Lecture 3

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, March 2019

Embryology: what does it cover?

Zygote

↓

Multicellular embryo

↓

Dynamic multicellular organism

CELL DIFFERENTIATION and MORPHOGENESIS

STABLE GENOME

↕

Genomic equivalence

(= equal amount of DNA and the same nucletide sequence in all cells of a organism – cloning)

X

VARIABLE TRANSCRIPTOME

↕

Transcription Regulators

Embryonal x Fetal Development

Early embryo
before implantation

All the organs
are established

Parturition

Embryo

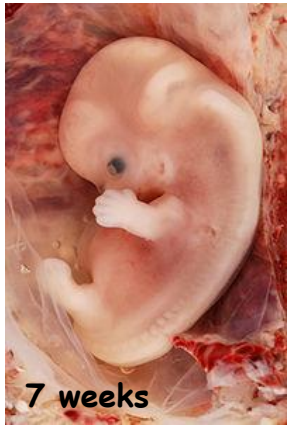
Fetus

Fertilization

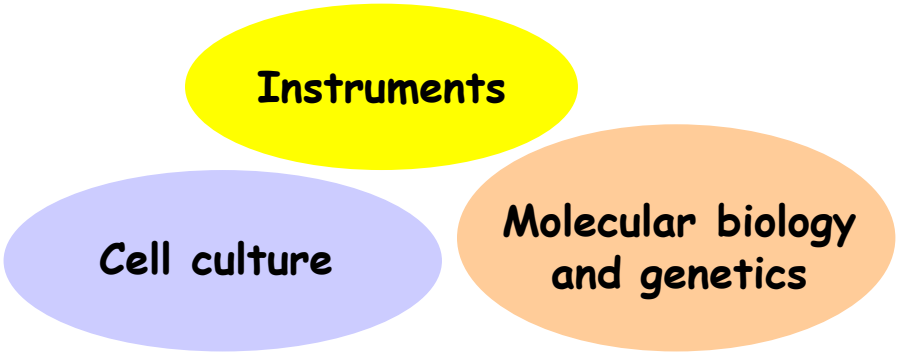
Week 8

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

Indviduals 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.

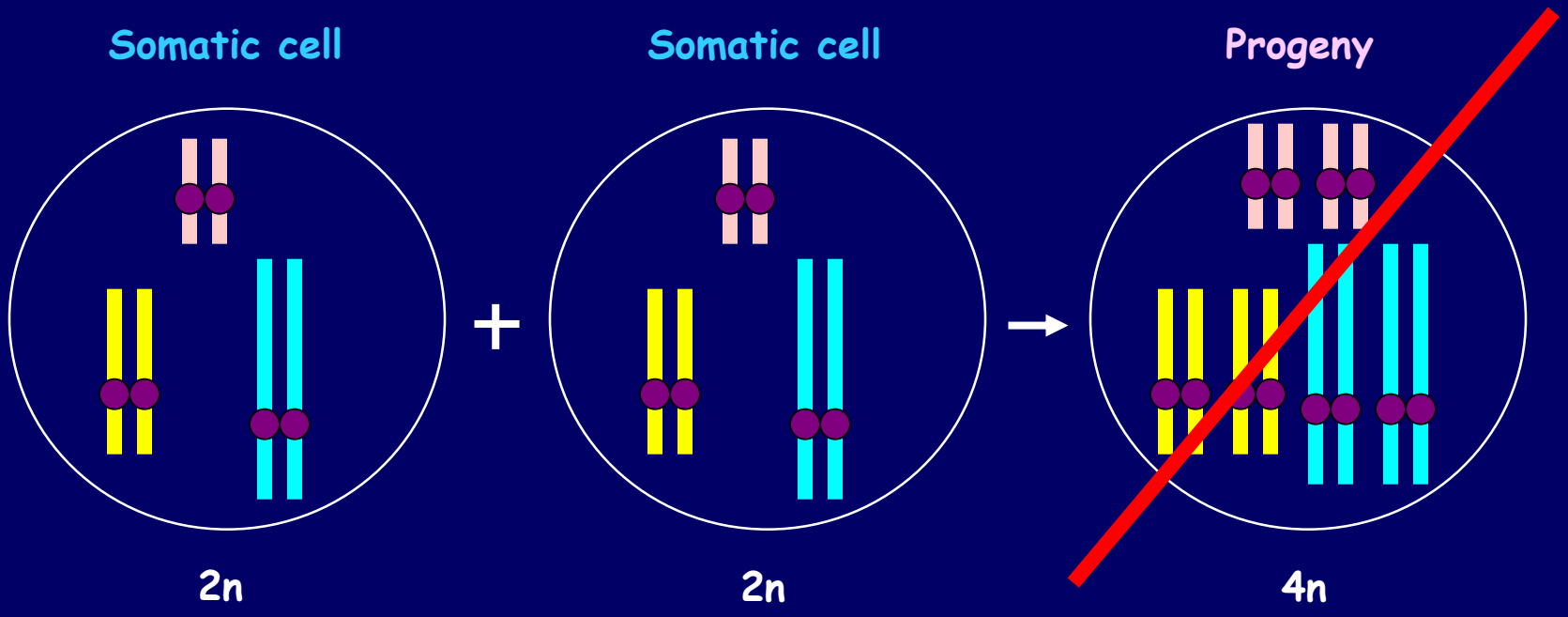
Although development of eggs and sperm differ in many morphogenetic details,
key genetic processes taking place in both types of gamates are principally the same.

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

These genetic processes include:

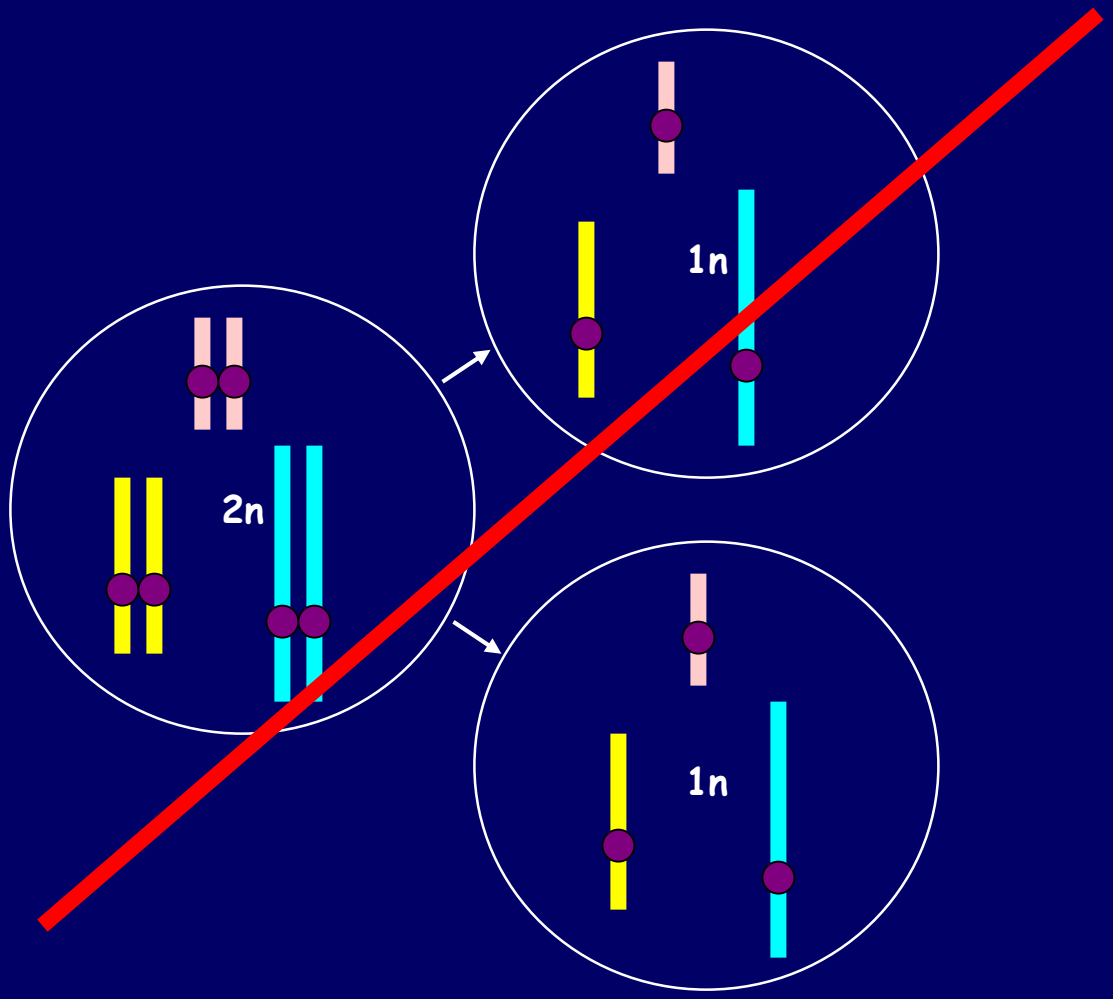
- **„Crossing over"**
- **Independent segregation chromosomes**
- **Reduction of the number of chromosomes**

