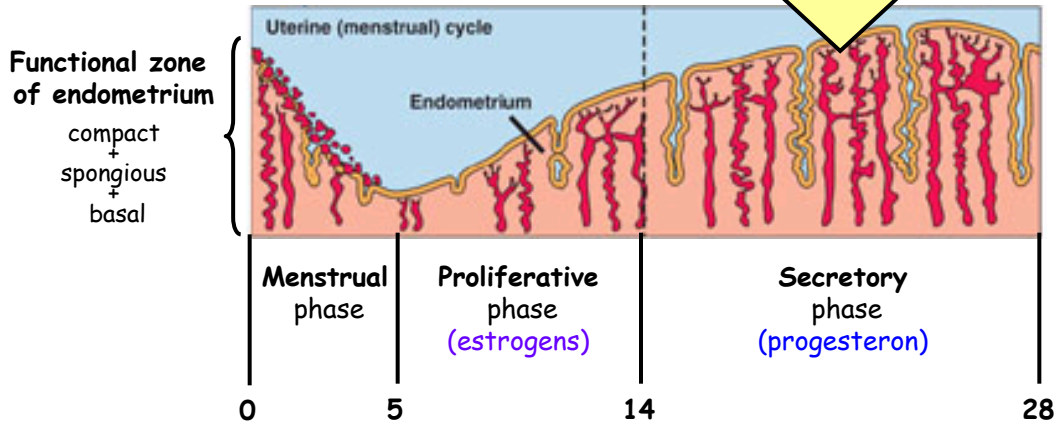
Effectivity of reprogamming depends on many factors (type of somatic cells, position in cell cycle phase, ...)

# Blastocyst implantation

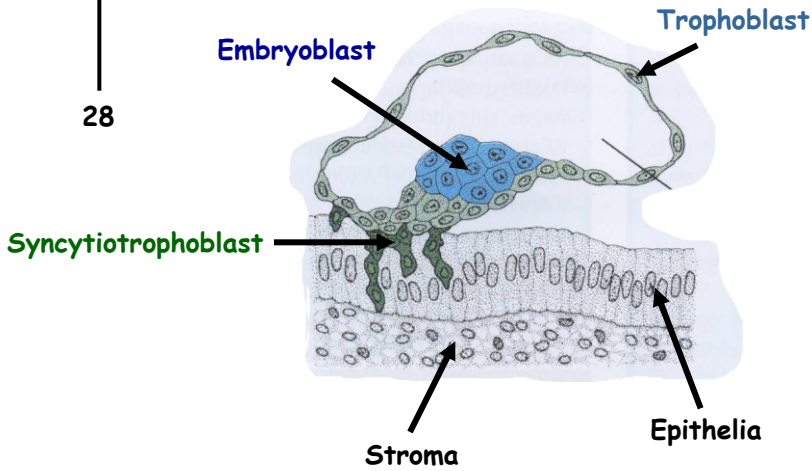


**Molecular players**

- Selectin on trophoblast + glycoproteins on epithelia
- Integrins + Laminin, Fibronectin
- IGF1, IGF2, VEGF
- Metalloproteinases + their tissue inhibitors
- Progesteron
- hCH
- Prostaglandins

