

# C8545 Developmental Biology

## Lesson 1

### Introduction into the Study of Development

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# Outline of Lesson 1

- The course at a glance and literature
- Repetitorium of basic terms
  - Major body directions
  - Mitosis vs. Meiosis
- Overview of development
  - Asymmetrical cell division as a major force of the development
  - Genetic integrity of the differentiated cells
  - Concept of stem cells
- Gametogenesis, fertilization and lineage tracing



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# The Course at a Glance

## □ Lesson 1

- Introduction into the Study of Development
  - Repetitorium of Basic Terms
  - Overview of Development
  - Gametogenesis, Fertilization and Lineage Tracing

## □ Lesson 2

- Oogenesis and Early Development of *Drosophila*

## □ Lesson 3

- Early Development of Amphibians and Amniotes

## □ Lesson 4

- Vertebrate Organogenesis
  - Development of Ectodermal Derivatives



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# The Course at a Glance

- **Lesson 5**
  - Vertebrate Organogenesis
    - Development of Mesodermal and Endodermal Derivatives
  
- **Lesson 6**
  - Plant Reproduction
  
- **Lesson 7**
  - Plant Embryogenesis
  
- **Lesson 8**
  - Postembryonic Plant Development



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# The Course at a Glance

- **Lesson 9**
  - Morphogenesis in Animals and in Plants
  
- **Lesson 10**
  - Regulation of Gene Expression in the Development



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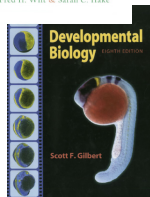


# Literature

PRINCIPLES OF  
DEVELOPMENTAL  
BIOLOGY



Fred H. Wilt & Sarah C. Hake



- **Fred H. Wilt and Sarah Hake, Principles of Developmental Biology** (W.W. Norton & Company, New York, London, 2004)
- **Scott F. Gilbert, Developmental Biology**, eighth edition (Sinauer Associates, Inc., Publishers Sunderland, Massachusetts, USA, 2006)
- Zdeněk Vacek, Embryologie (Grada Publishing, 2006)
- Dubová J., Hejátko J., Friml J. (2005) Reproduction of Plants, in Encyclopedia of Molecular Cell Biology and Molecular Medicine (ed, R. A. Meyers), pp. 249 – 295. Wiley-VCH, Weinheim, Germany
- Selected original papers in scientific journals



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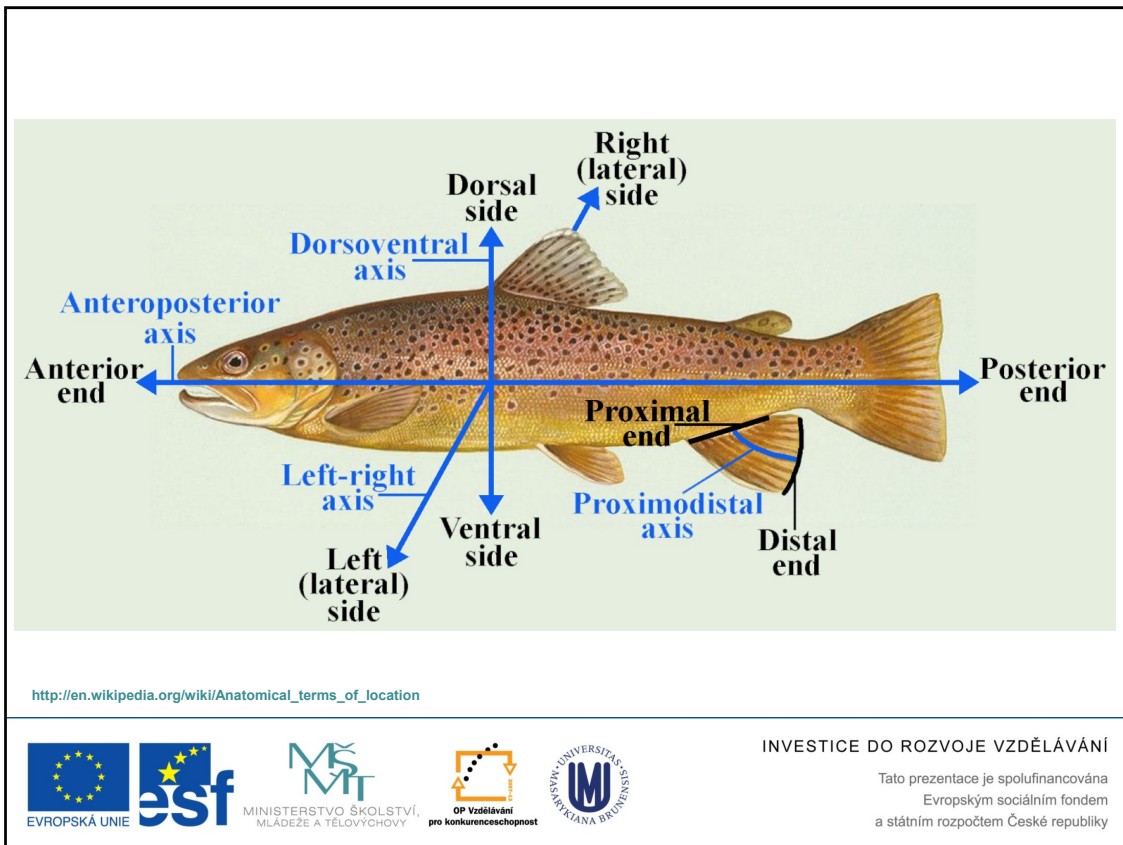
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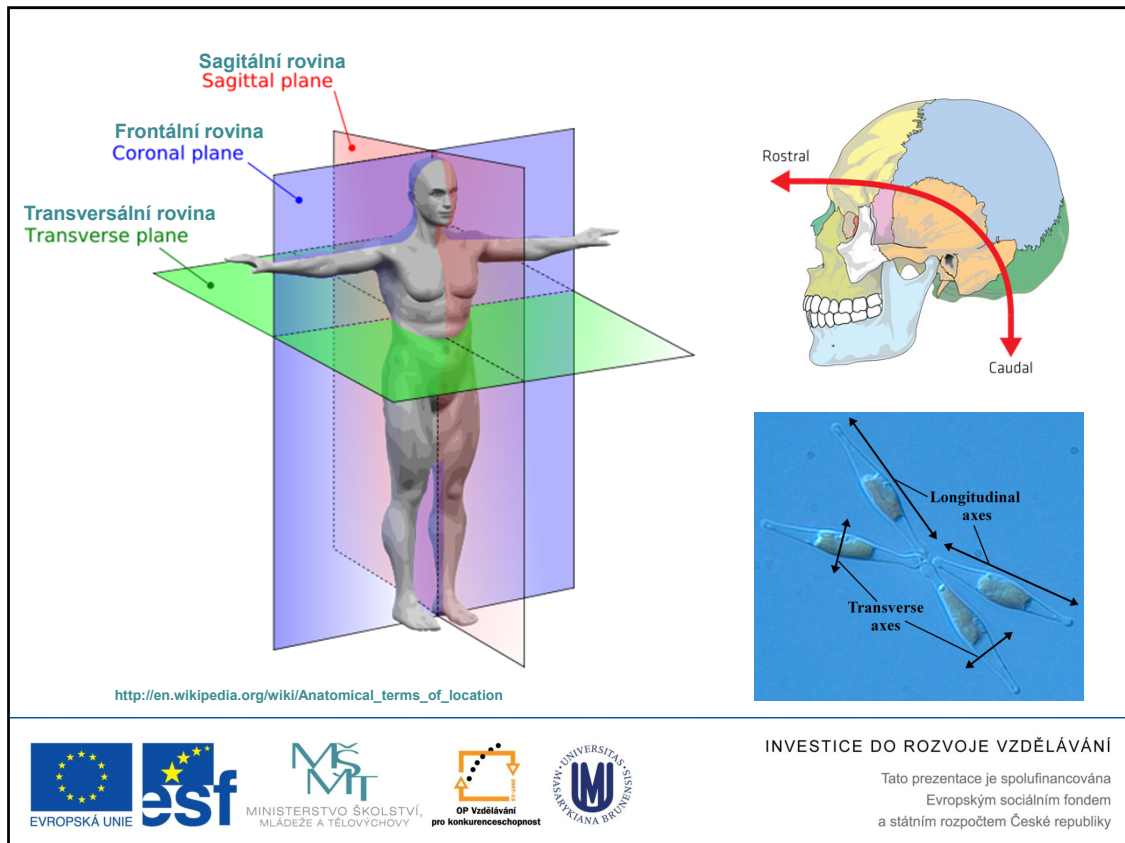


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The terms describing the directions and relative positions in the body were introduced that are independent of the body position or movement (see the figure).



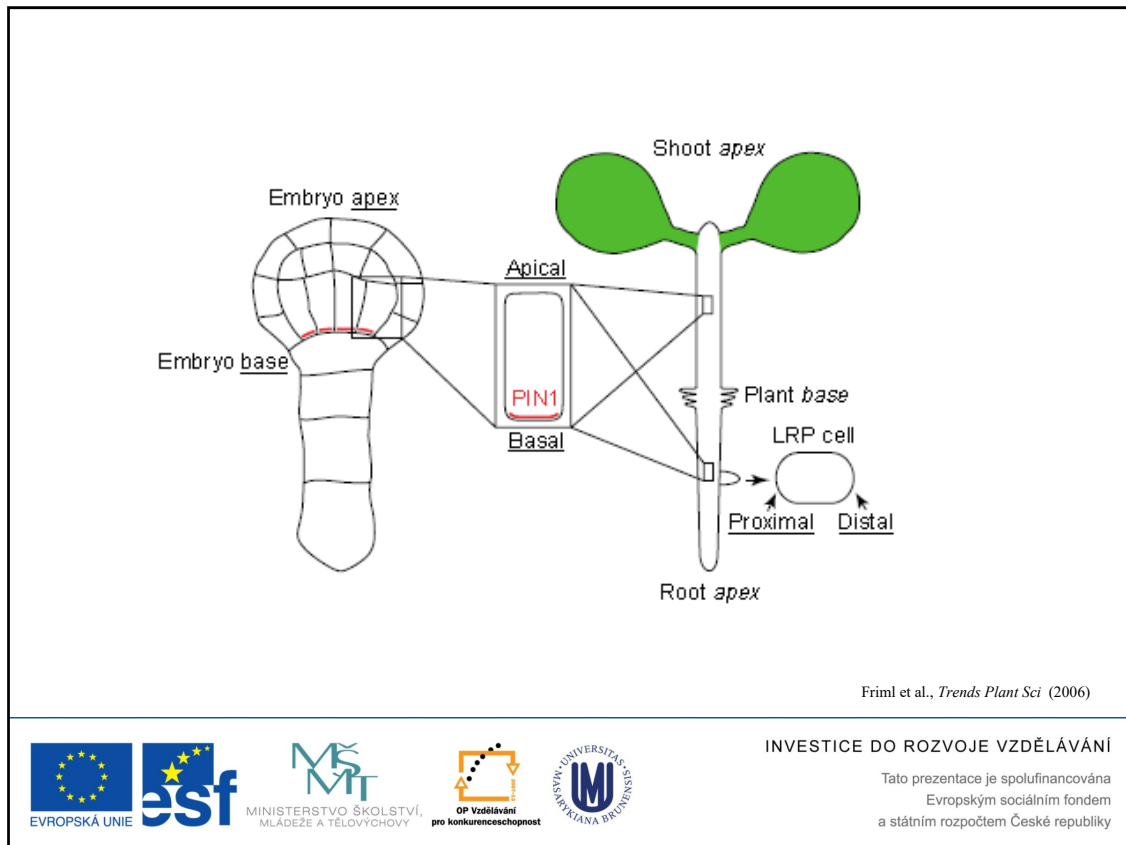
The terms describing the directions and relative positions in the body were introduced that are independent of the body position or movement (see the figure).

**Sagittal plane** goes through or is parallel to the antero-posterior axis and divides the body into *sinister* and *dexter* (left and right) portions.

**Coronal plane** is perpendicular to sagittal plane and divides the body to the dorsal and ventral portions.

Finally, **transversal plane** is perpendicular to both aforementioned planes and divides the body into cranial and caudal (head and tail) portions.

In organisms that maintain a constant shape and have one dimension longer than the other, at least two directional terms can be used. The **long** or **longitudinal axis** is defined by points at the opposite ends of the organism. In higher organisms, this axis might refer to e.g. section of a specimen in an antero-posterior or apical-basal axis. Similarly, a perpendicular **transverse axis** can be defined by points on opposite sides of the organism.