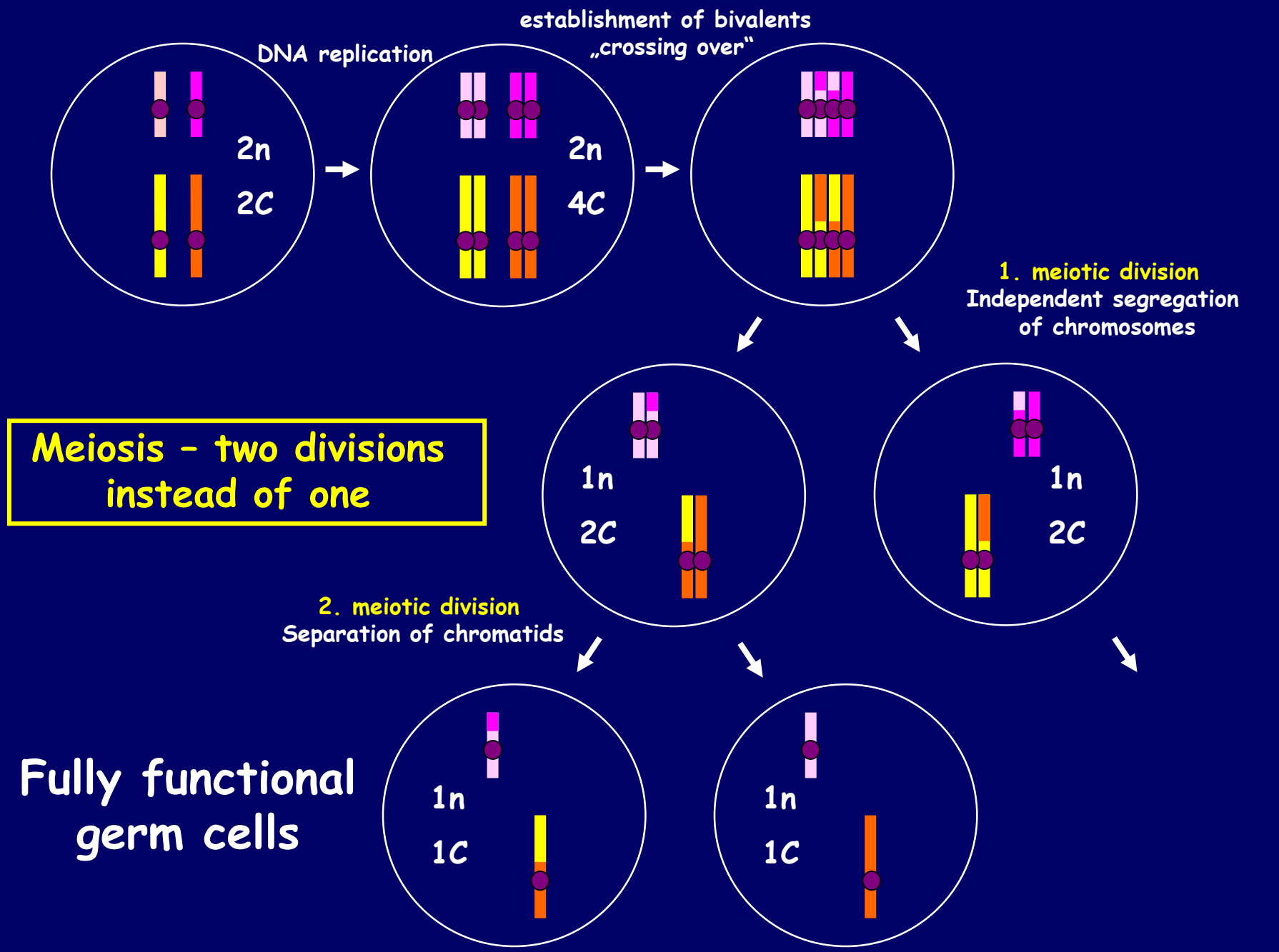
Reduction of the number of chromosomes
Why?



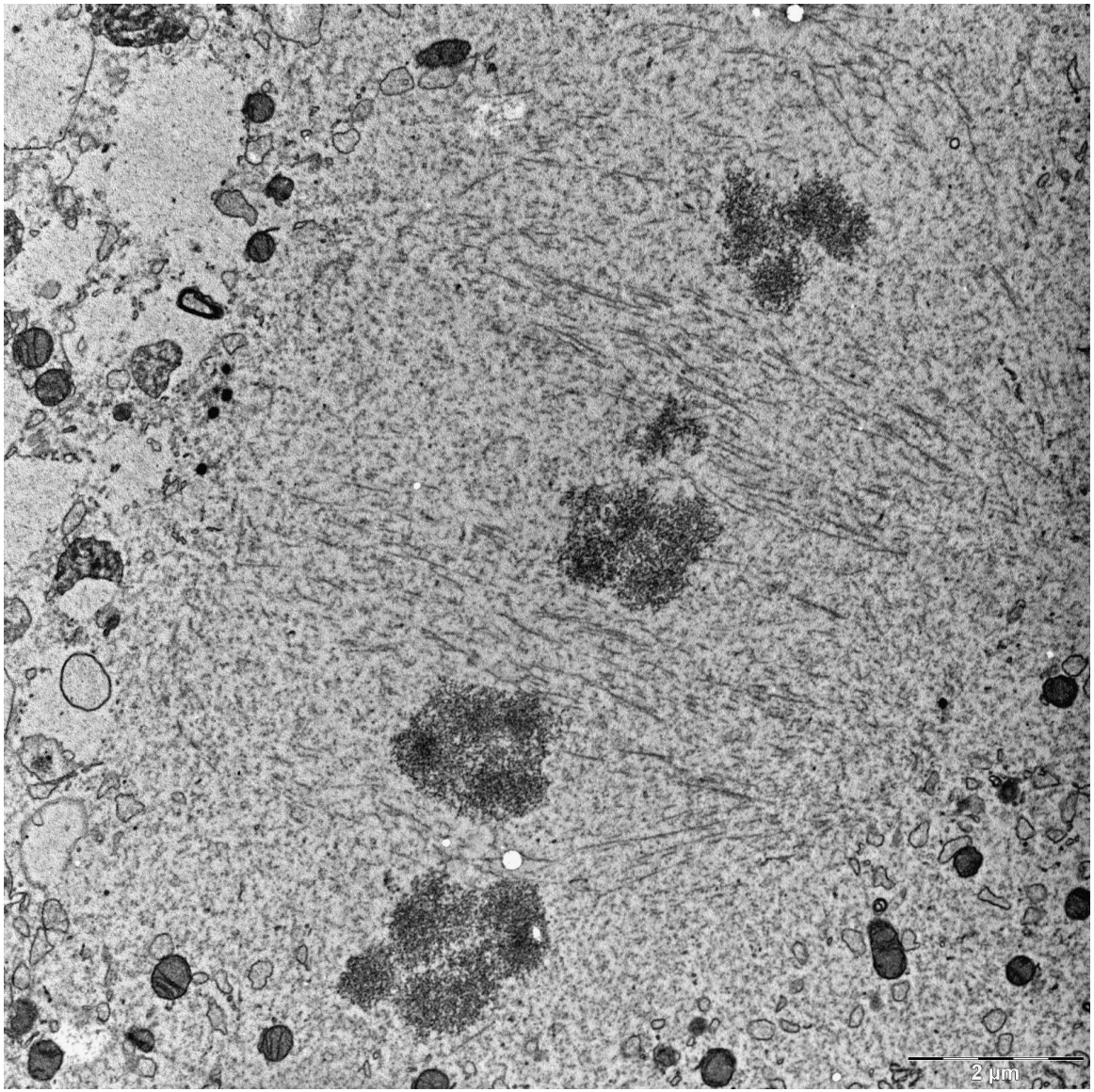
Gametes have to contain haploid number of chromosomes (n) in order to prevent multiplication of chromosomes in progeny above a diploid number (2n)

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




DNA replication
establishment of bivalents
„crossing over"
2n
2C
2n
4C
1. meiotic division
Independent segregation
of chromosomes
Meiosis – two divisions
instead of one
1n
2C
1n
2C
2. meiotic division
Separation of chromatids
Fully functional
germ cells
1n
1C
1n
1C

MI oocyte - tetrades



Dr. Zuzana Holubcová, June 2018

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

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

SAME

Genetic function

♂ Sperm X ♀ Eggs

DIFFERENT

Significance for embryogenesis (reproduction)

Morphological and physiological properties

Development and underlying regulatory mechanisms

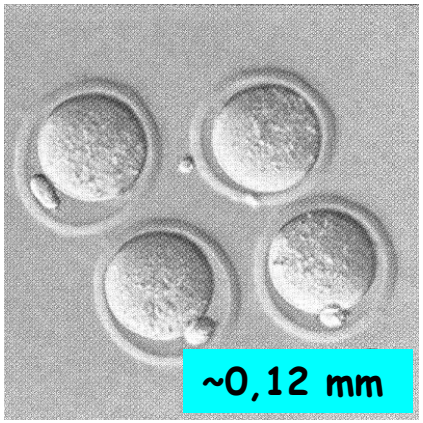
Primordial germ cells – PGC

- 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 continuosly until high age (two testes of man produce about 1000 spermatozoa every second)

- numbers of ocytes (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

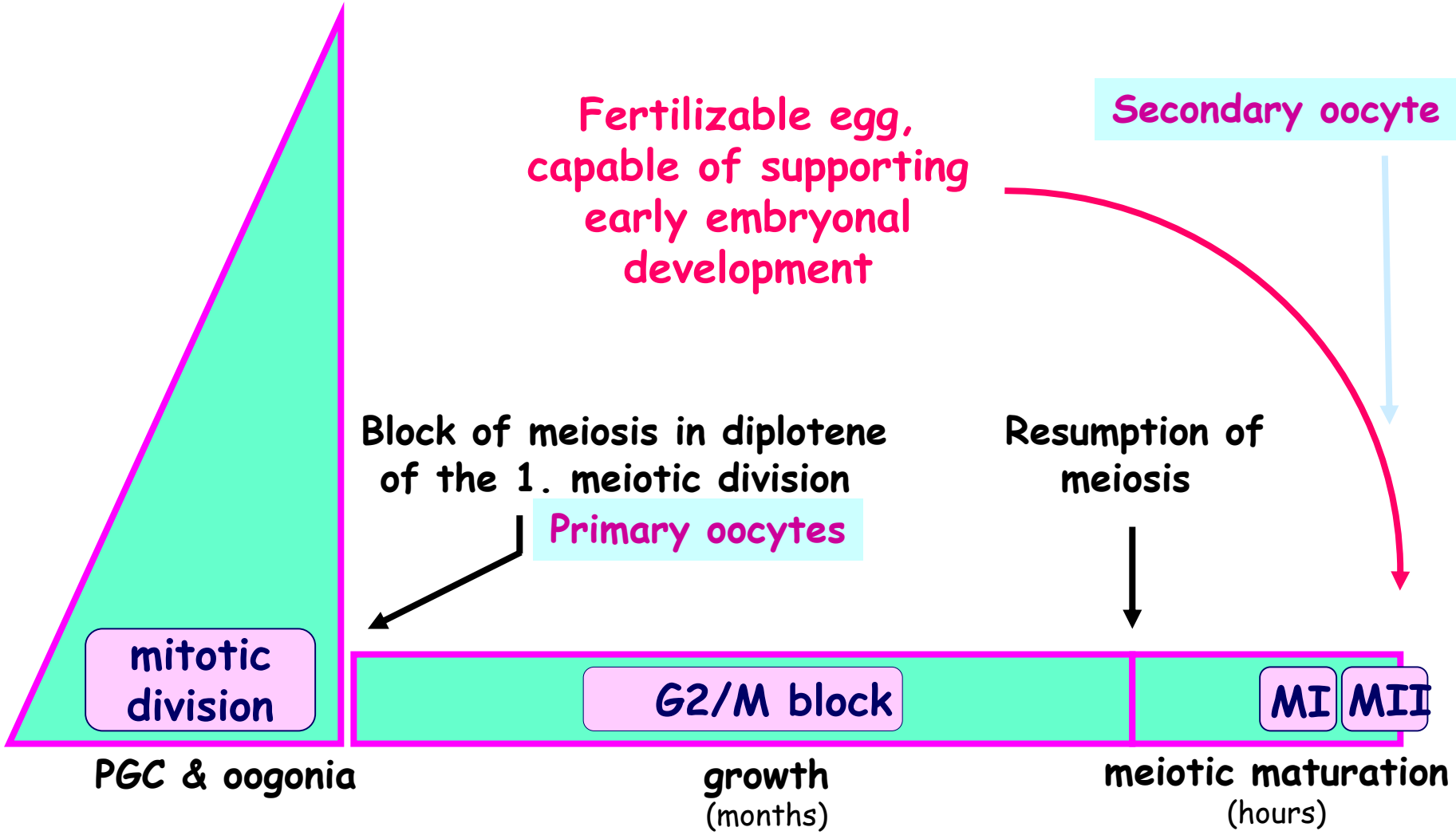
Highly specialized cell.
The only cell in the female body that can undergo meiosis and fertilization, and thus give rise to a new individual.