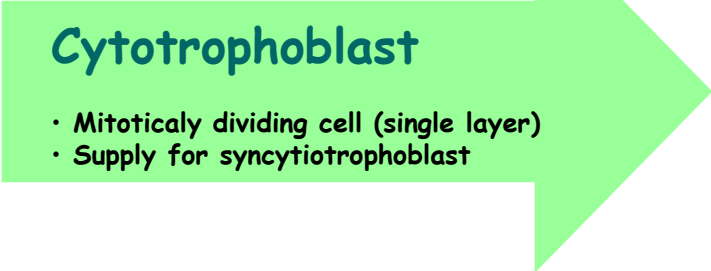
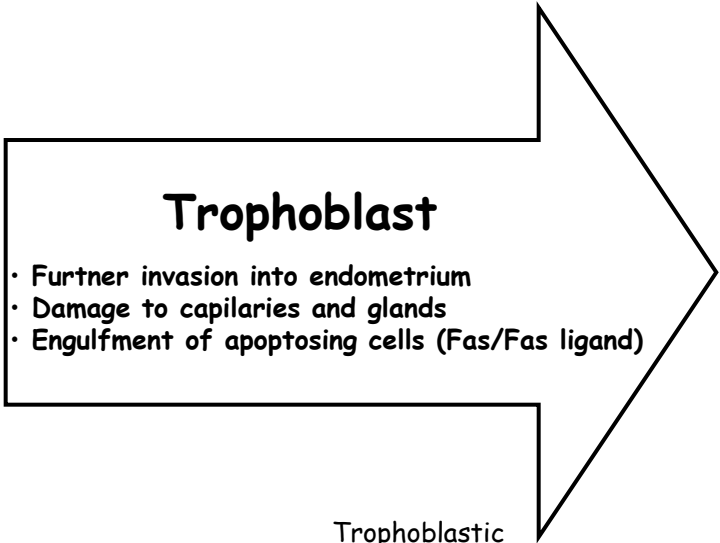