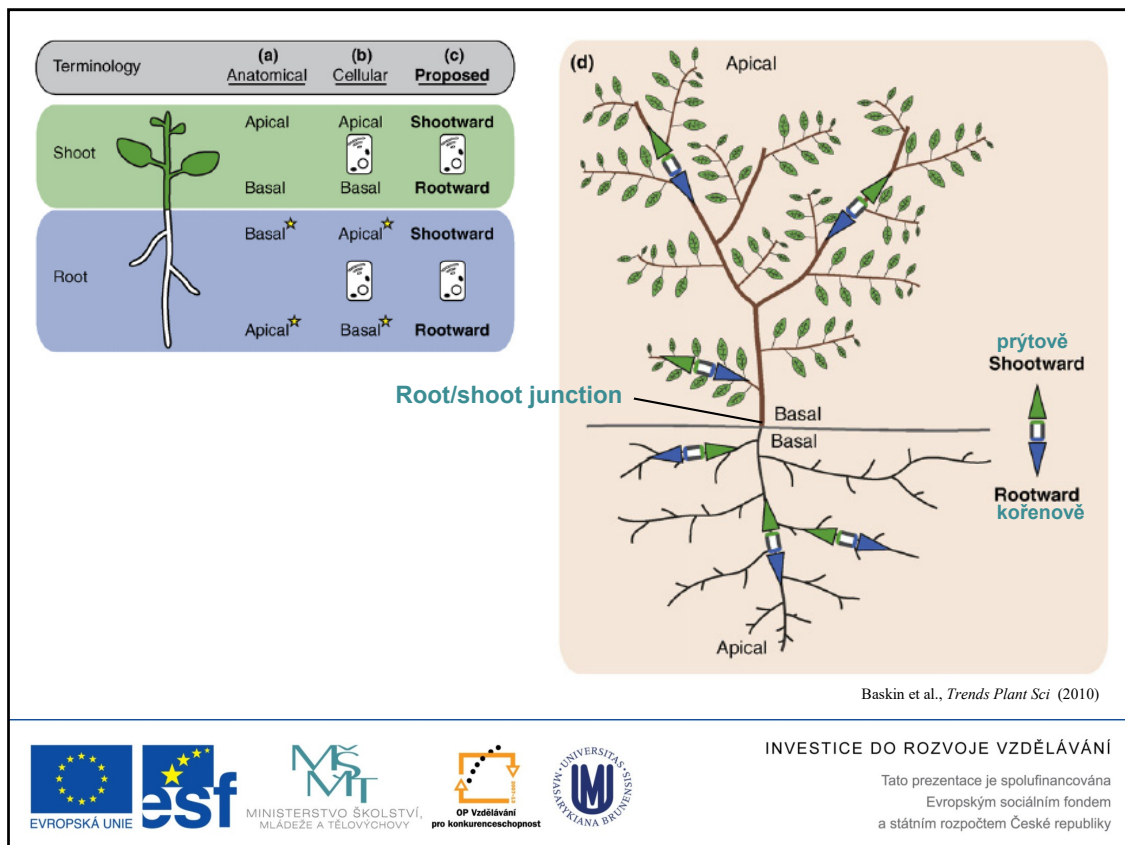
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In plants, the proper terminology undergoes substantial changes recently, as there are differences between embryonic and postembryonic development.

During embryonic development, the **apical-basal axis** is established that refers to the shoot (aerial portion of the plant) and root. This polarity is specified via polar auxin transport that is driven by polar localization of specific proteins, auxin efflux carriers.

These proteins maintain their polarity throughout the postembryonic development, specifying thus the apical-basal directions.



During postembryonic development, however, the anatomical term “apical” refers to two potential directions-apex of the shoot and apex of the root. In this sense, the base is being considered as the root/shoot junction (see the figure).

Therefore, to avoid potential misunderstanding, the novel terms **shootward** and **rootward** were introduced that are being used for the clear determination of **cell polarity**, while the terms apical and basal are suggested to be used only for anatomical purposes.



# Outline of Lesson 1

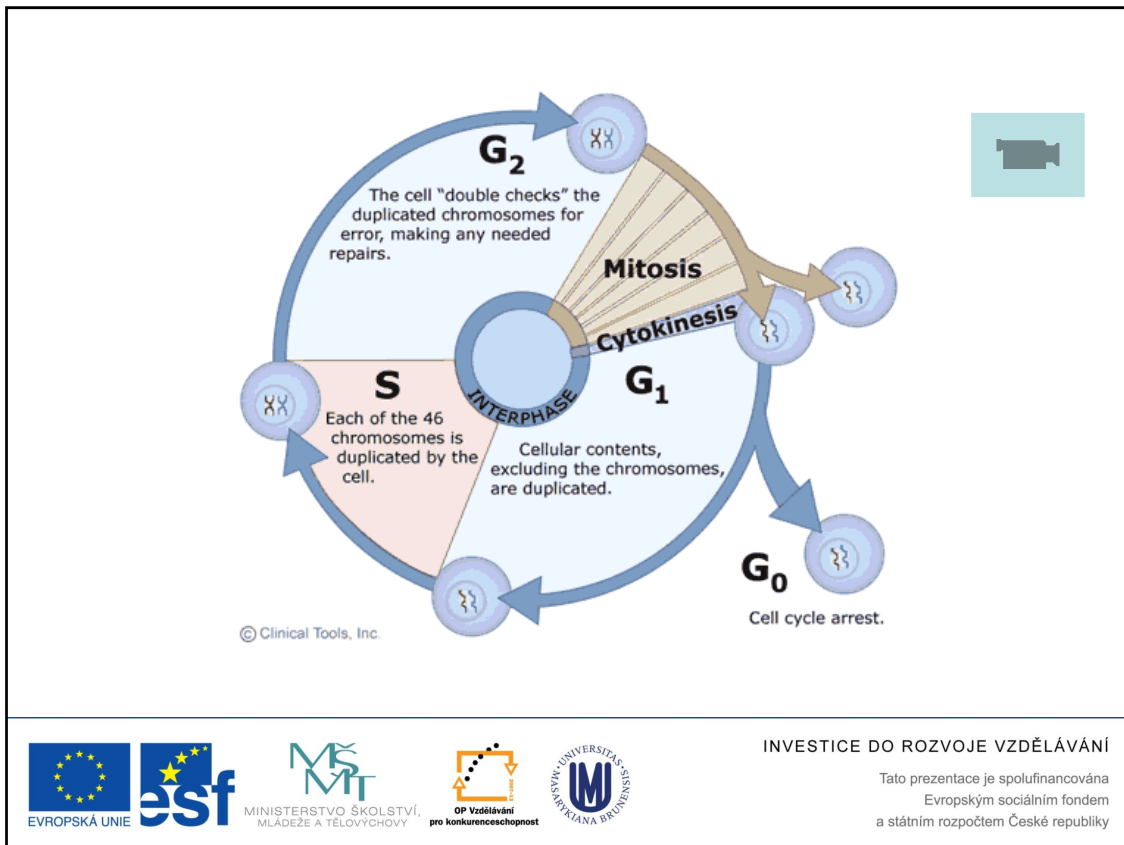
- The course at a glance
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  - Mitosis vs. meiosis



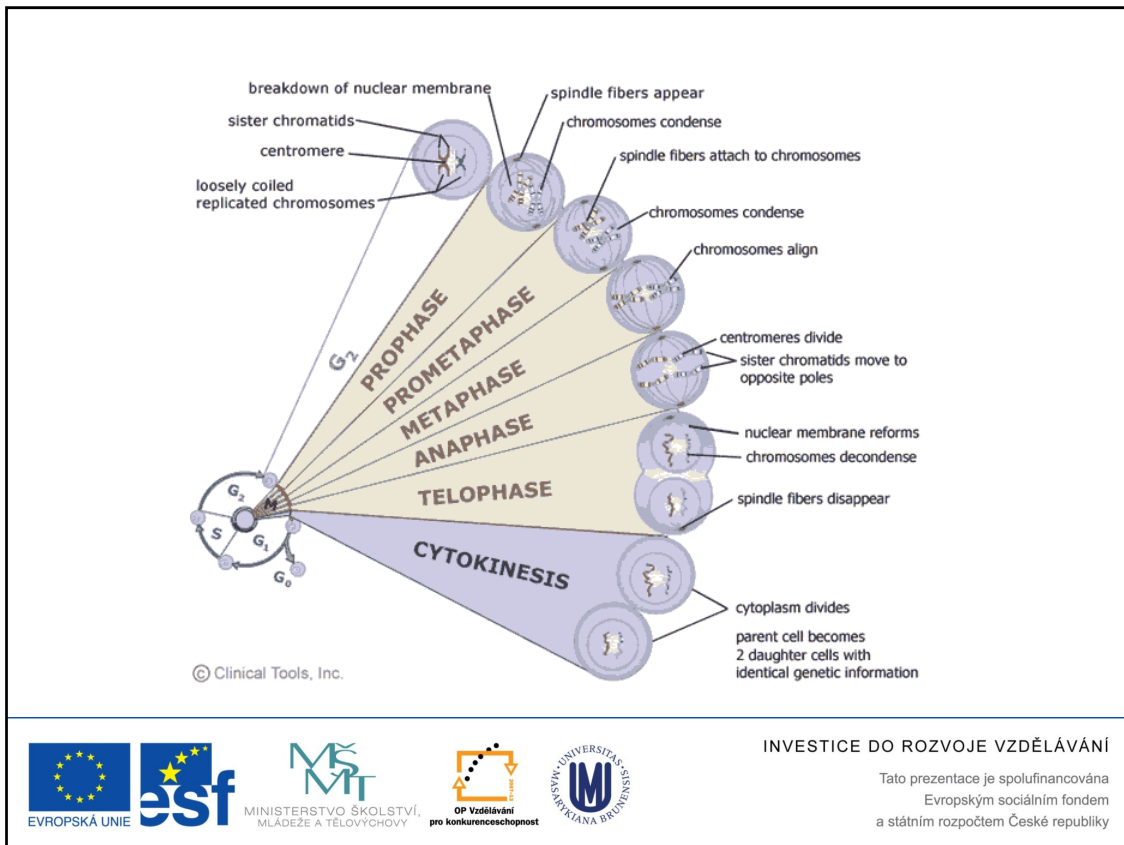
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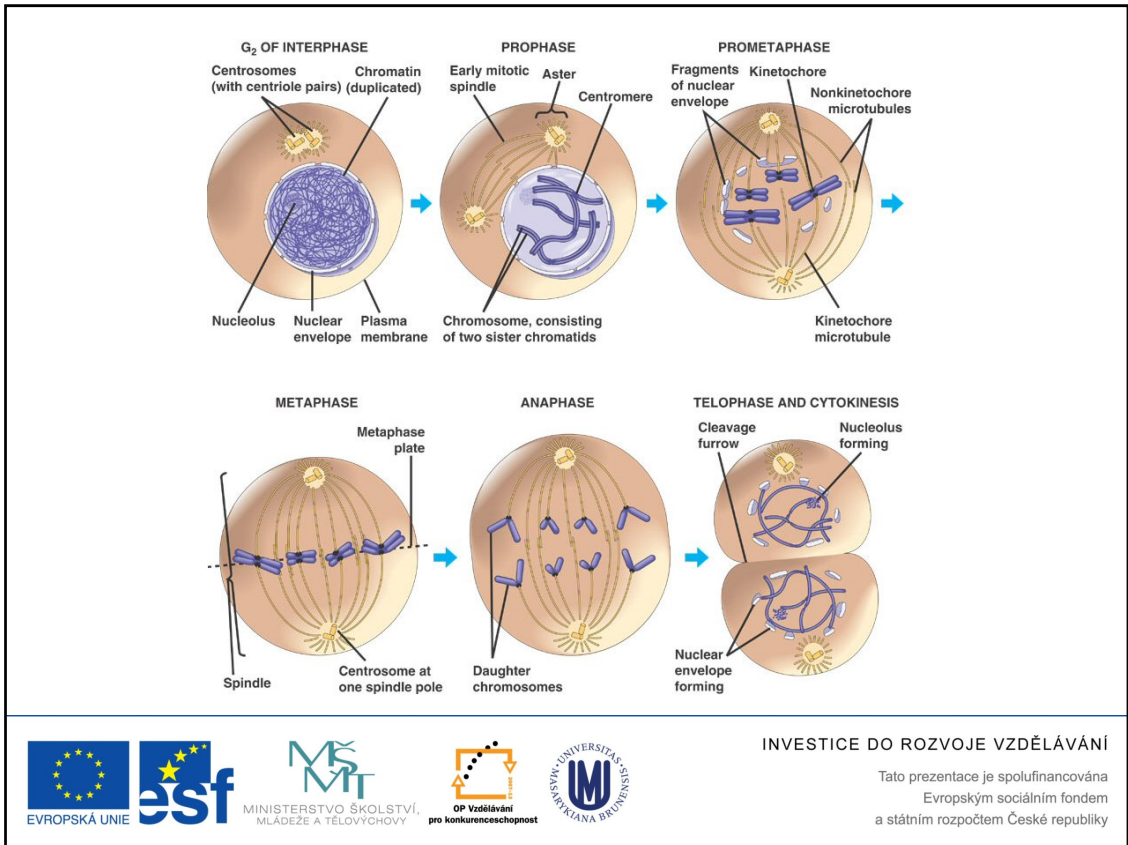
Cell life cycle and commented cell cycle video.



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In the course of mitosis, individual stages could be distinguished.