&

„Totipotent" cell
It can generate all the cellular diversity that si typical for multicellular organism.

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

Key periods of oocyte development



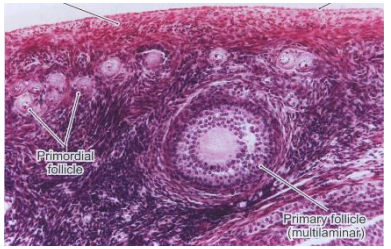
Where and how the oocyte development is achieved ? (1)

Diplotene of the Meiosis I

Inside the ovary

30 µm

20 mm



Primordial follicle

Recruited follicle (unilaminar)

Preantral follicle (multilaminar)

Primary follicles

Growth

Secondary (antral) follicle (medium size)

Selection of the Dominant follicle (the one most sensitive to FSH)

~14 days

Tertiary (Graafian) follicle

Massive hormone production

Androstendione
Theca folliculi interna

↓

Estradiole
Granulosa cell

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

Ooocyte growth

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

&

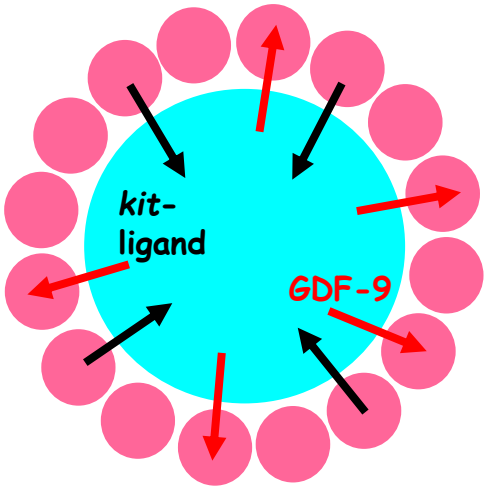
Signal that initiates growth is not known
(it is not FSH - hypophysectomy does not prevent growth)

&

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)

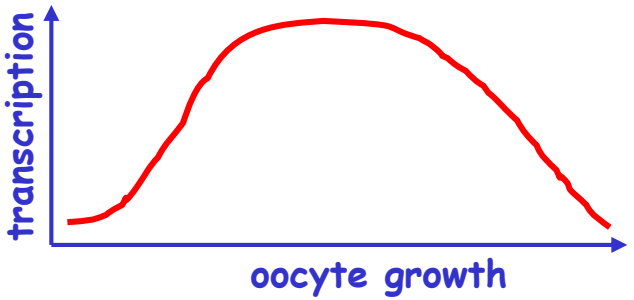
Ooocyte growth

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



Intensive translation – many proteins (very limited knowledge)
Example: ZP1, ZP2, ZP3 – proteins of *zona pellucida*
Fully grown oocyte – ~120 ng total protein

Transkriptome and proteome – underlie unique properties of oocyte

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

Epigenetic changes occuring 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, X0 results in an abnormal development of ovaries)

&

Genomic imprinting

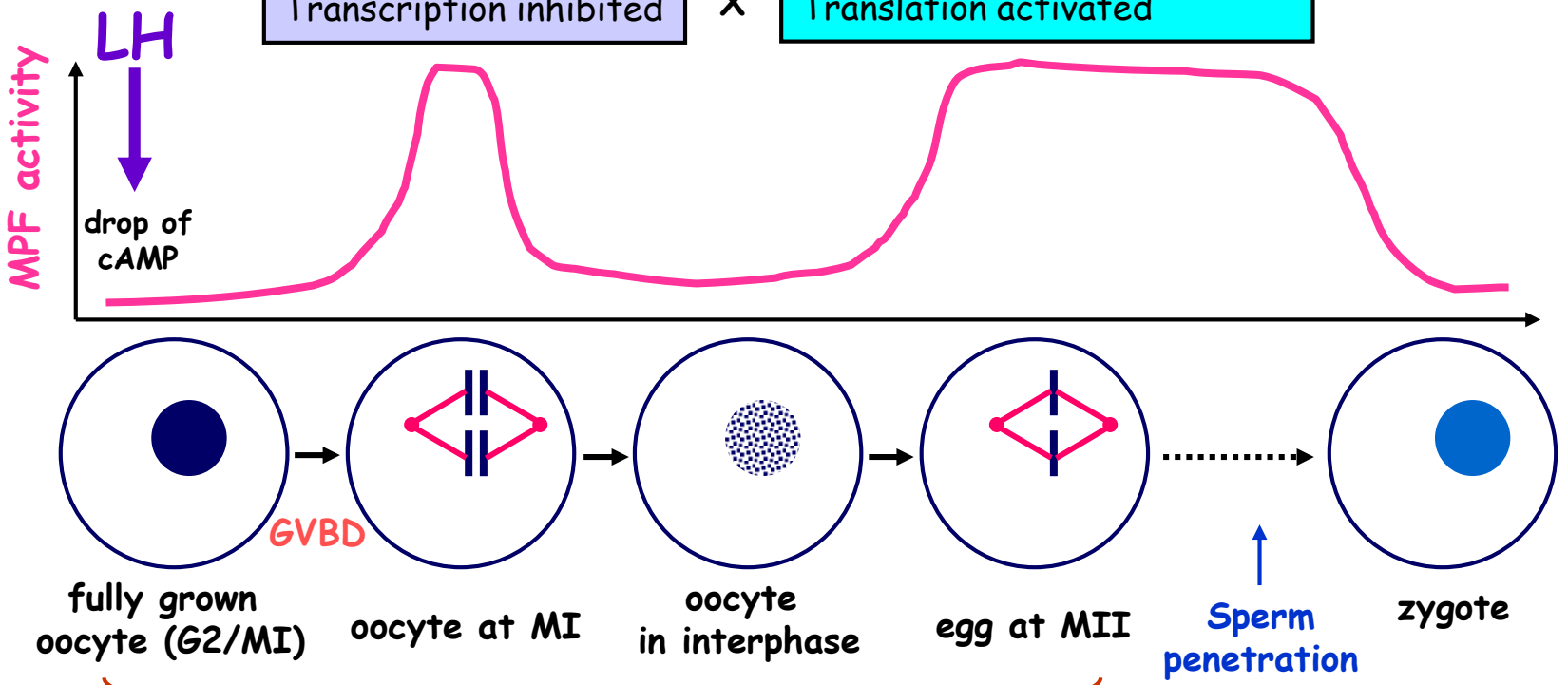
- epigenetic modification of autosomal chromosomes that leads to monoallelic expression of genes – due to activity of enzyme DNA methyltransferase
- PGCs are globally demethylated
- imprinting is newly established during oocyte growth (about 70-80 genes)

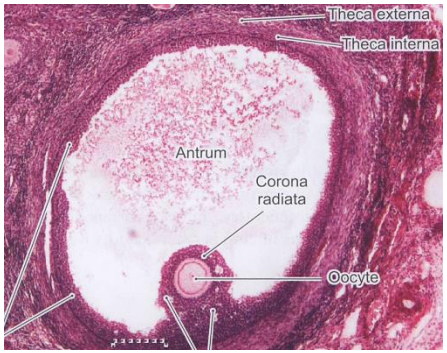
Abnormalities in imprinting may result in spontanneus abortions in assisted reproduction !!!
(*in vitro* manipulation with gametes and embryos may produce abnormalities in imprinting)

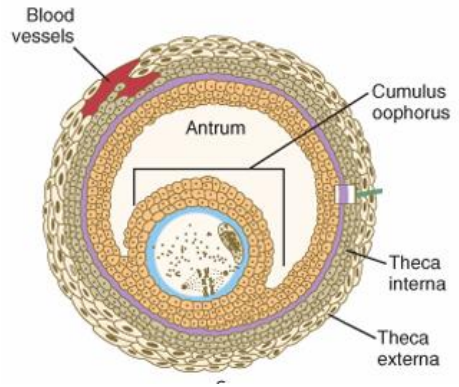
Where and how the oocyte development is achieved ? (5)

The last hours before ovulation – meiotic maturation

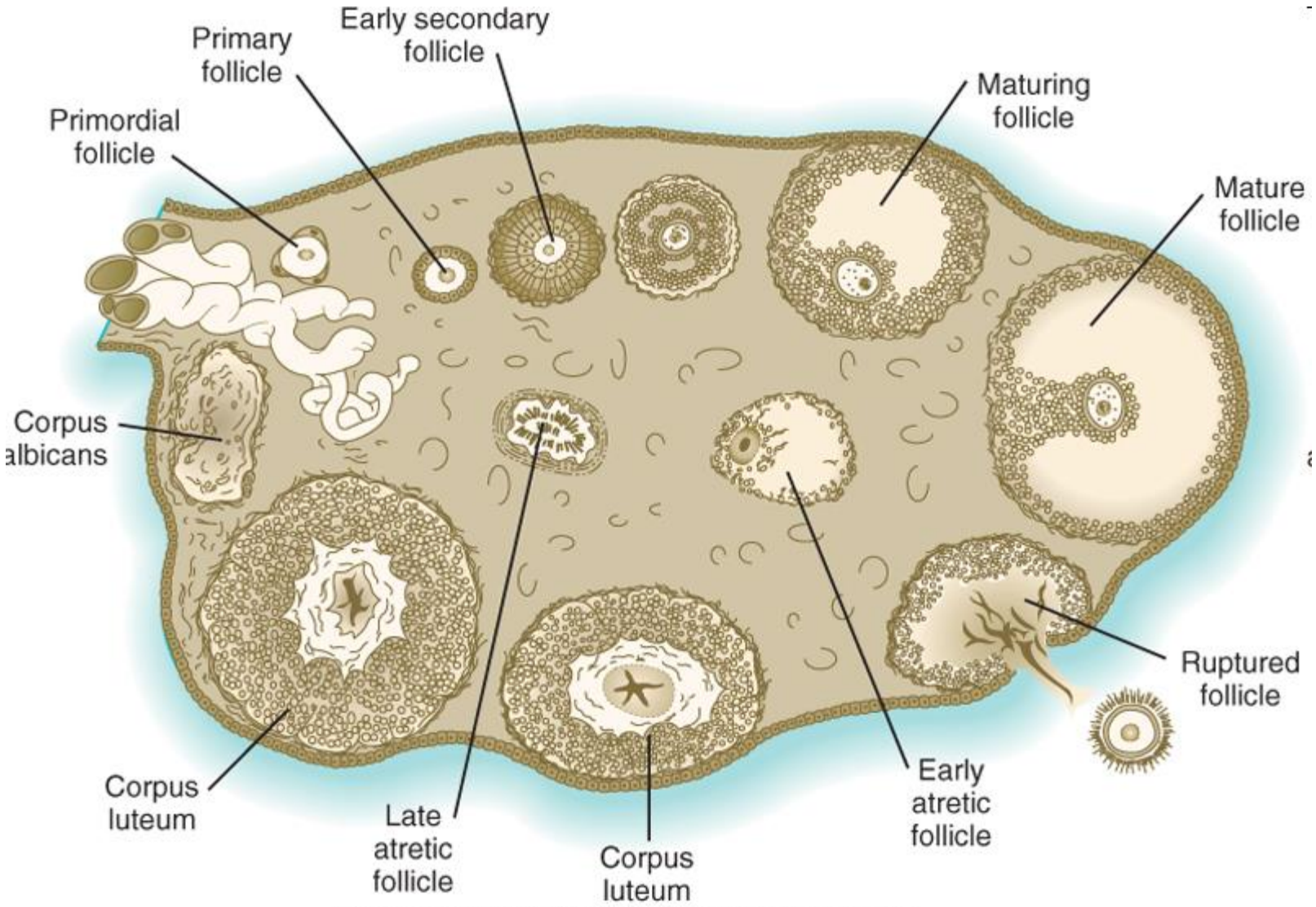
Transcription inhibited X Translation activated