**Ovulation followed by Fertilizaion**

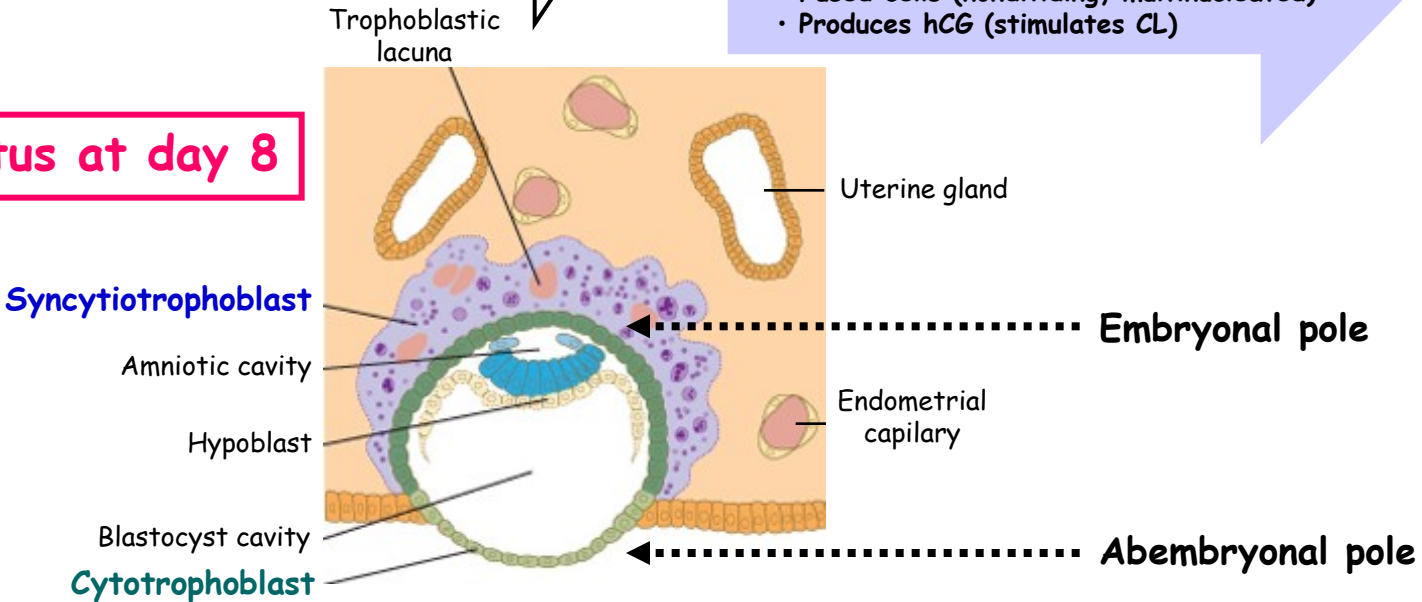


# Early development - The second week (1)

Completion of implantation + Further embryo development



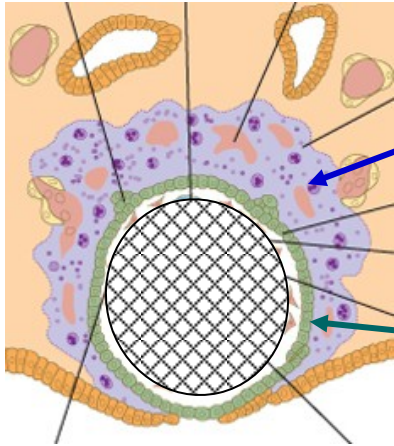
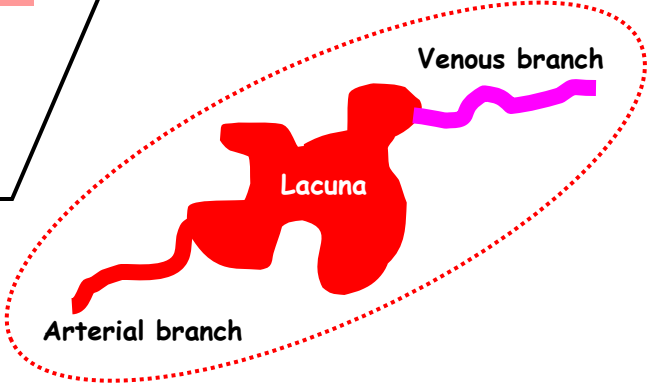
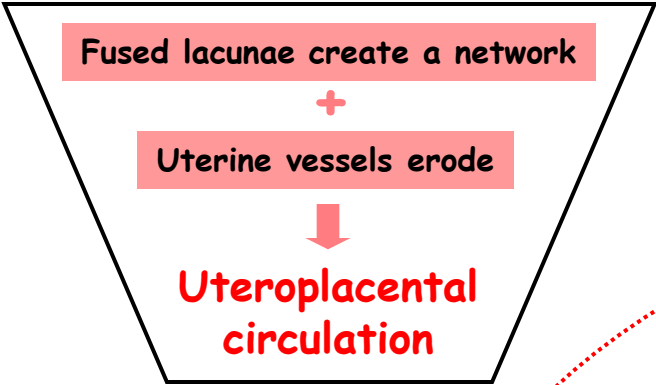
**Status at day 8**



# Early development - The second week (2)

**Decidual reaction of endometrium**

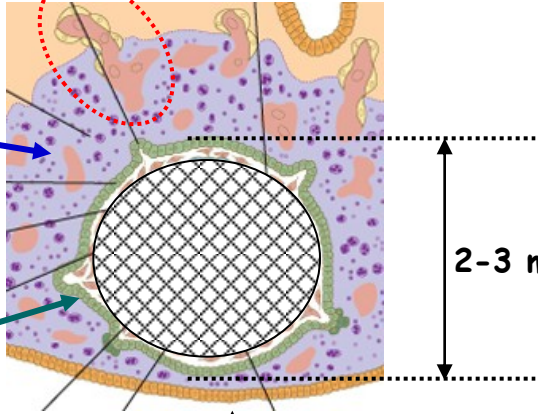
- Vessel number/density increases
- Fibroblasts differentiate into glycogen- and lipid-containing cells