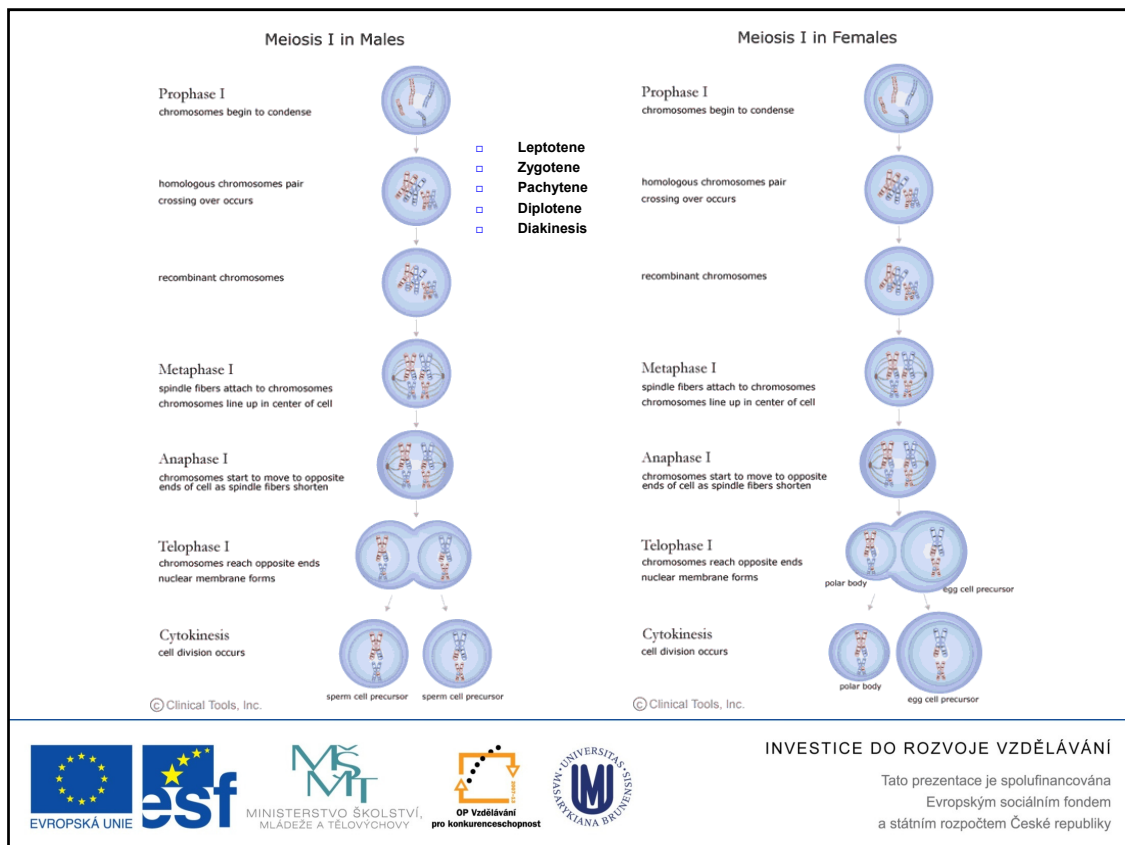
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Meiosis leads to reduction of the chromosome content and formation of gametes, carrying segregated alleles.

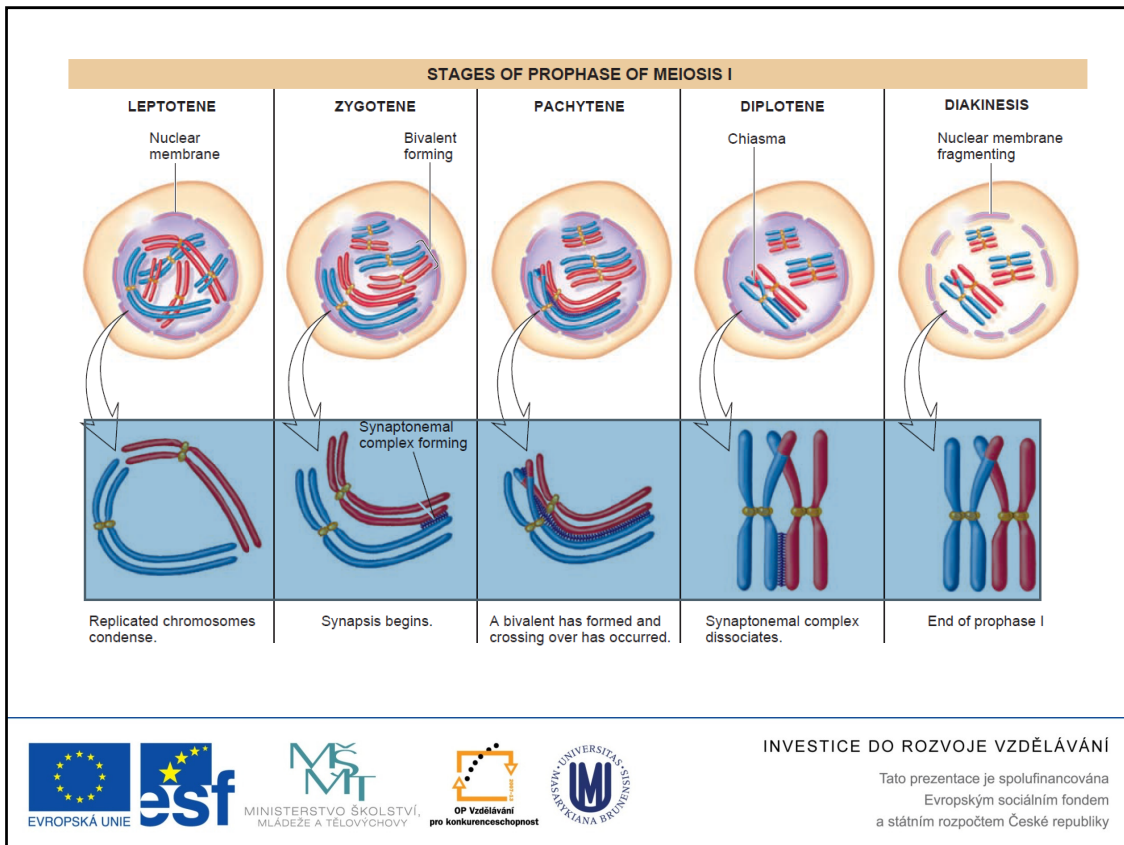
In contrast to males, meiosis in females is **asymmetric**, leading to formation of **single egg cell** and three polar bodies (see the next slide).

Source of the figures: <http://www.le.ac.uk/ge/genie/vgec/he/cellcycle.html>

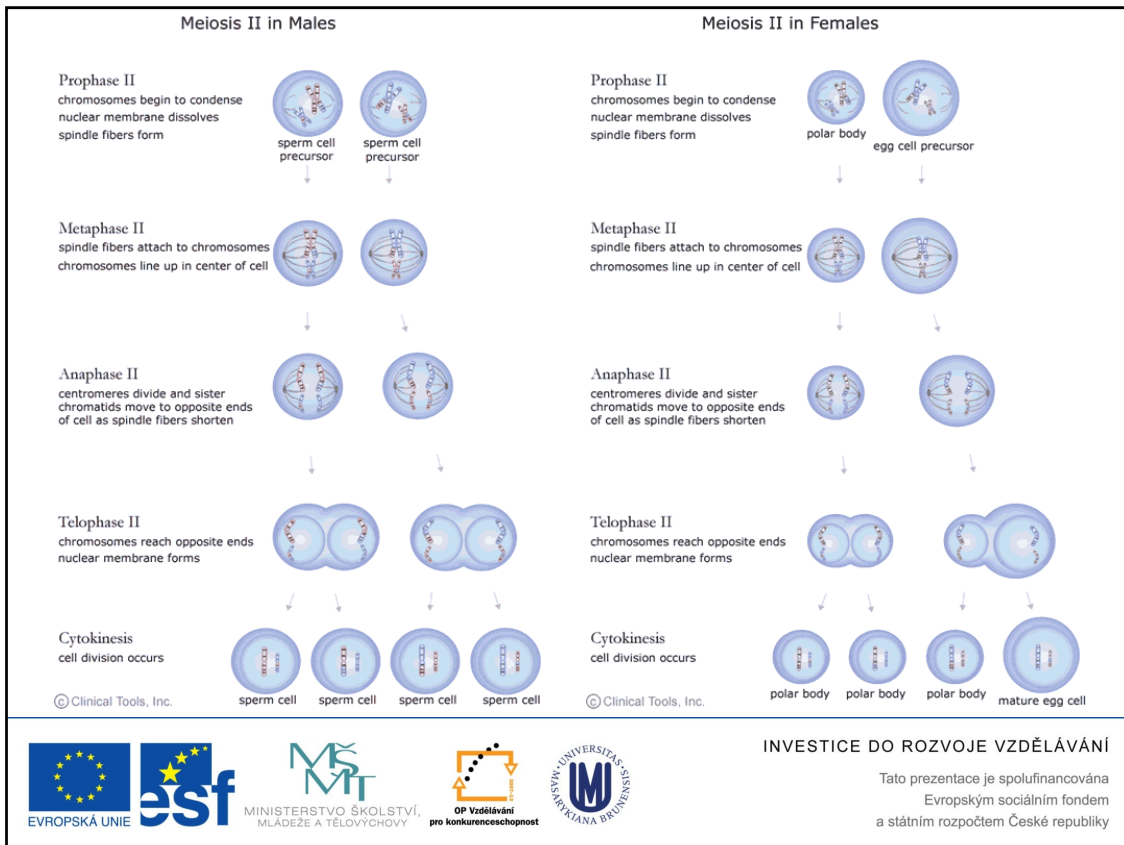


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The prophase I could be further subdivided into five phases: **leptotene**, **zygotene**, **pachytene**, **diplotene** and **diakinesis** (see the figure above).



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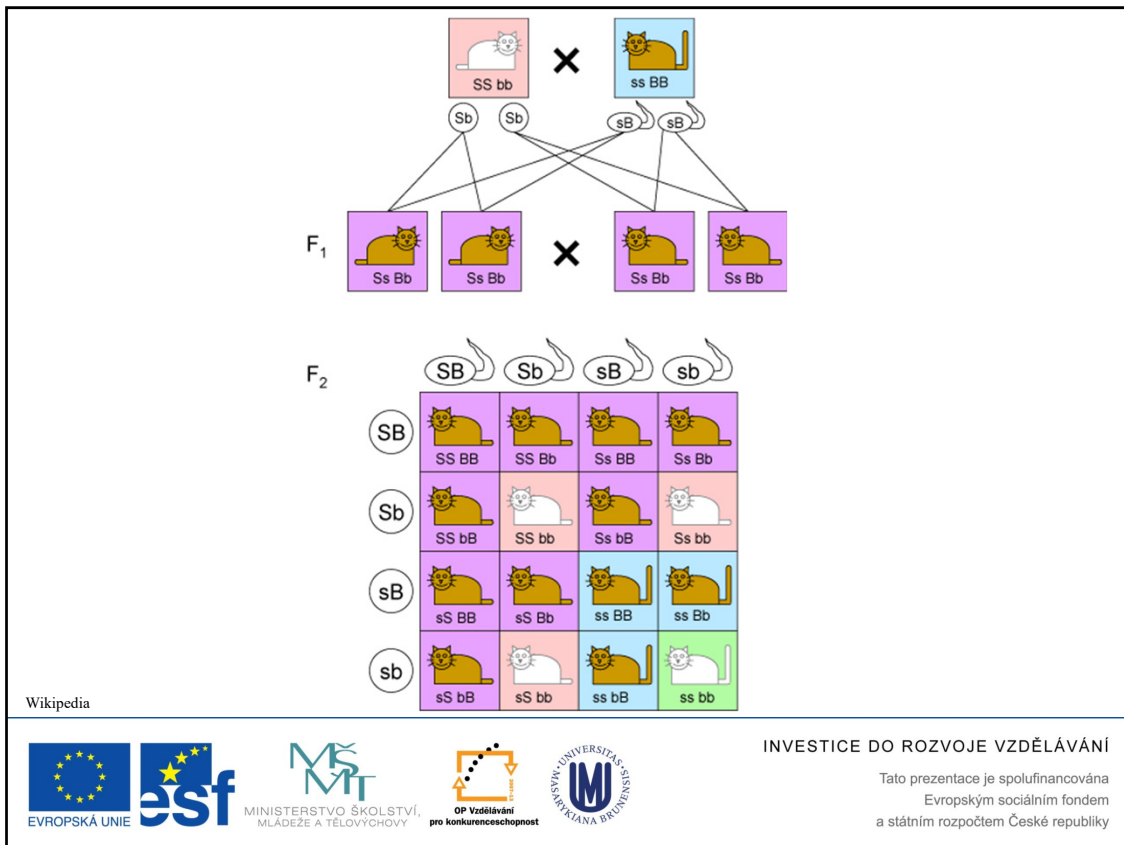
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Gametes formation and their independent and random combination allows novel quality formation via **distribution of individual alleles** and their **new combinations**.



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Hejrátko et al., *Mol Genet Genomics* (2003)

Wikipedia and Bio-Image

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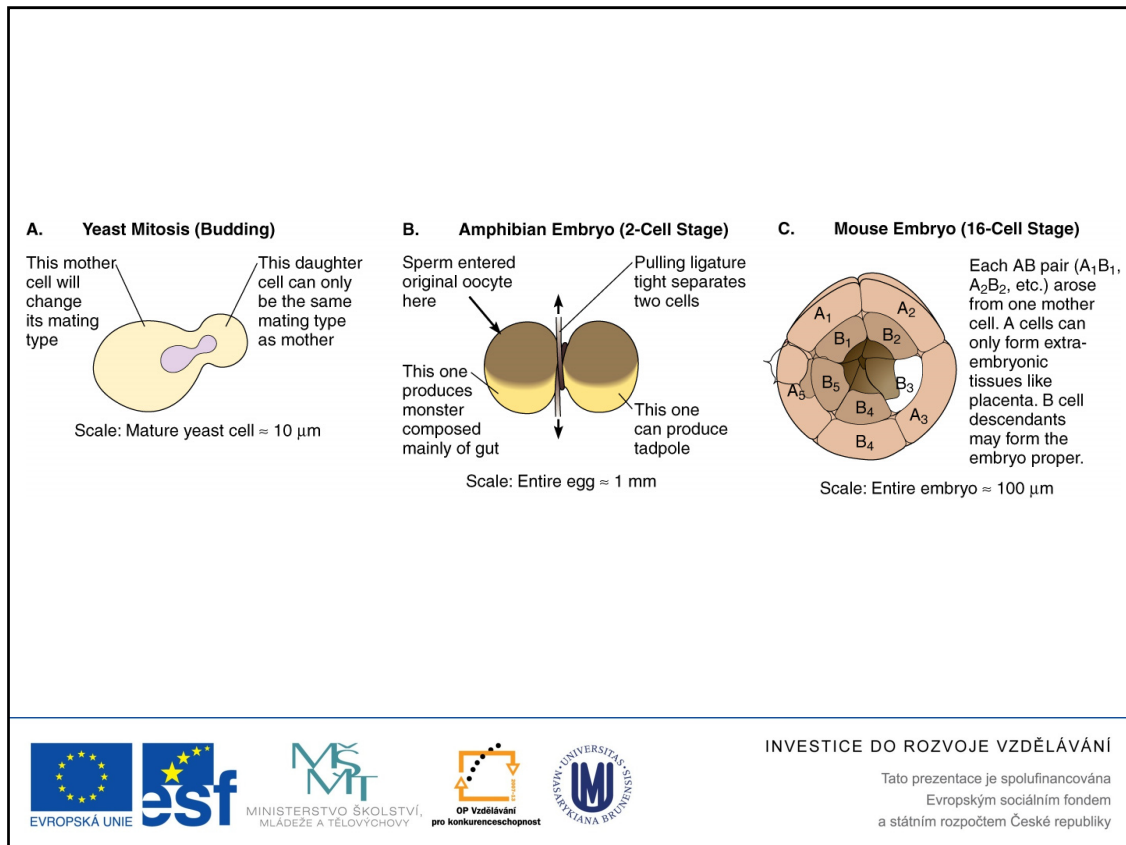
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The fundamental question of developmental biology is formation of multicellular, differentiated organism from a single-cell zygote.