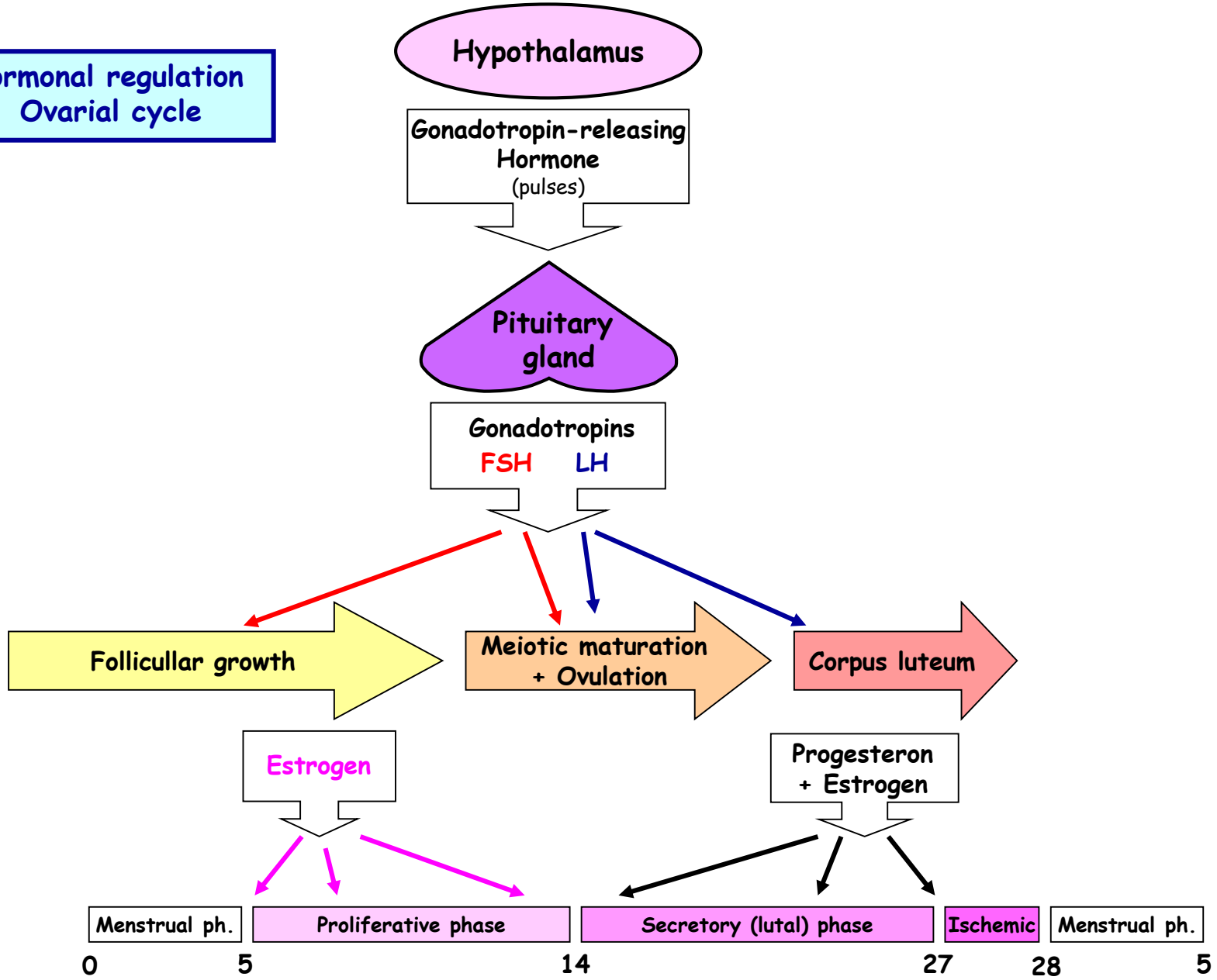


The final look into the ovary

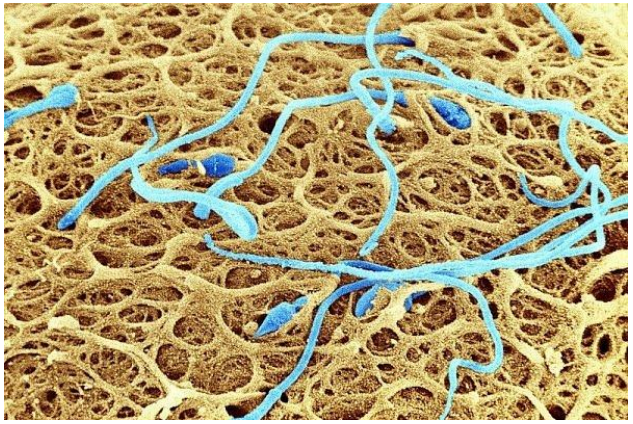


Where and how the oocyte development is achieved ? (6)

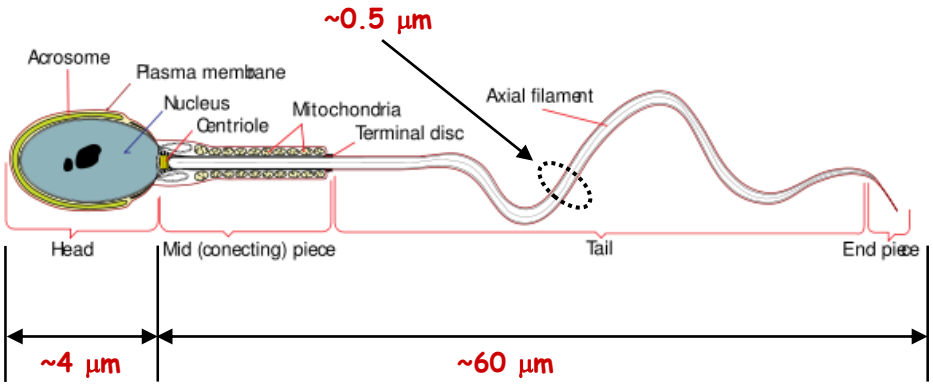
Hormonal regulation
Ovarial cycle



Sperm cell development - Spermatogenesis (1)



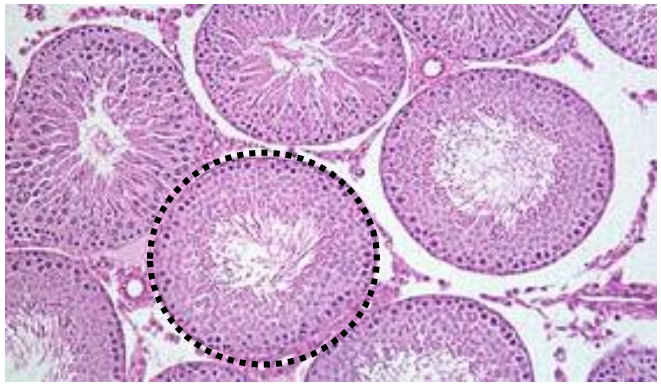
Sperms on the oocyte



Minimal ejaculate (WHO)

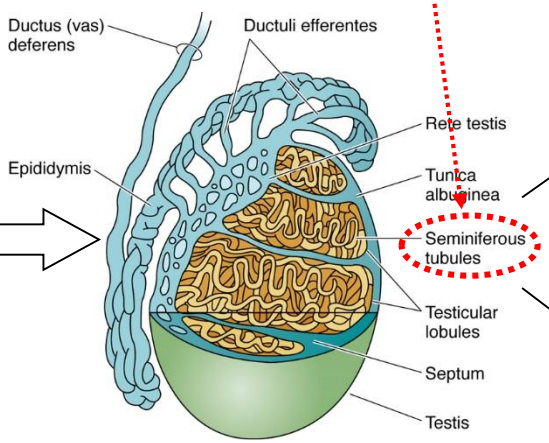
- Volume - 1.5 ml
- Sperm number - 15.1 millions/ml
- Motility - 40%

Sperm cell development (2)

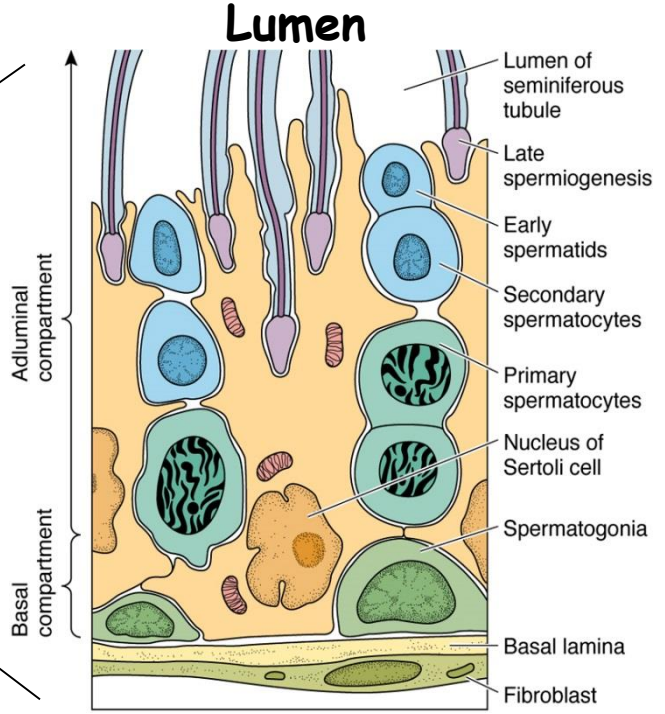


Before puberty → Slowly mitotically dividing spermatogonia in **seminiferous tubuli**