**Status at day 10**

**Syncytiotrophoblast**  
Sponge-like appearance

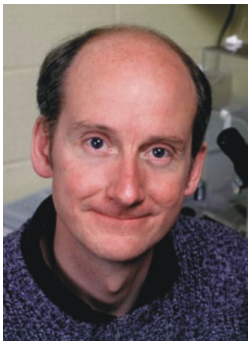
**Cytotrophoblast**



**Status at day 12**

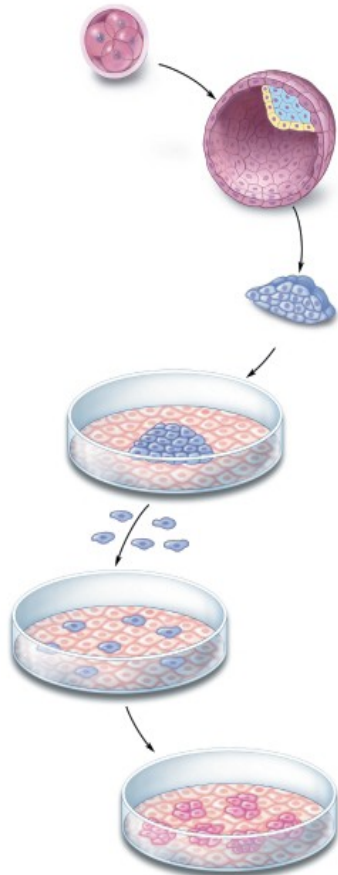
↑  
Epithelization of the implantation site





# Human Embryonic Stem (hES) Cells

(Thompson et al, 1998)

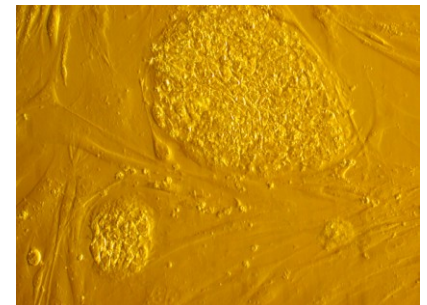
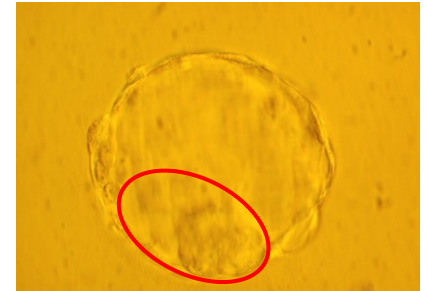


Early embryo at blastocyst stage

Isolated embryoblast (ICM - Inner Cell Mass)

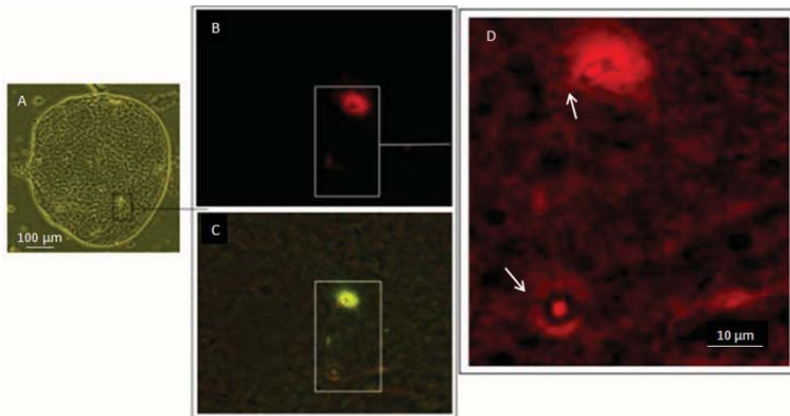
Isolated embryoblast after placing to *in vitro* conditions (+ feeder cells + FGF2)

Propagation in culture by enzymatic disaggregation (repeated passaging)