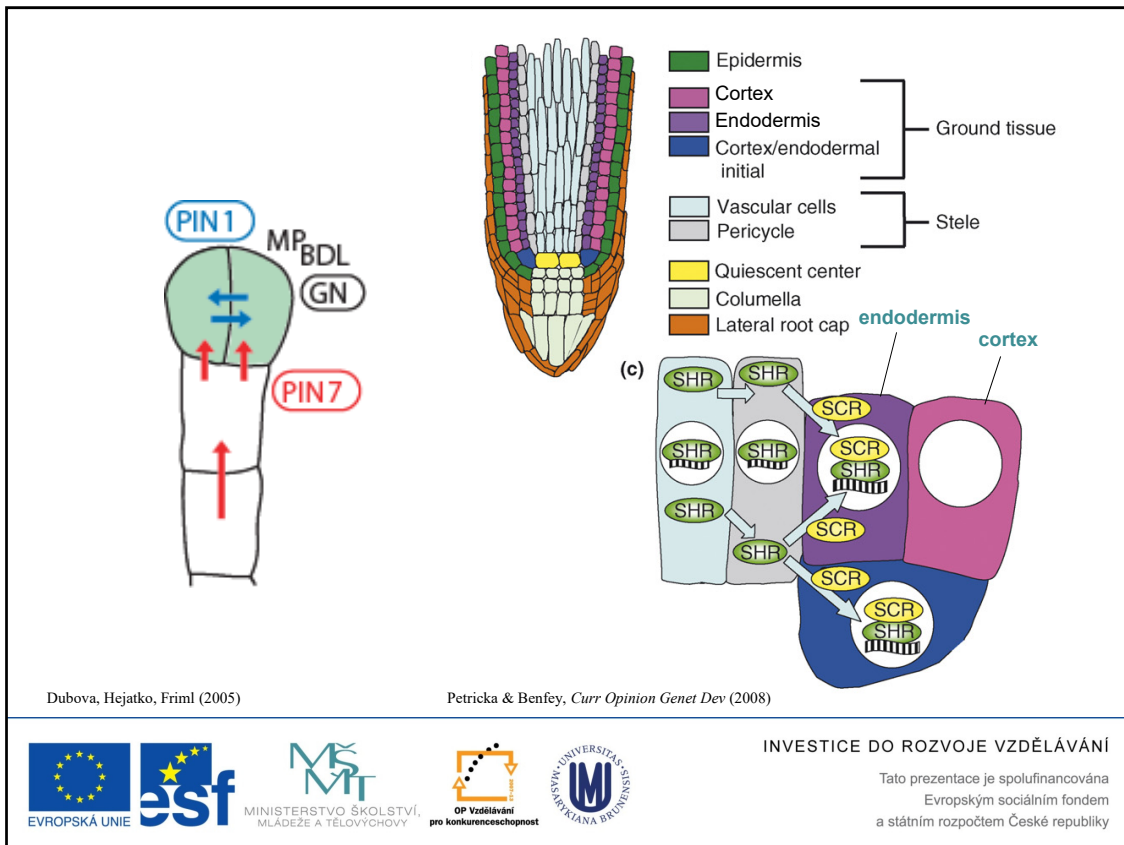
The answer to that question is precise regulation of development via **asymmetrical cell division**. The asymmetrical cell division is defined as a division of the mother cell that results into two, non-equivalent daughter cells.



Demonstration of the non-equivalent cell division.

The molecular mechanisms involved are either **non-equivalent distribution of the cell content** and/or **changes in the cell milieu** (environment) that could be e.g. changes in the light intensity or chemical composition produced by the surrounding cells.

There are basically **two major principles** involved in the regulation of the nature of the cell progeny-the **origin of the cell line** that is mostly important in animal systems and the **position of the cell**, i.e. its environment that is important in both animal and plant systems.



An example of the asymmetric cell division in plants.

Early zygote division (left) or asymmetric division of the stem cell in the root of *Arabidopsis* (right). The movement of the SHORTROOT (SHR) into adjacent cell layer induces expression of SCARECROW (SCR) that is sequestering the SHR into the nuclei of the SCR expressing cells. That allows differentiation of two cell types (endodermis and cortex, respectively) from the common ancestor, the initial (stem) cell. This mechanism will be discussed in more detail in later lessons.

Thus, different cellular environment leads to the nonequivalent differentiation of the two daughter cells.



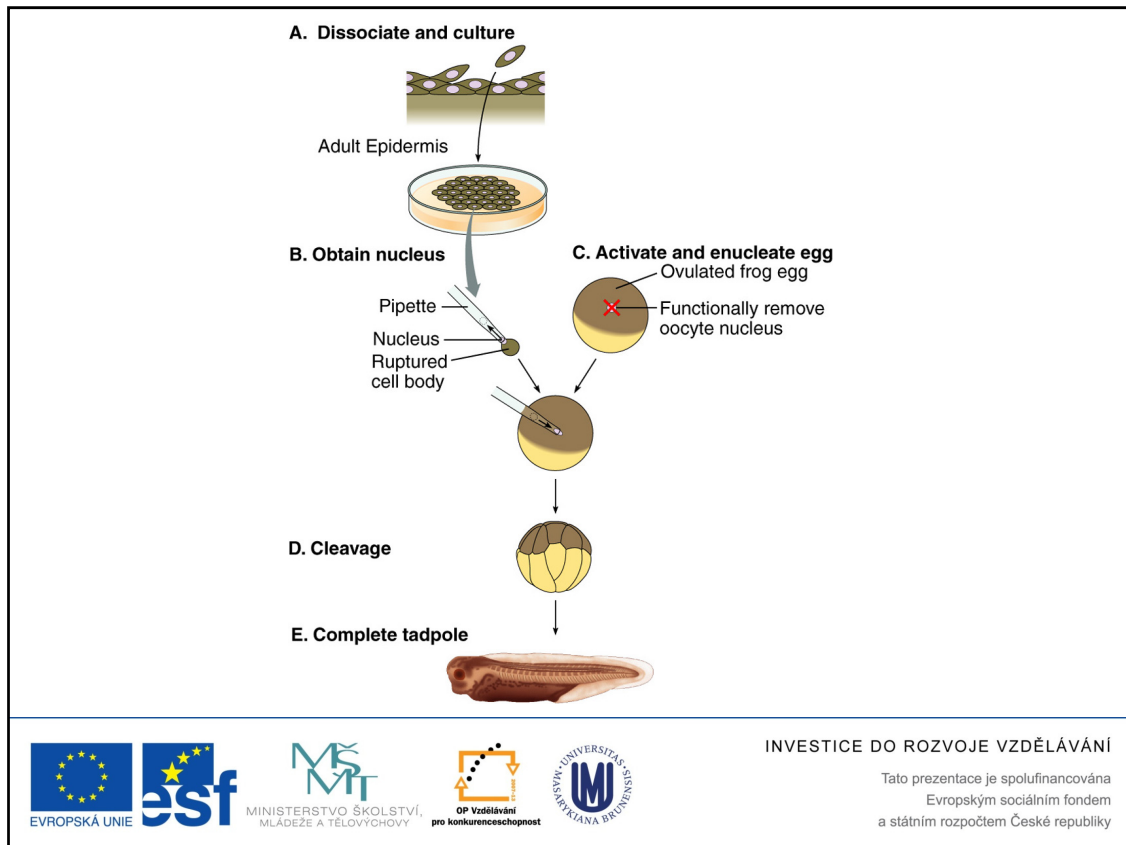
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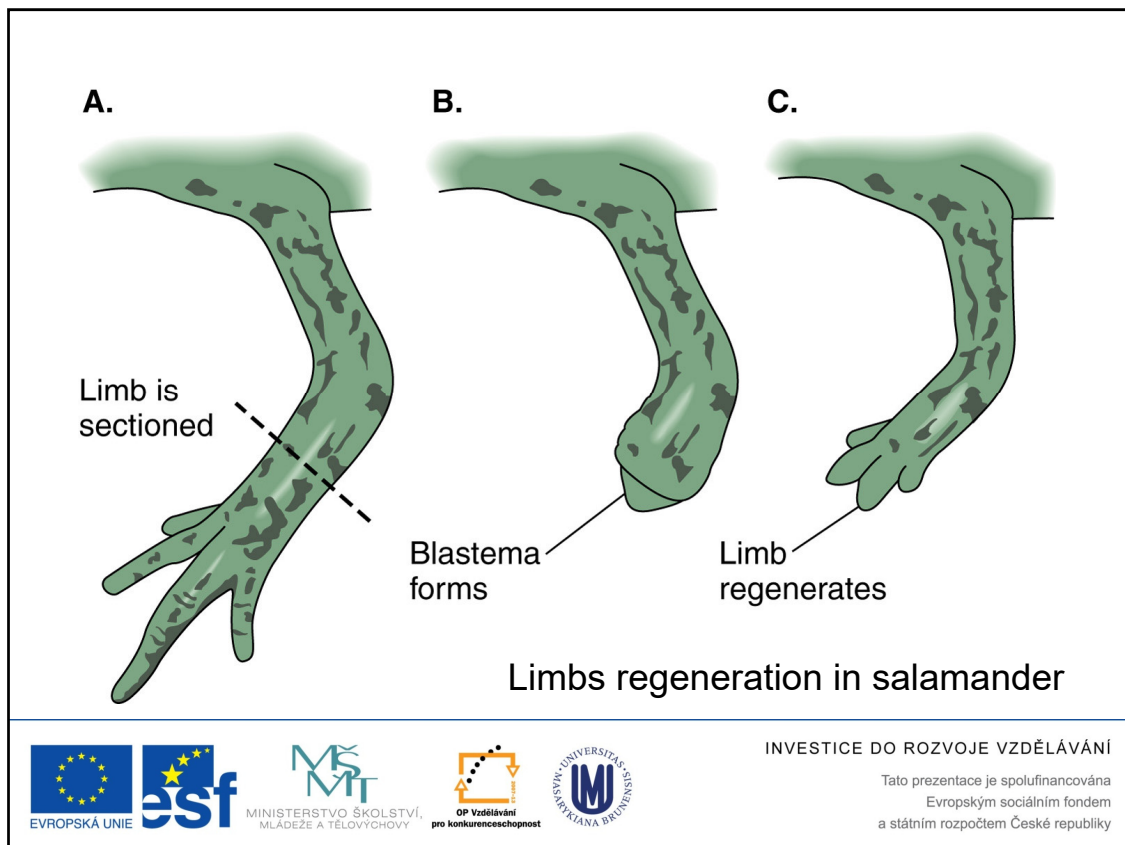
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The experimental evidence for the genetic integrity of the differentiated cells (John Gurdon, 40's-50's, Oxford).

The cells of differentiated tissue (e.g. epithelial cells in this case) have been used as a donor of the genetic material (nuclei) for enucleated oocytes in amphibians (*Xenopus laevis*). That allowed formation of complete tadpole.

However, the older (more differentiated) the donor cell is, the lower efficiency of that process and the tadpoles often fail undergo normal morphogenesis. Similar principles were employed for the mammals cloning (the Dolly sheep).