After puberty

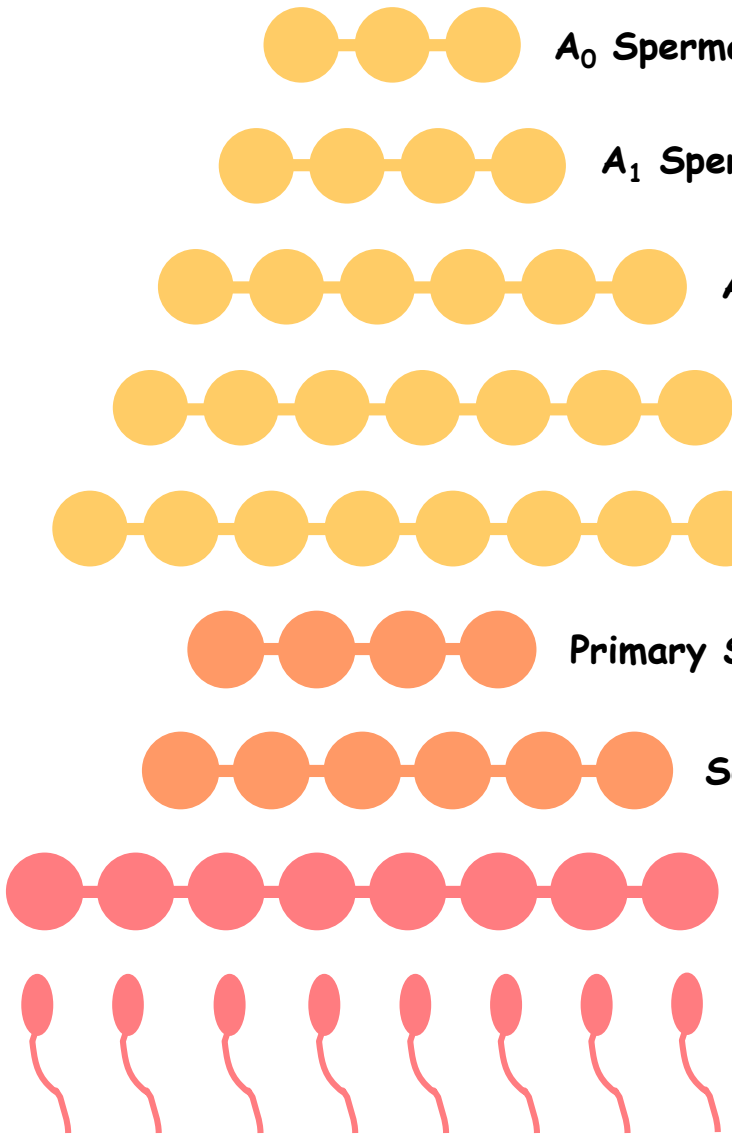


~0.25 mm
~0.5 km



Spermatocytogenesis (mitotic) → **Meiotic phase** → **Spermiogenesis**

Sperm cell development (3)



A_0 Spermatogonia – Stem cells

A_1 Spermatogonia

A_2 Spermatogonia

A_3 Spermatogonia

B Spermatogonia

- Mitotic divisions
- Connected to basal membrane
- 2N, 4C – primary spermatocytes

Primary Spermatocytes

1. Meiotic division

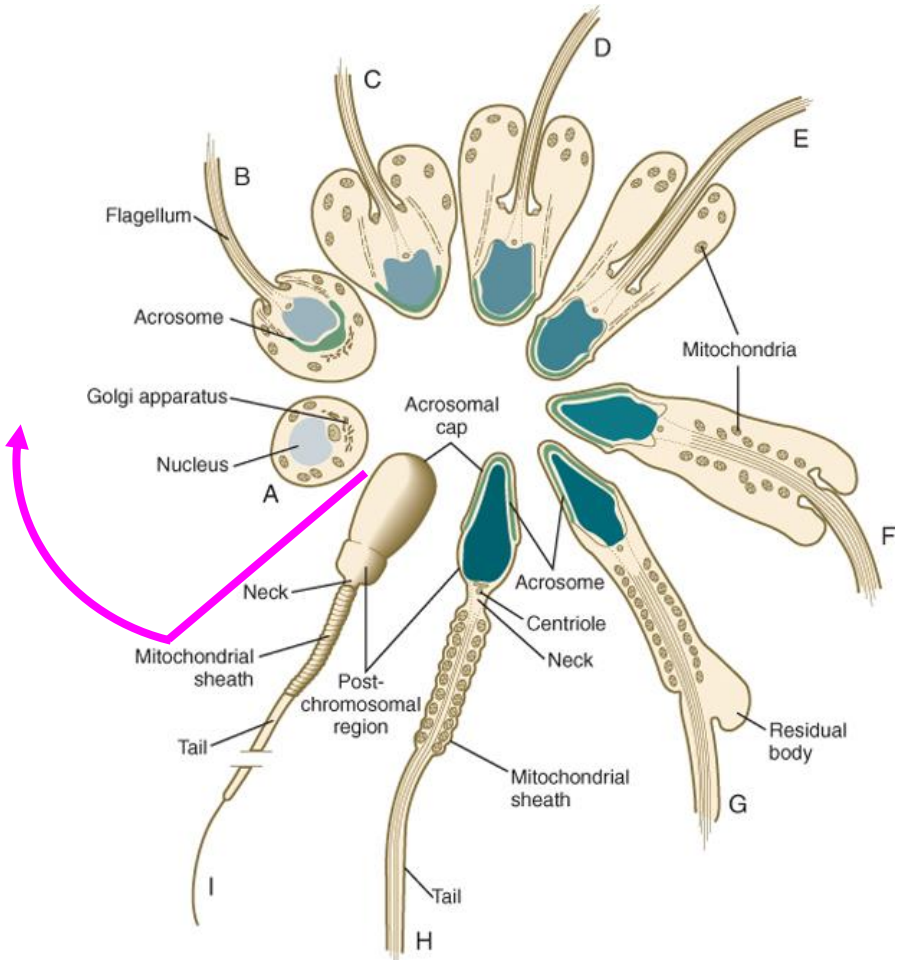
Secondary Spermatocytes - 1N, 2C

2. Meiotic division

Spermatides – 1N, 1C

Spermiogenesis
- No division
- Differentiation

Sperm cell development (4) - Spermiogenesis



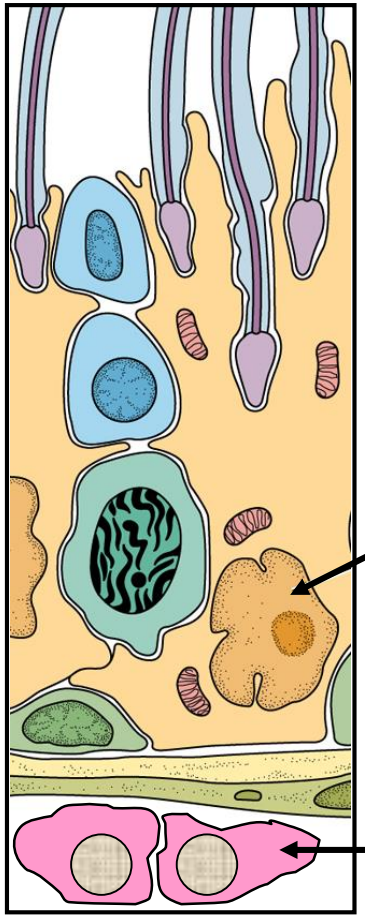
Histones to Protamines
Genome inactivation
Loss of cytoplasm