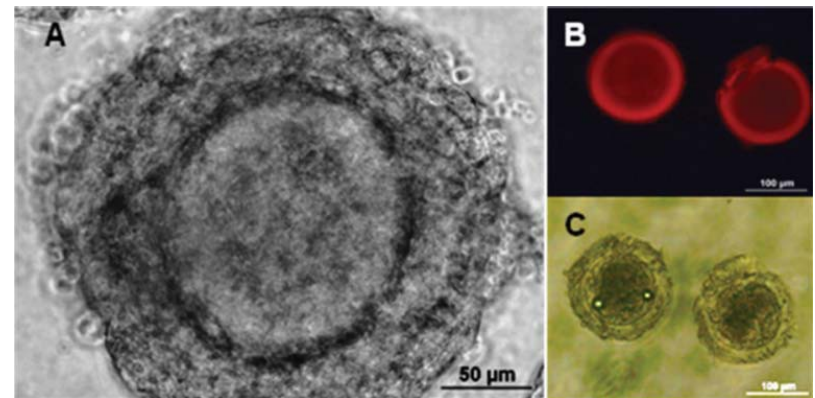


# Derivation of postmeiotic germ cells from hESC

Prof. Harry Moore, University of Sheffield, 2009



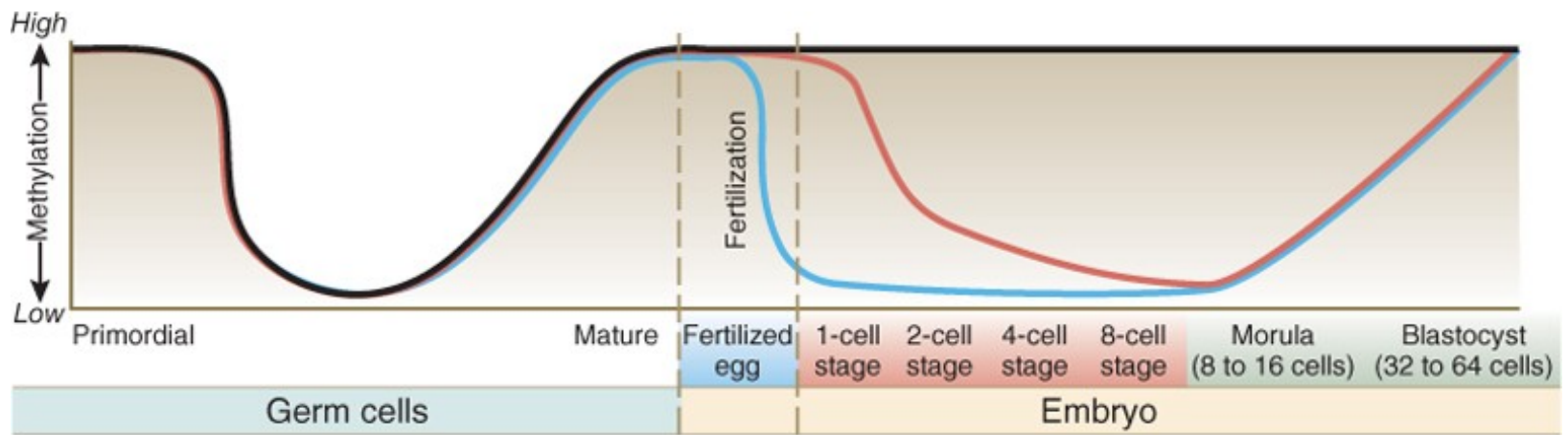
- B) C-KIT
- C) I-97 antigen
- D) Cells with condensed chromatin and signs of flagellum

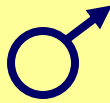


Structures that are highly reminiscent to oocyte-granulosa complexes (zona pellucida is not developed)

**Thank you for your attention !**

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





Primordial germ cell - PGC  
 Embryonal mitosis  
Block of mitosis

Spermatogonia

Postnatal mitosis

Initiation of meiosis

Primary spermatocyte

Finalization of the 1. meiotic division

Secondary spermatocyte

Finalization of the 2. meiotic division

Spermatide

SPERM

Mitosis

1. meiotic division

2. meiotic division

Primordial germ cell - PGC

Embryonal mitosis

Oogonia

Embryonal mitosis

Entry into meiosis

Block of meiosis in diplotene of the 1. meiotic division

Primary oocyte

Reinitiation of meiosis

Block of meiosis in M-phase of the 2. meiotic division

EGG

Finalization of the 2. meiotic division

Fertilized egg

Embryonal and fetal development

Sperm penetration

# Egg - attractive target for manipulation