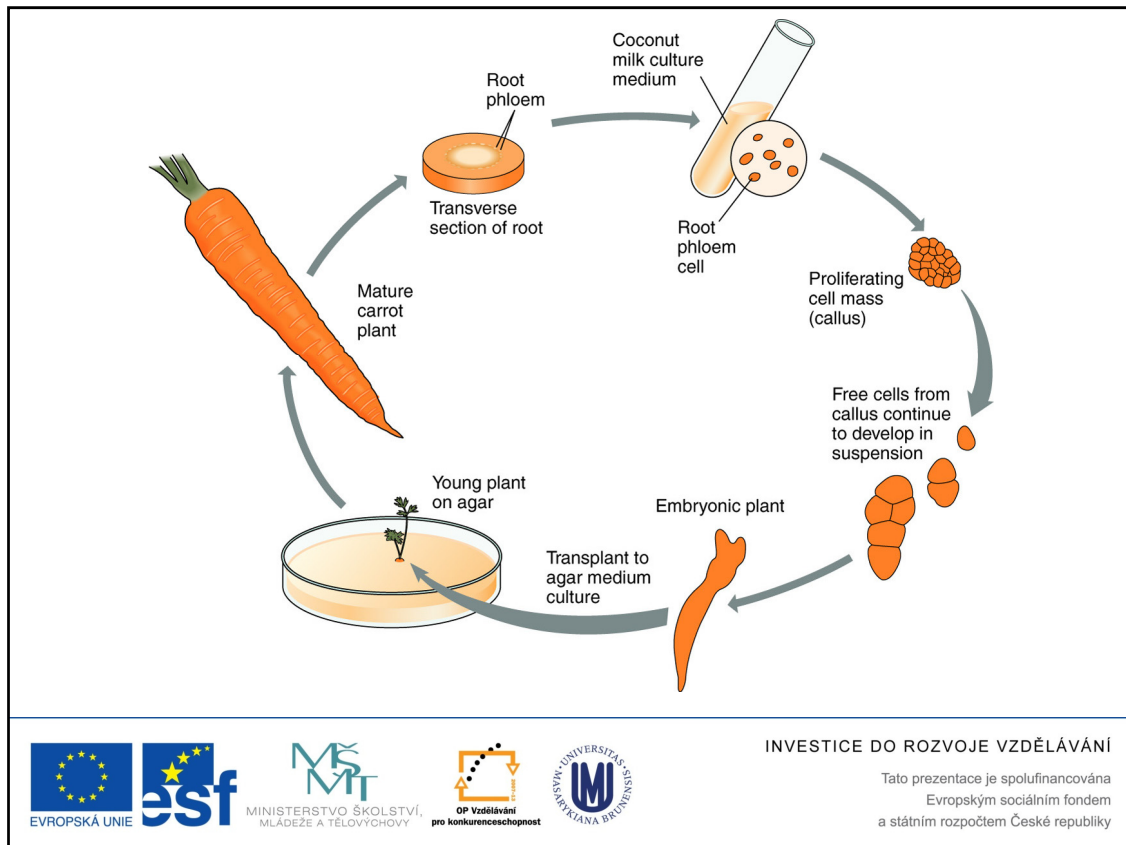


Another evidence of the genetic integrity during cell specialization, i.e. during cell differentiation, is the opposite process, i.e. cell dedifferentiation and the ability of regeneration in animals and particularly in plants.

In some animals, e.g. in some amphibians, there is high level of regeneration developed. E.g. in newt that can regenerate iris cells into functional lens cells (removed by trauma or experiment) or limbs regeneration in salamander (above).

The question remains, whether the new limb is being regenerated from the pool of undifferentiated cells. However, there is considerable body of evidence suggesting that at least a part of the limb is created via dedifferentiation and subsequent re-differentiation of the cells of the connective tissue underlying the epidermis (limb dermis). Similar principles seem to operate in plants (next slide).





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The ability of differentiated plant cells to regenerate the whole plant was experimentally proven by Frederick Steward (50's) at the Cornell University.

He managed to isolate phloem cells from the carrot and by the coconut milk treatment (source of the plant hormone cytokinins) he regenerated the entire new carrot plant.



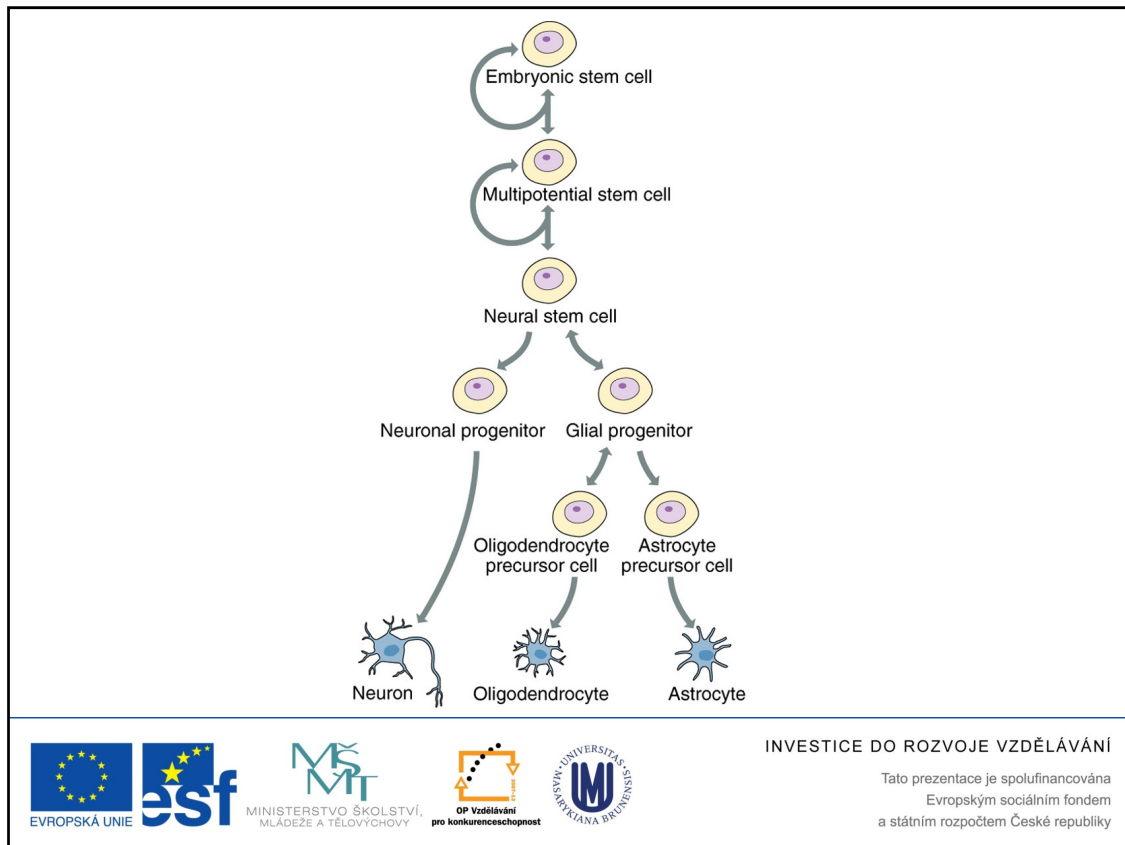
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The concept of stem cells.

Cells in the body undergo differentiation. During that process, they are losing the ability to form different cell types and their developmental faith becomes more and more restricted (see the figure). Under normal circumstances, this process is more less unidirectional, i.e. more differentiated cells cannot dedifferentiate.

The highest developmental potential has a zygote, that we call is *totipotent*, i.e. it can give rise to all cells in the organism.

There are specialized cell types in the later development, called stem cells. These cells maintain their self-renewal capacity, providing thus a source of the cells at the certain differentiation level.

*Embryonal stem cells* are one of the most pluripotent stem cells know so far. However, recent findings suggest a presence of stem cell populations also during later development in different tissue types.

For a long time, neural cells were believed to be non-renewable. However, recently, stem cells were identified even in the neural tissues, e.g. in the bone marrow. In spite of there are still some unclarities in the experimental data provided, the experiments with bone marrow cells tagged with GFP and injected into donor mice suggest the possibility of the presence of stem cells in the neural tissue.

GFP labeled cells were injected in the mice mutants lacking the immune system (*nude* mutant). These cells not only developed immune system, the GFP tagged cells were found in the brain and some of them were producing the neuron-specific proteins suggesting that they acquired neuron identity.

There are also reports of the stem cells in the brain that have a glial appearance and might be reprogrammed to form neurons.



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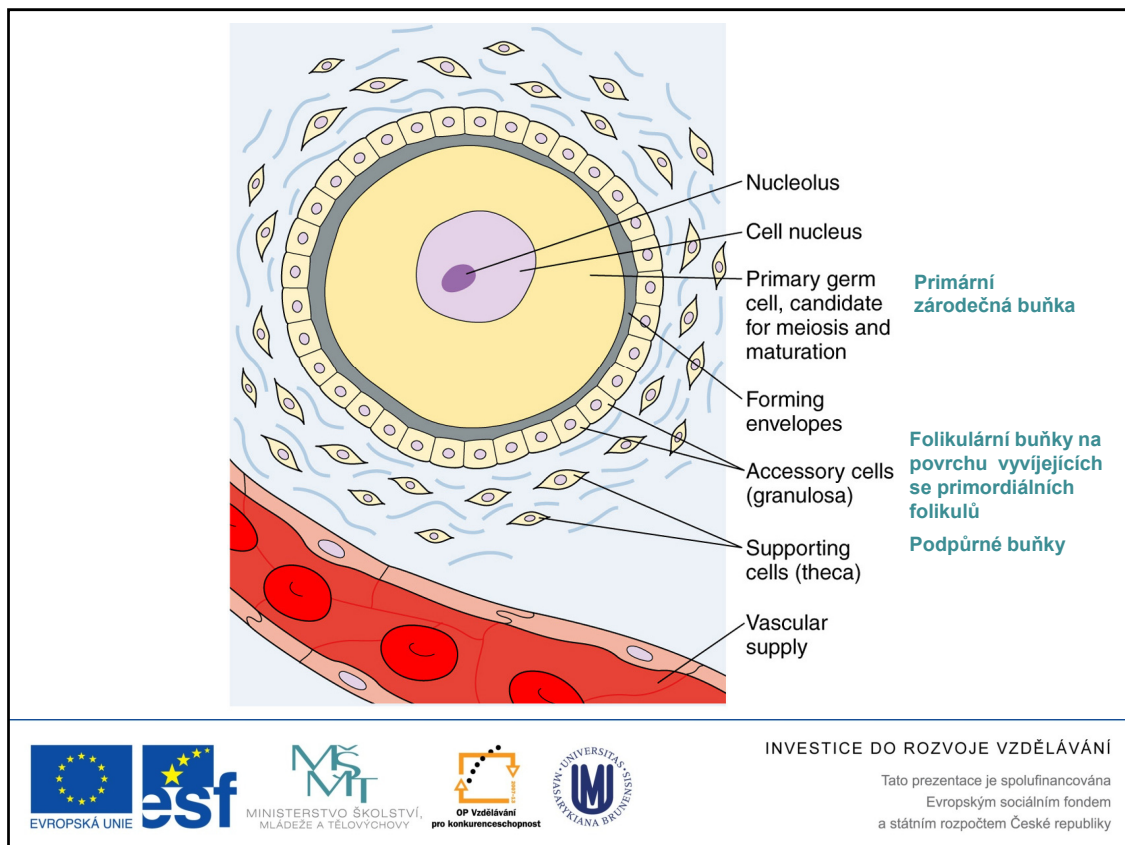


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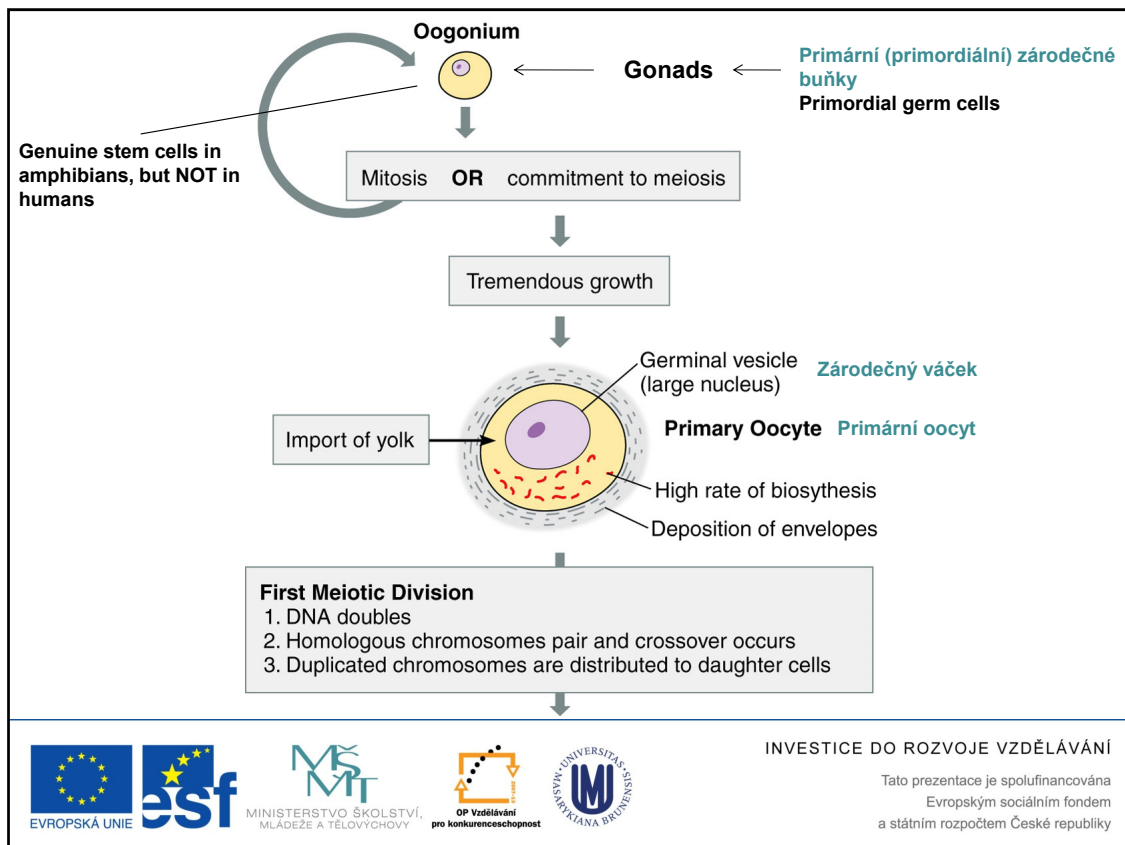
The development starts before fertilization.

During gametogenesis, the gene set is reduced to the haploid level. Fusion of the gametes allows formation of totipotent zygote after fertilization.

In most of the animals, there are common principles of gametogenesis, therefore, we will discuss them in a general form at the outset. The individual differences among different organisms will be discussed in separate lessons.

In animals, the **primary (or primordial) germ cells** are formed during development and these cells are able to undergo meiosis to form female gametes, the eggs. Ones the primordial cell reside in female gonad, they are called **oogonia**.