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

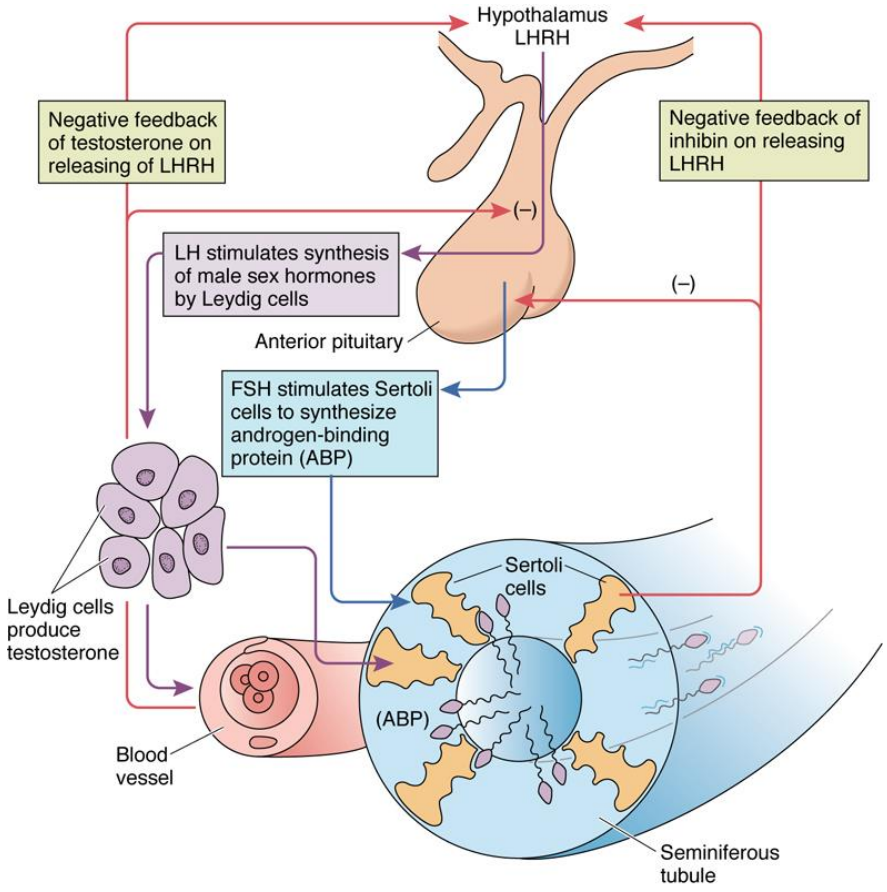


Sertoli cells

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

Leydig cells

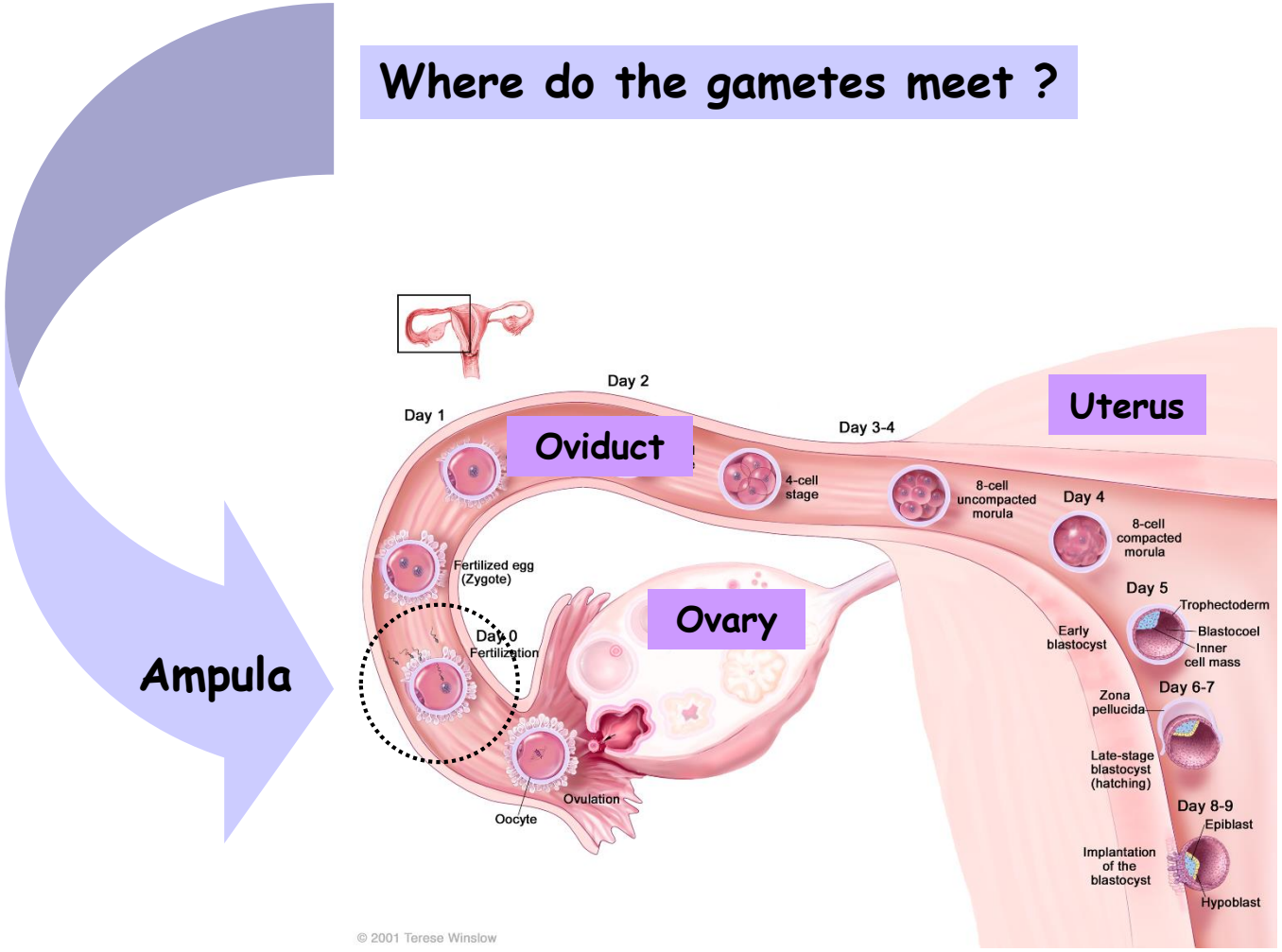
- In interstitium
- 10 % of testis
- Produce testosteron



Fertilization (1)

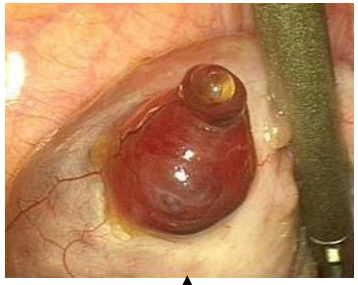
= the process that culminates in the union of one sperm nucleus with the egg nucleus within the activated egg cytoplasm

Where do the gametes meet ?

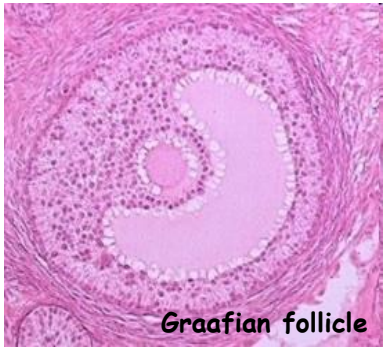


Fertilization (2)

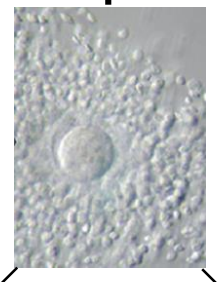
Oocyte makes itself ready for being penetrated



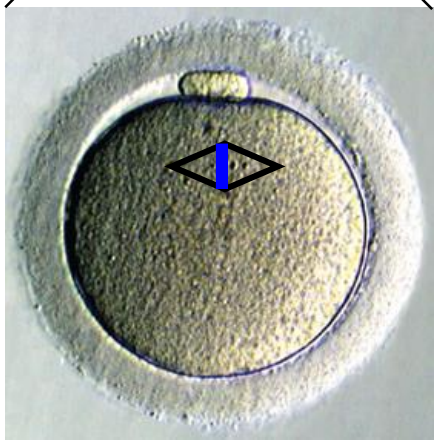
LH surge



35-40 hrs

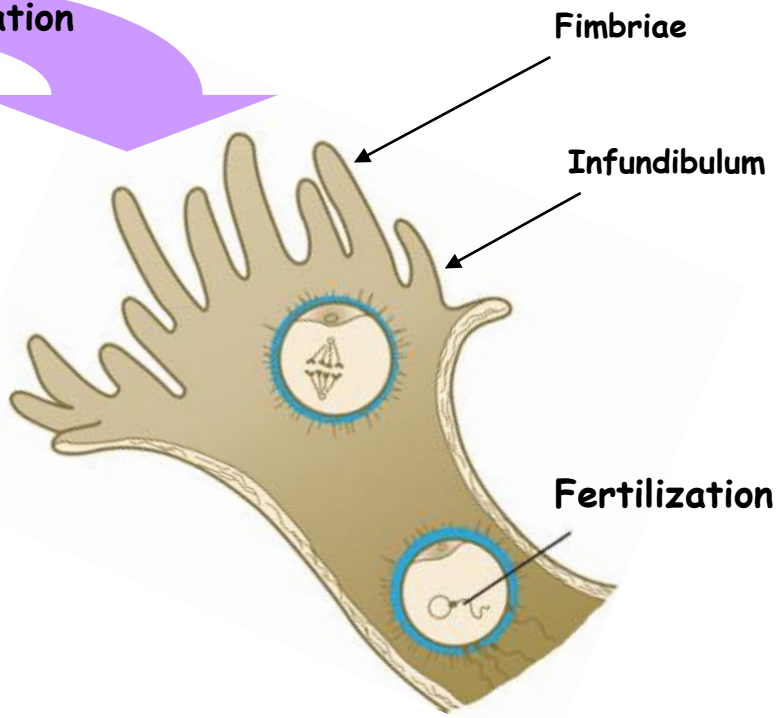


Preovulatory stage



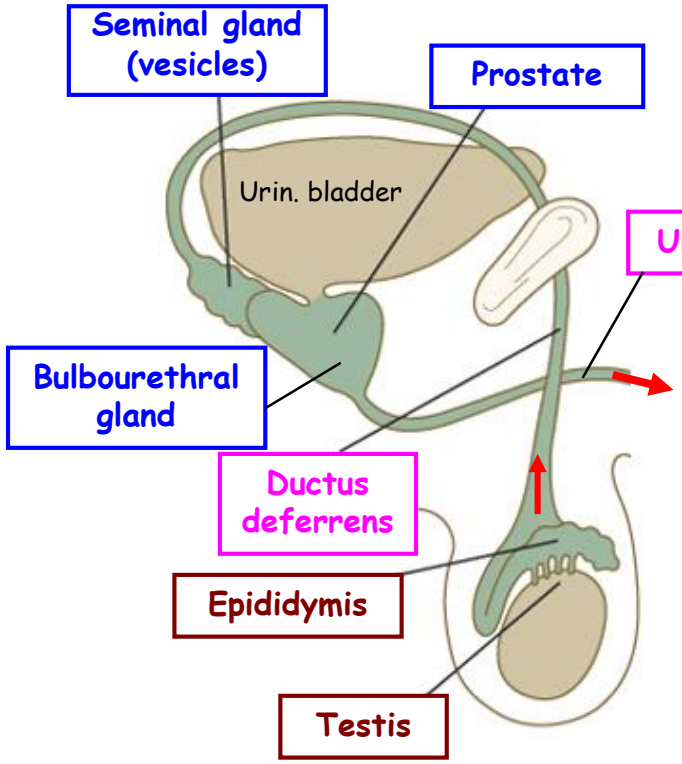
Ovulation

Oocyte is fertilizable for only 12 to 16 hours



Fertilization (3)

Travel of sperm to the site of fertilization



EJACULATE
(2-6 ml)

Sperms

200 – 600 million

Products of accessory sex glands

- Cholesterol (in prostasomes)
- Prostaglandin
- Fructose
- Vesiculase (coagulates semen)

Fertilization (4)

Travel of sperm to the site of fertilization

2 – 7 hours

Vagina
200-600 millions of sperms

Cervix

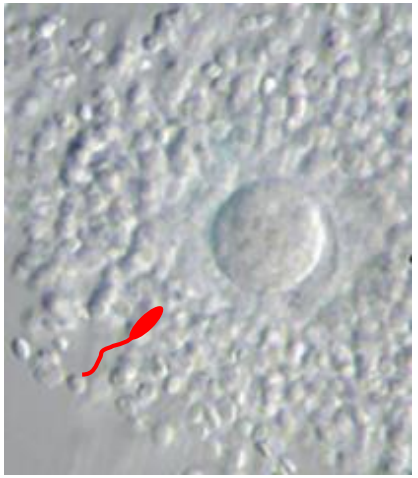
Uterus

Oviduct
100-1000 sperms

- Acid environment
- Sperms move actively

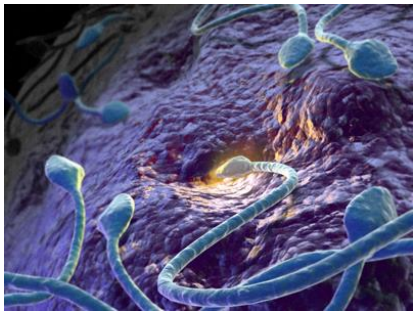
- Cleans sperm
- Cervical mucus stabilizes sperms
- Initiates capacitation