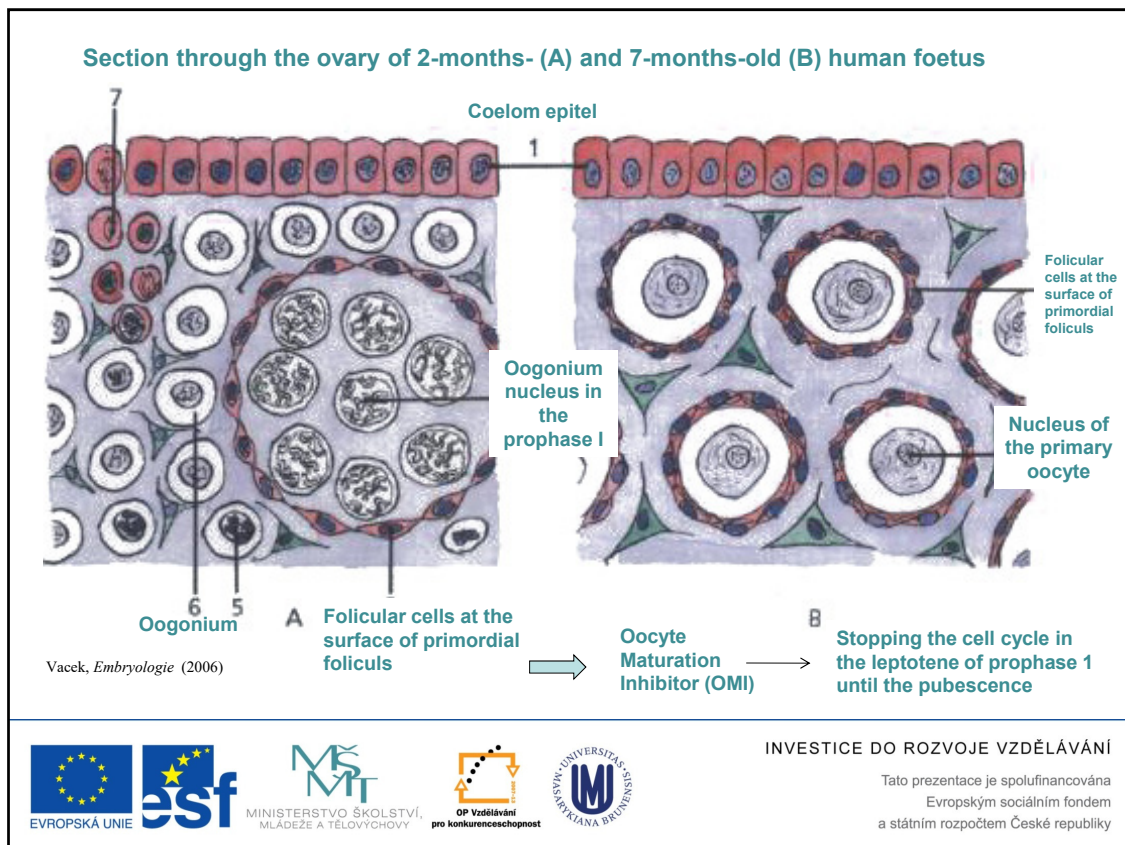
In humans, the oogonia form limited population that does not undergo mitosis, while in frogs, the oogonia divide, allowing thus formation of many thousands of eggs during annual breeding season. Thus, the frogs oogonia are real **stem cells** that is not the case in humans. The other cell types are of supporting function.



One of the hallmark of the oogenesis is an extensive growth. That allows stockpiling of all material that will be necessary during embryogenesis. In contrast, there is no growth (increase in the dry mass) during embryogenesis. The growth results from both the biosynthetic activity of the oogonium and by the import of the **yolk** that is produced in mother's liver. DNA replication occurs.

Growing germ cell is now called **primary oocyte**, and the **meiosis** is started (enters prophase I).

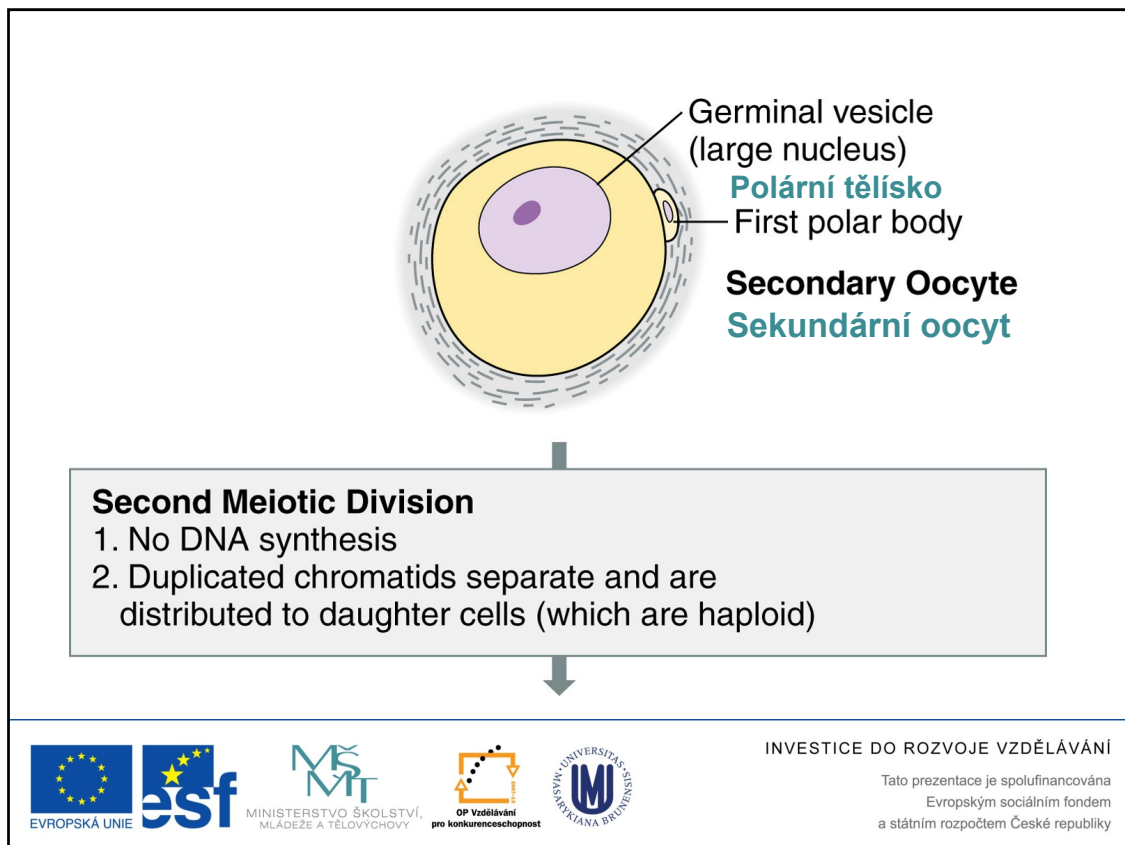
The ability to undergo meiosis is another important characteristics of germ cells. This stage is characteristic by the formation of large nucleus that is called **germinal vesicle**. The growth stage of the primary oocyte may take very long (4 months in *Xenopus*, several weeks in humans). Often environmentally induced hormonal signals might be necessary to continue the further development.



Sections through the human foetal ovary at the age of 2 (A) and 7 month (B). The invasion of coelom epithel in between the primary germ cells that will develop in to follicles is apparent.

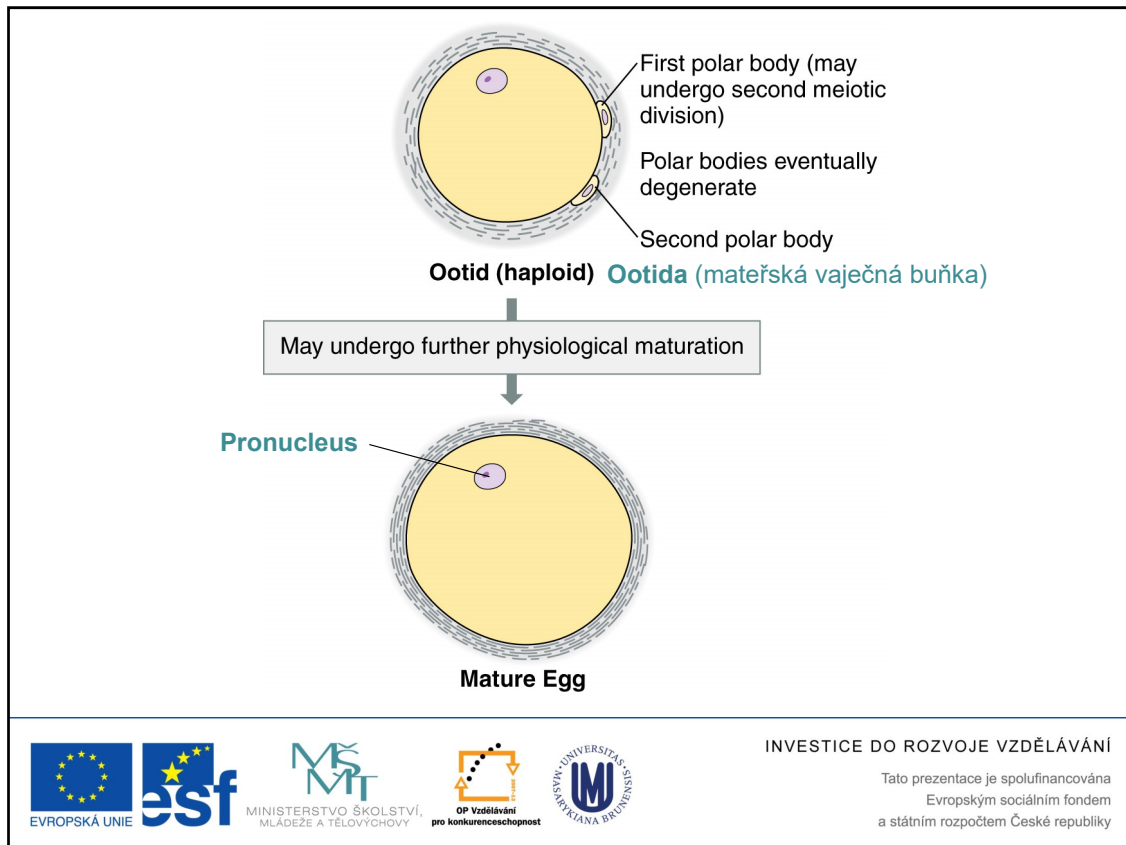
After coelom epithelial cell surround the oogonium, they will become follicular cells. The nuclei of oogonia surrounded by the follicular cells will enter the prophase I and further proceeding of the cell cycle is stopped at the leptotene of prophase I until reaching the pubescence.

In some oocytes, this stage of development might take up to 30-40 years (from the second month until climacterium) and during that stage, an intense synthesis of RNA of all types and proteins take place.



During **cytokinesis**, the centrosomes are formed close to the perimeter of the cell, leading thus to **assymetric cell division**-formation of **secondary oocyte** (retains about 95% of the cytoplasm and all the stockpiled material) and very small **polar body**. The polar body might undergo second meiotic divisions, but in every case later on degrades.



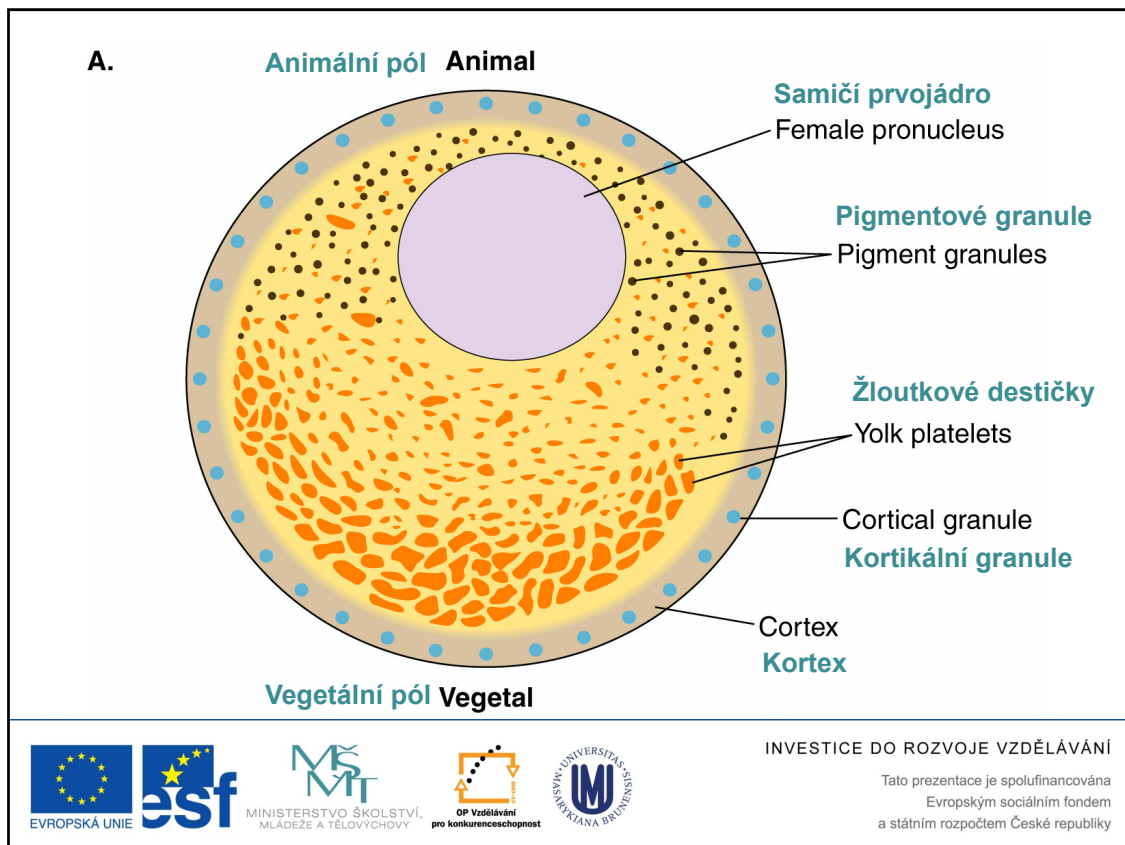


Second meiotic division of the secondary oocyte leads to the formation of another polar body and the egg that might be called **ootid**.

The nucleus of the egg is called **pronucleus**. The completion of meiosis takes place at the moment of fertilization and at a rapid pace, when the sperm cell is in the cytoplasm of the egg.

More frequently, the primary oocyte will finish the first meiosis and enters the prophase of the meiosis II, where it stops. Similarly to the previous case, the hormonal signal or fertilization might induce further development.

The fertilization occurs in the stage of the arrested primary or secondary oocyte, dependent on the species. In that case the fertilization is a “hurry-up” signal for the finalization of meiosis. In the egg urchin, the meiosis is completed before fertilization.



The egg is a complete cell, although of exceptional size. The egg has a specific intracellular structure that ensures that the cytoplasm is not mixed and progeny of the egg mitosis after fertilization will “inherit” differentially composed cytoplasm. It is important for the next development.

1. **Pigment granules** are located at the **animal pole**, while
2. Yolk platelets are located on the **vegetative pole**
3. The peripheral cytoplasm has different ultrastructure, especially in terms of the **cytoskeleton organization** and is called **cortex**
4. Cortex contains special abundant vesicles called **cortical granules**

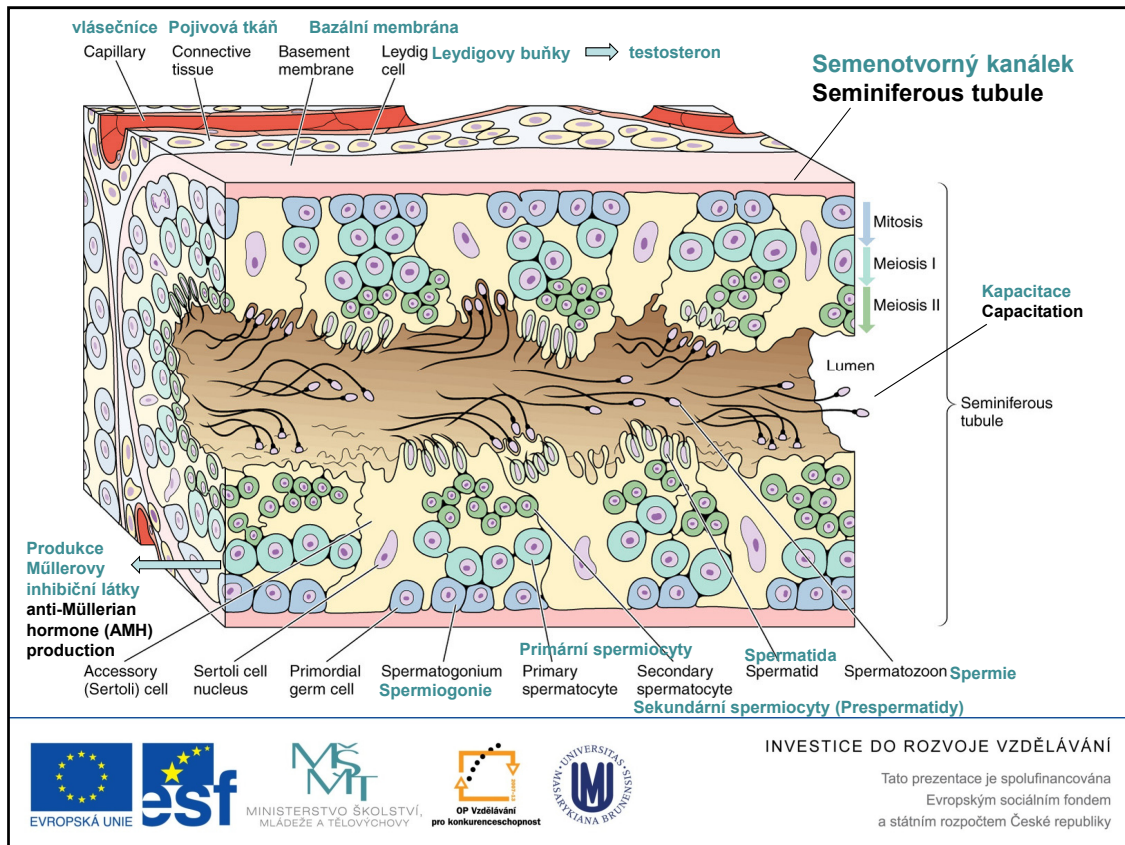


Ostrich's egg...the largest cell in the world 😊.



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Male germ cells in animals lie along the perimeter of the **seminiferous tubules**.

These are blind tubular structures, connected with other tubules into a common duct. Between tubules is connective tissue that contains supporting cells. In mammals these are **Leydig cells**, producing testosterone.

The duct system is connected with secretion system of other glands, e.g. **prostate** that helps formation of seminal fluid.

One of the most apparent differences in comparison to the female gametogenesis is the **absence of stockpiling** of the cell material. The spermatid cells contain only few of the cytoplasm and are designed to deliver the haploid nucleus to the egg cells.

The male primordial germ cells are real stem cells as they can undergo mitosis. Their progeny is called **spermatogonia** and is located close to the perimeter of tubule, close to the **Sertoli cells**.

Sertoli cells nurture the developing sperm cells through the stages of spermatogenesis and produce several important regulators, among them anti-Müllerian hormone (AMH) that is important during early stages of fetal development (see later in Lesson 5). Translocation of germ cells from the base to the lumen of the seminiferous tubules occurs by conformational changes in the lateral margins of the Sertoli cells.

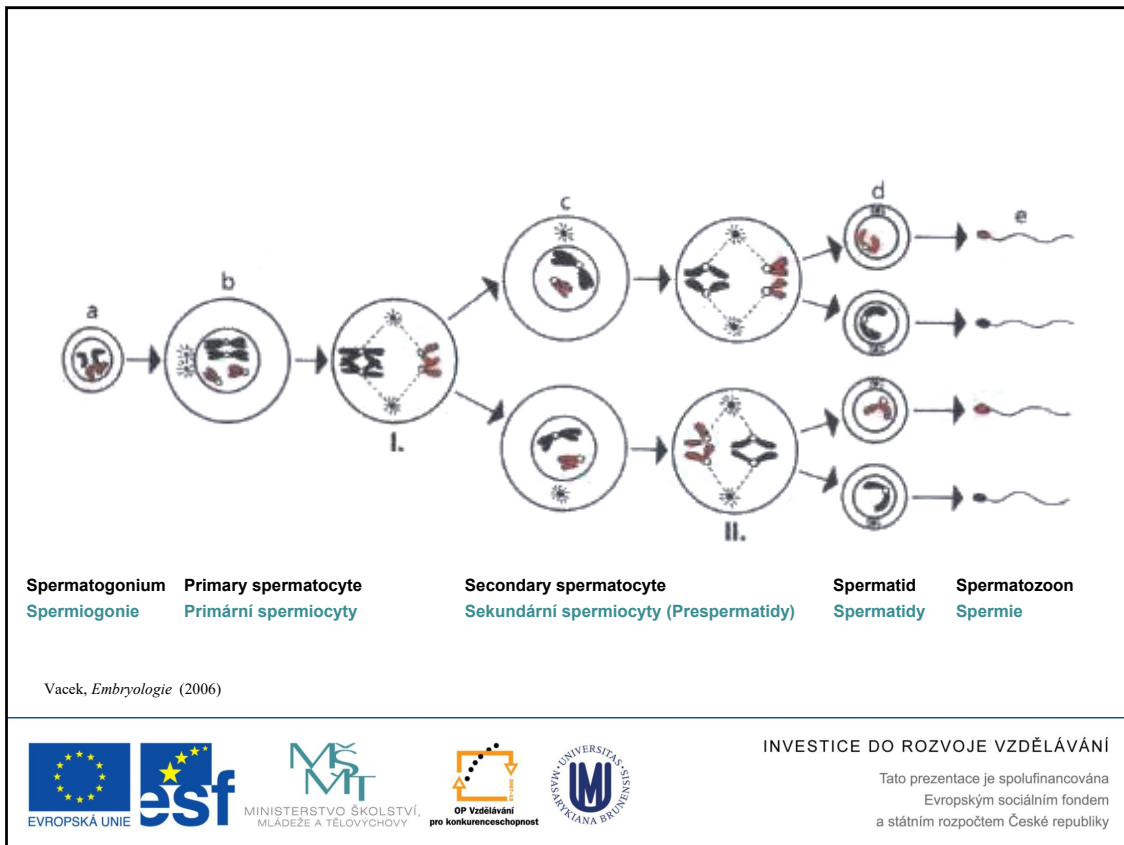
Spermatogonia grow a bit, and form **primary spermatocytes** that undergo meiosis I.

That leads to formation of **secondary spermatocytes**.

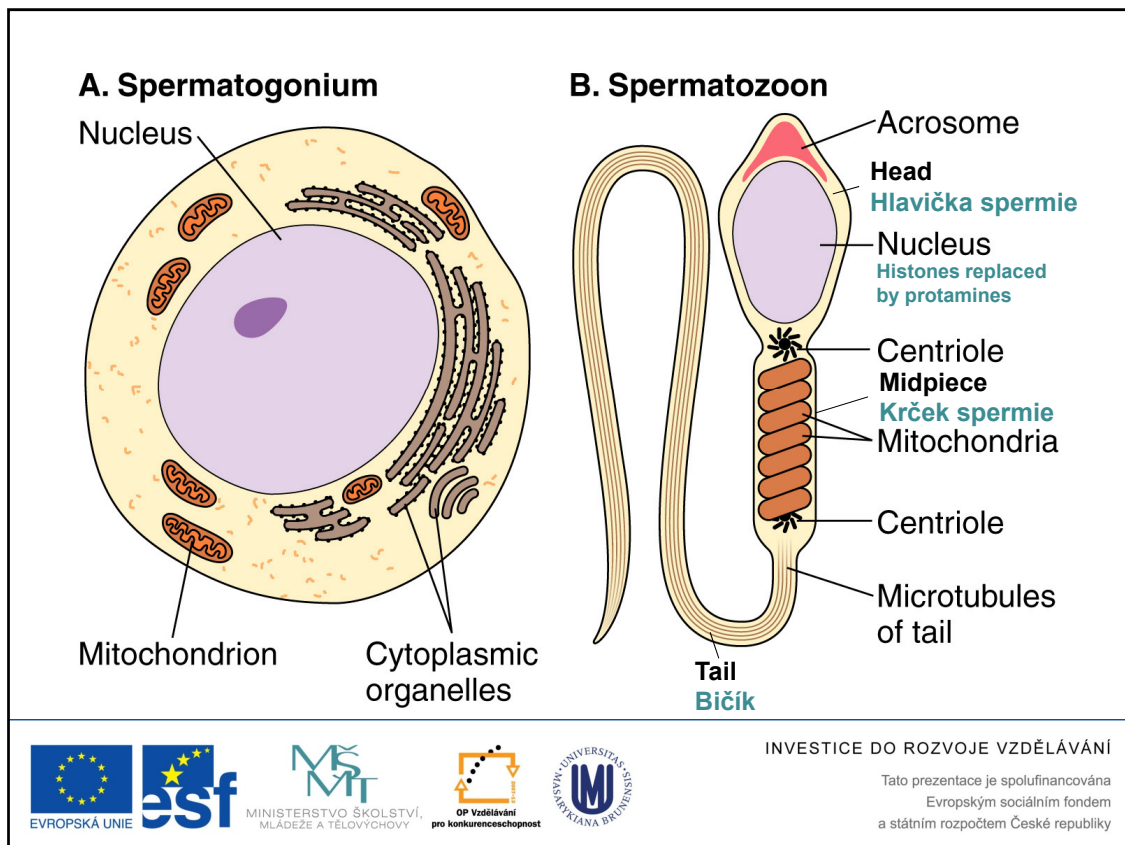
Meiosis II leads to production of four **spermatids**.

In contrast to egg cell formation, all four spermatids survive. The spermatids are connected with cytoplasmic bridges and are located in Sertoli cells close to the lumen of the seminiferous tube, where they undergo **spermiogenesis**, i.e. formation of **spermatozoa** (singular spermatozoon), commonly called **sperm**.

The process of spermiogenesis results in dramatic subcellular changes (see the slide # 40).



Overview of the meiotic division during human sperm development.



During spermiogenesis, the most of the cytoplasm and cellular organelles are sloughed-off, including cytoskeleton and vesicular and membranous systems like the Golgi apparatus and endoplasmic reticulum. Before its disappearance, Golgi apparatus participates in the formation of acrosome, a new type of vesicle.

Spermatozoa are composed of the **head**, **midpiece** and **tail**.

In the **head**, there is nucleus (no nucleolus apparent), the histones disappear and are replaced by the *protamines*, other basic proteins that facilitate dense packing of the chromatins. At the tip, there is acrosome. In invertebrates, the chromatin is not condensed and adjacent to the acrosome.