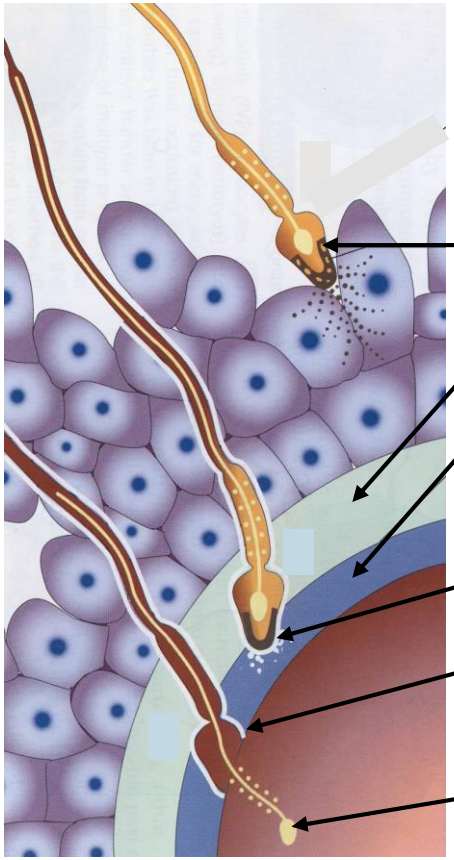
- Removal of glycoproteins from the head
- Change to composition of cell membrane
- Increase of motility



Fertilization (5)

Entry of sperm into the oocyte





Acrosome reaction

Initiated by the contact with ZP proteins

Release of acrosin, neuraminidase, esterases

Release of hyaluronidase

Zona pellucida

Perivitelline space

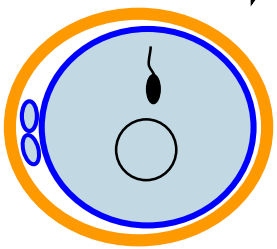
Penetration of ZP

Fusion with oolemma

Entry into ooplasm

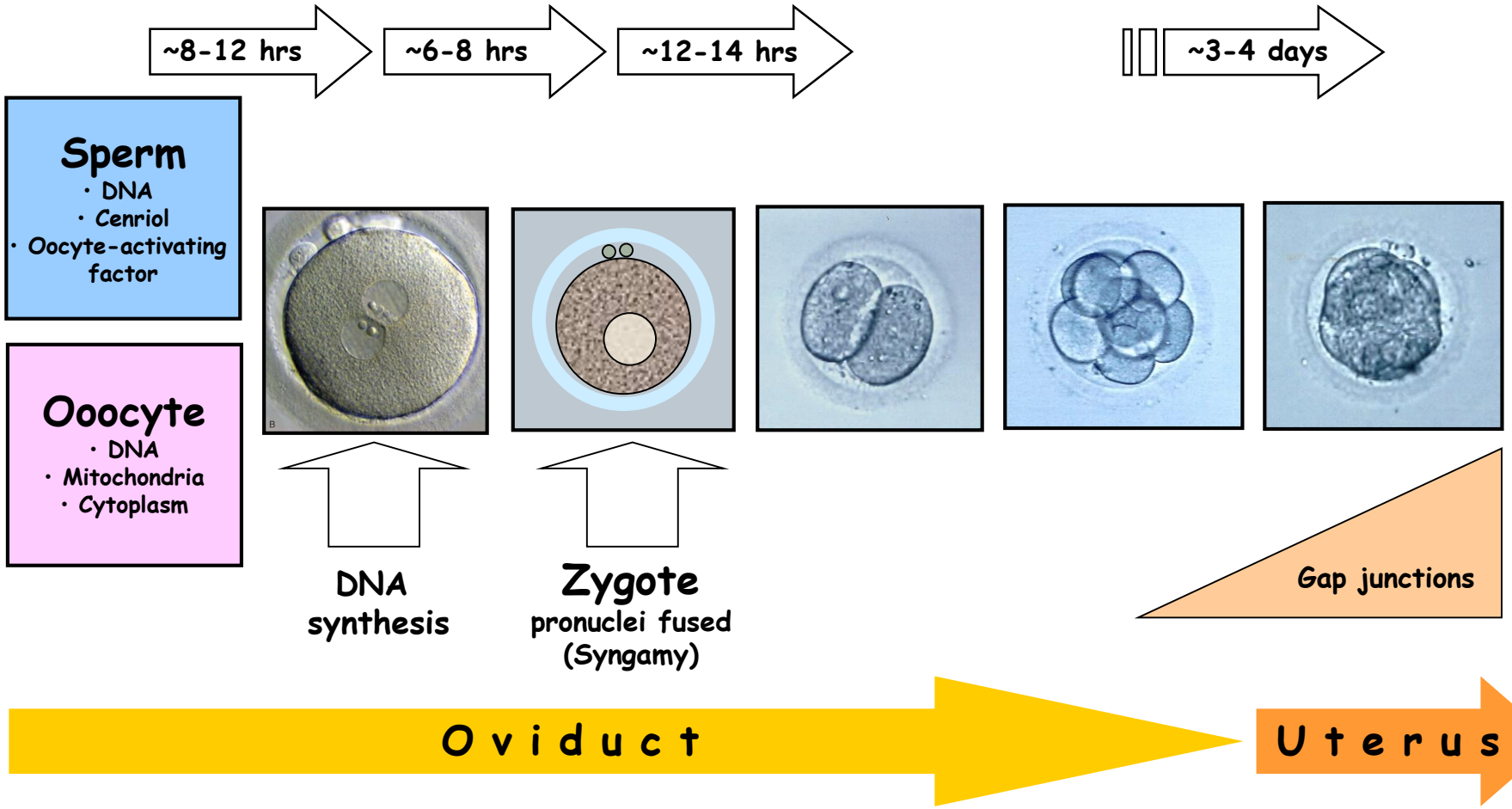
Release of lysosomal enzymes from cortical granules → Modification of ZP → Block of polyspermy

Completion of meiosis II

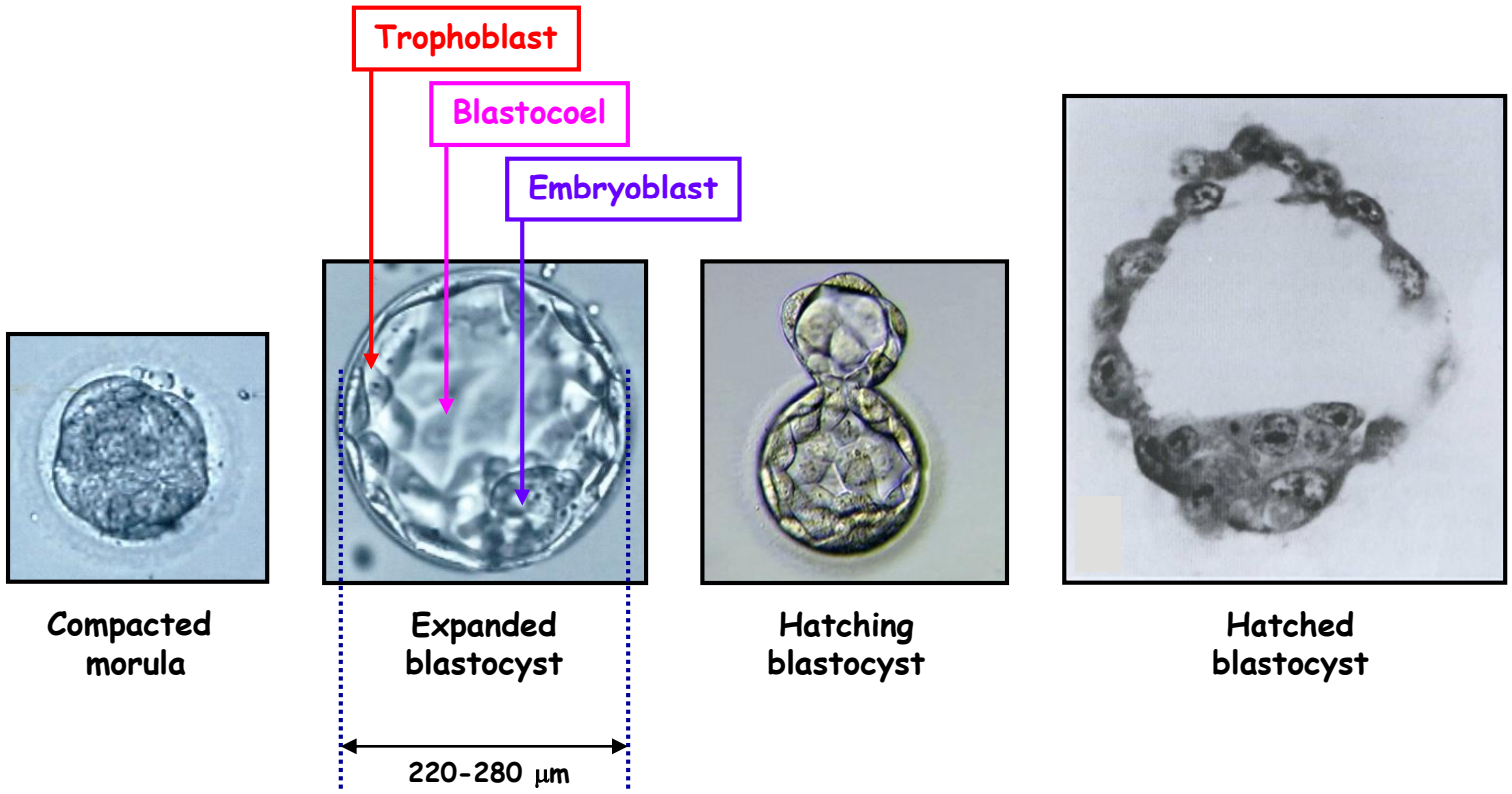


Fertilization (6)

Zygote formation and the first cleavages

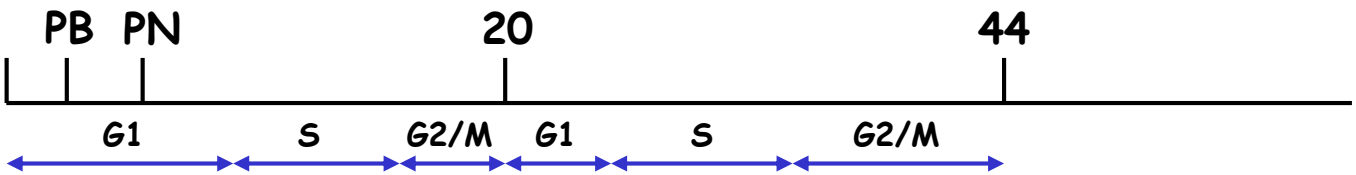


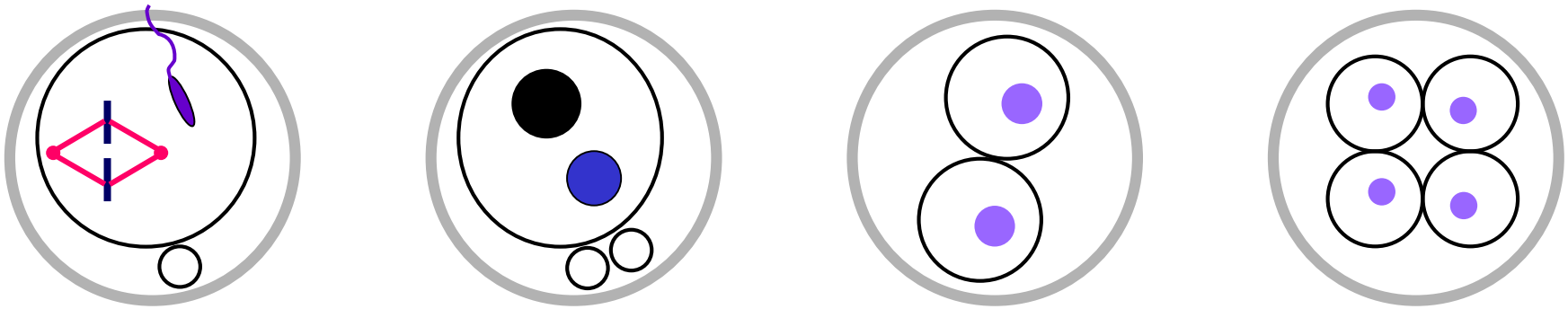
Blastocyst formation



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

Transcrips that replace degraded maternal mRNAs

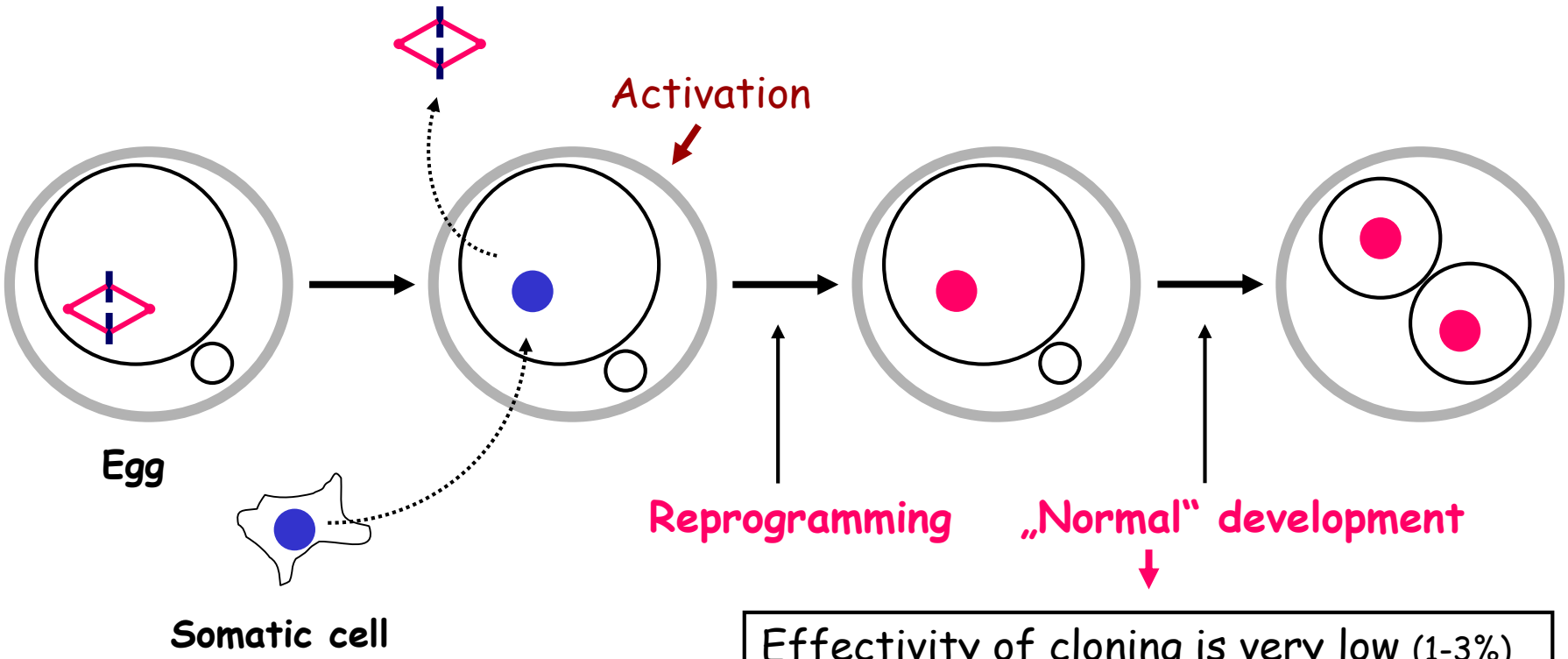
Novel transcripts that underlie new pattern of gene expression

It is „responsible" for establishment of totipotency of blastomeres

&

It represents phenomenon known as genome REPROGRAMMING

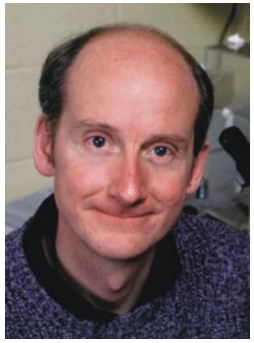
Nuclear transfer (cloning) - principle



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

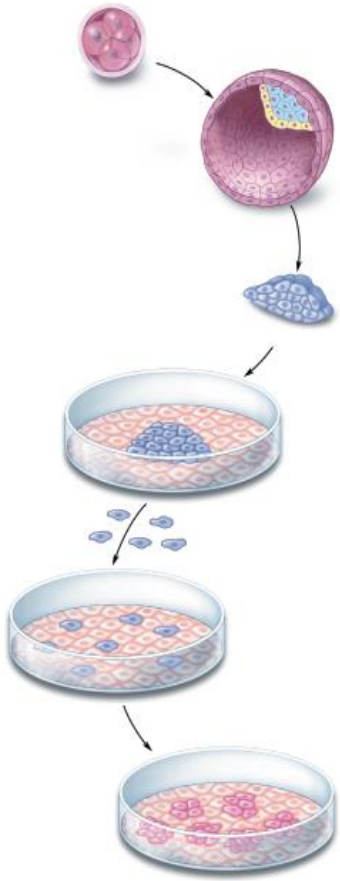
Reprogramming is slow and most likely incomplete (as the result, gene expression is often abnormal)

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

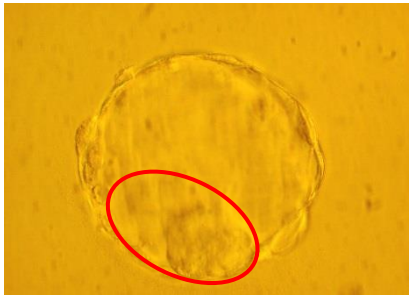


Human Embryonic Stem (hES) Cells

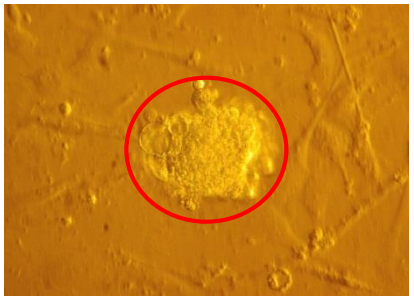
(Thompson et al, 1998)



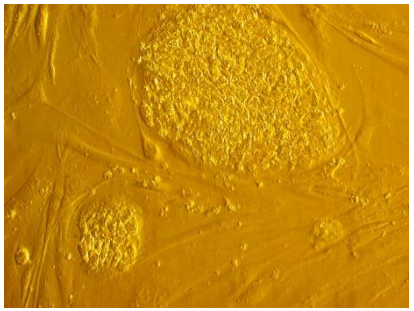
Early embryo at blastocyst stage



Isolated embryoblast (ICM - Inner Cell Mass)



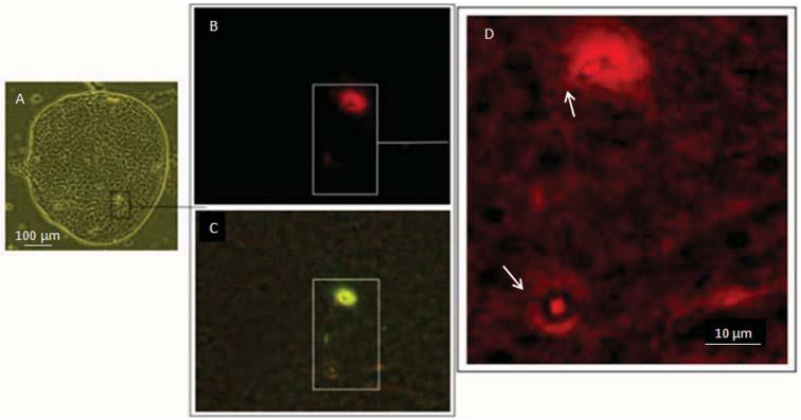
Isolated embryoblast after placing to *in vitro* conditions (+ feeder cells + FGF2)



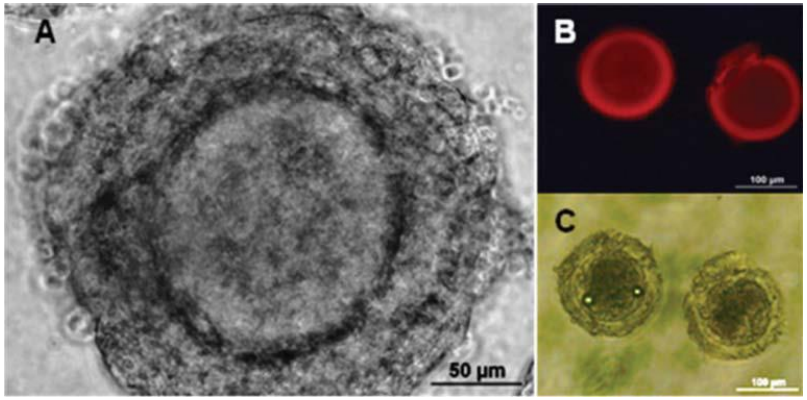
Propagation in culture by enzymatic disaggregation (repeated passaging)

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:
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