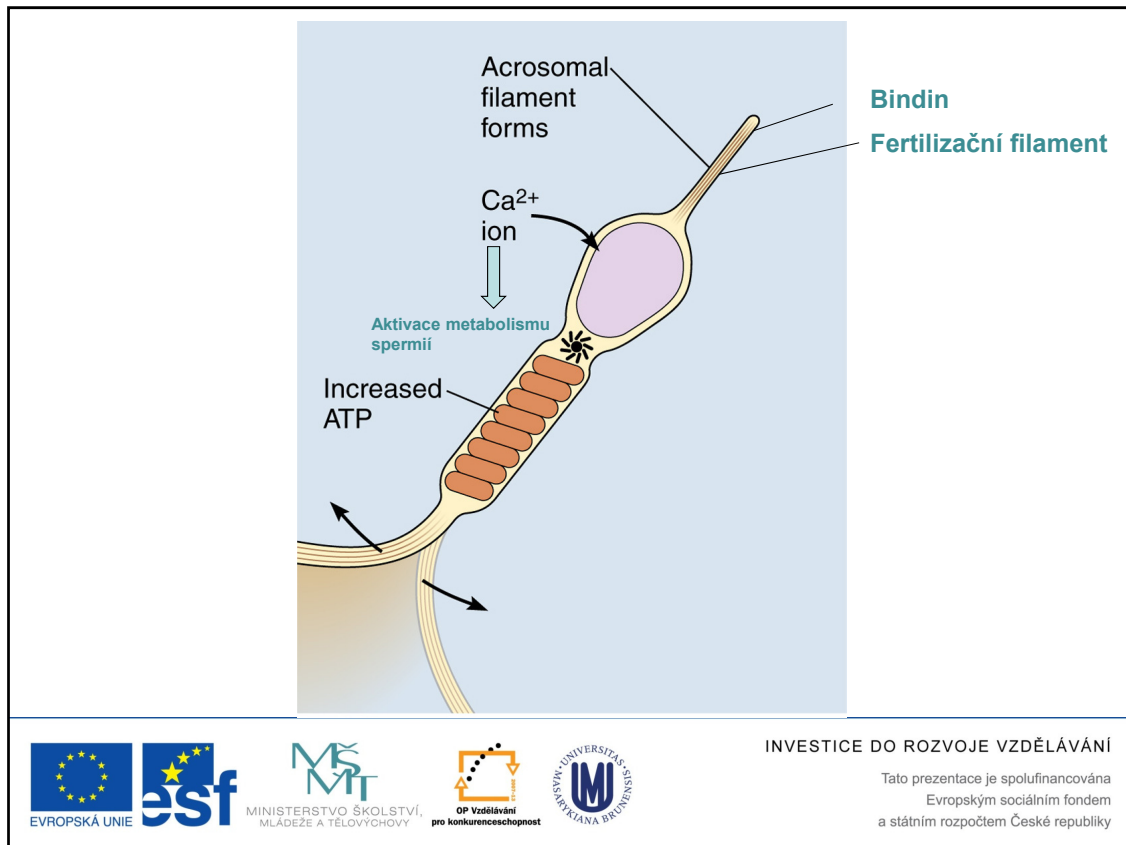
In the **midpiece**, there are located mitochondria that might fuse to form either one large mitochondrion or few larger mitochondria. There are located also centrioles, from the one located at the basal part of the midpiece emanate the microtubule array, the flagellum that allows wave like motions that propel the sperm.

For the competence of the sperm to fertilize the egg it is necessary a maturation process, called **capacitation** that occurs in the reproductive tracts via action of substances produced by other glands.

Fertilization results into:

1. Formation of the diploid zygote via **syngamy** (fusion of haploid nuclei of gametes).
2. Activation of the mitosis. The interaction of sperm and egg leads to the
  - Finalization of egg meiosis
  - Activation of the mitosis after syngamy





The sperm is activated after its release from the male reproductive organs.

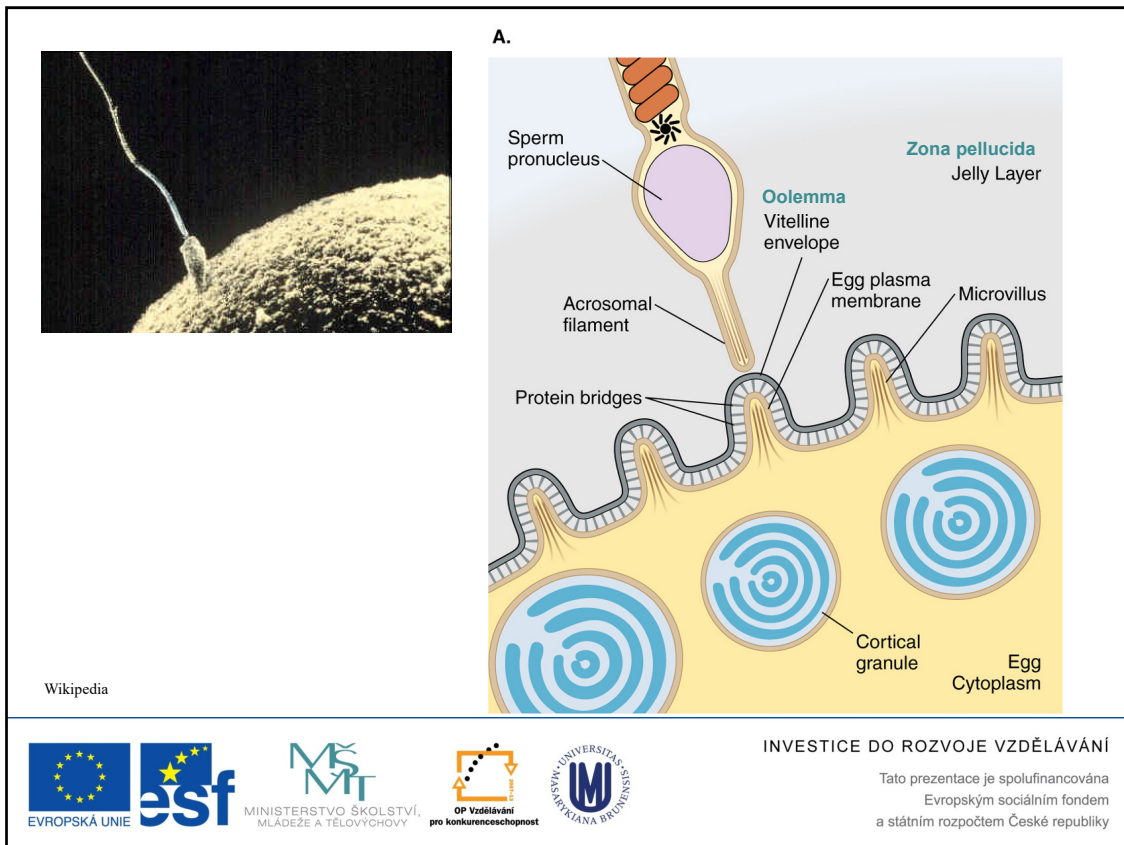
The mitochondria in the midpiece start respiration, allowing thus ATP production. That activates movement of the sperm via sinusoid flagellum beating.

Egg might produce chemoattractive substances that direct the movement of sperms. These substances may also further activate the spermatozoa.

A part of this activation in marine invertebrates is eversion of acrosome into a specific membraneous structure, called acrosomal filament that contains microfilaments (long fibrils of polymerised actin). However that is not the case in mammals.

For the metabolic activation of the sperm,  $Ca^{2+}$  is necessary.

The interaction of the sperm with egg is species-specific. *Bindins* are specific proteins involved in this interaction. The bindins of sperm in sea urchin interact with specific receptors at the surface of the egg. Though bindins evolved only in echinoderms, it is presumed that similar mechanisms are an important part of reproductive isolation in general.



The interaction of the sperm acrosome with the cell membrane of unfertilized egg leads to fast and dramatic changes in the egg membrane. These events were studied in the sea urchin and seem to be of general validity among other animal systems.

Among first events, release of the  $\text{Ca}^{2+}$  takes place (see later) and exocytosis of the cortical granules occurs.

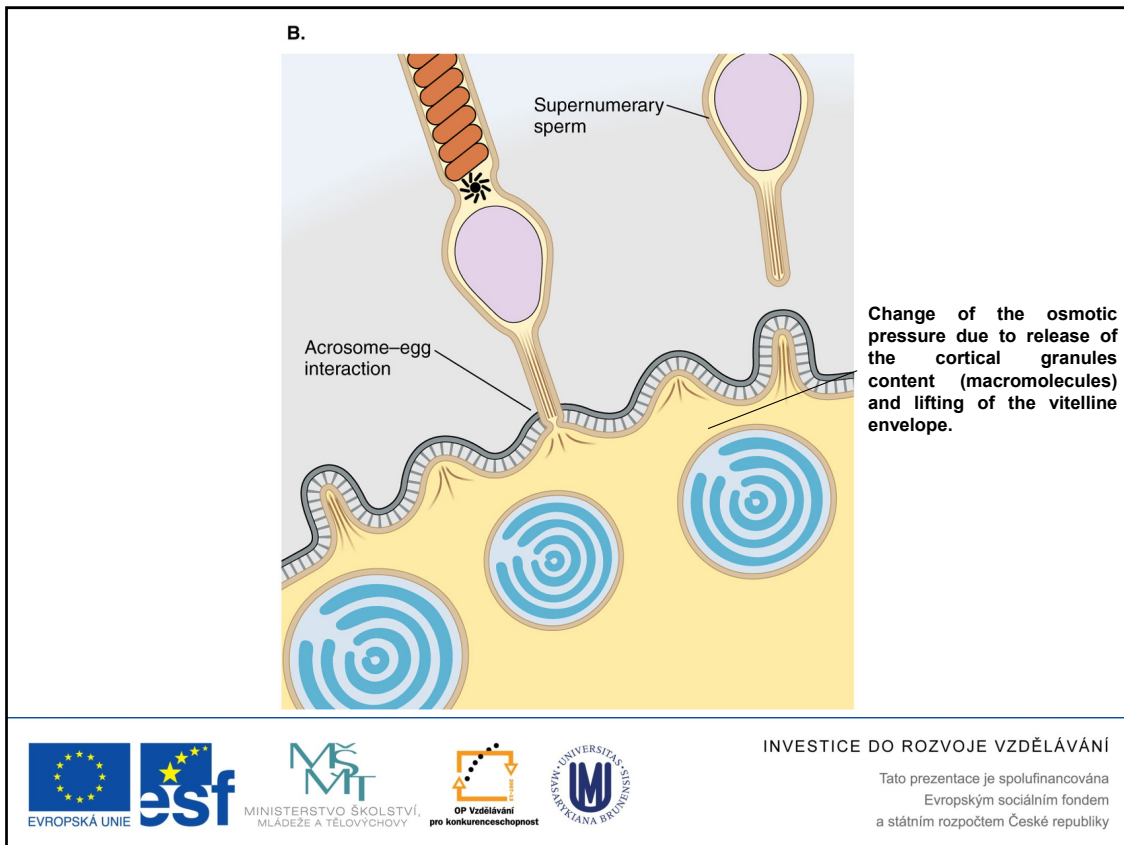
Vitelline membrane consists of proteins and becomes fertilization envelope upon fertilization (see later).



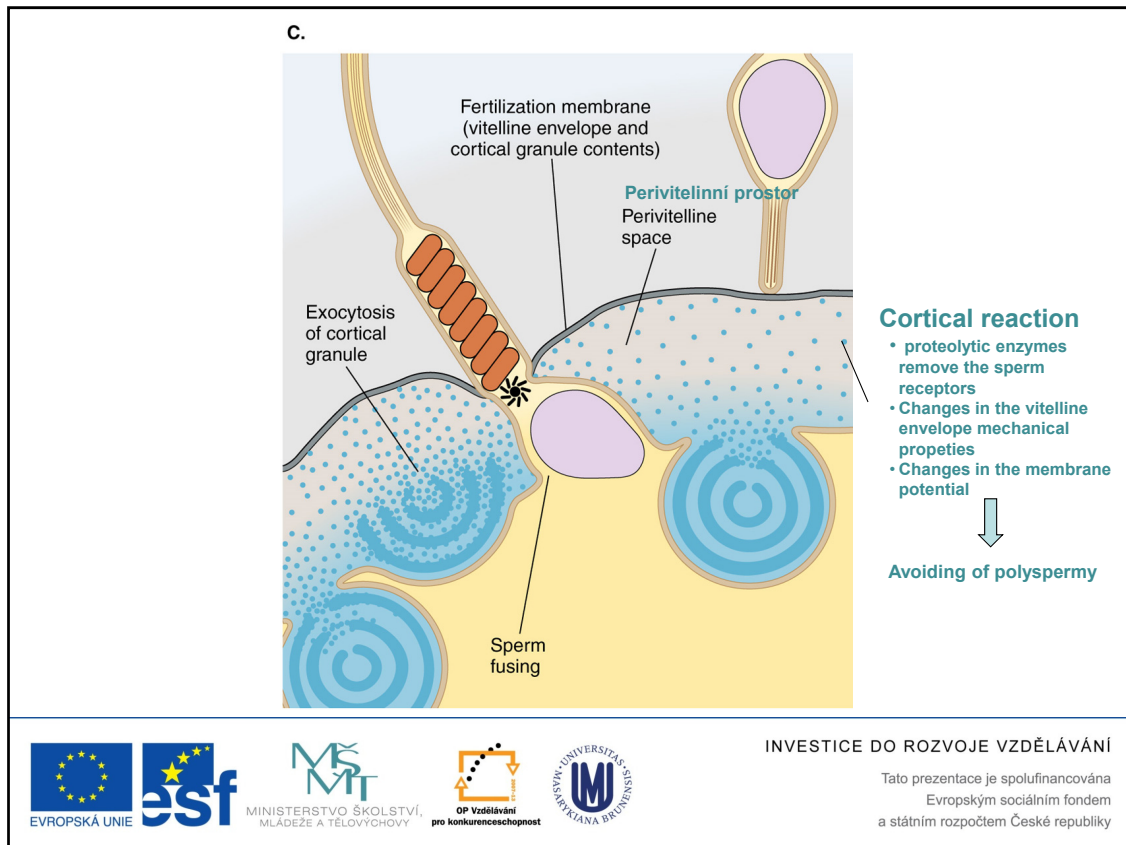
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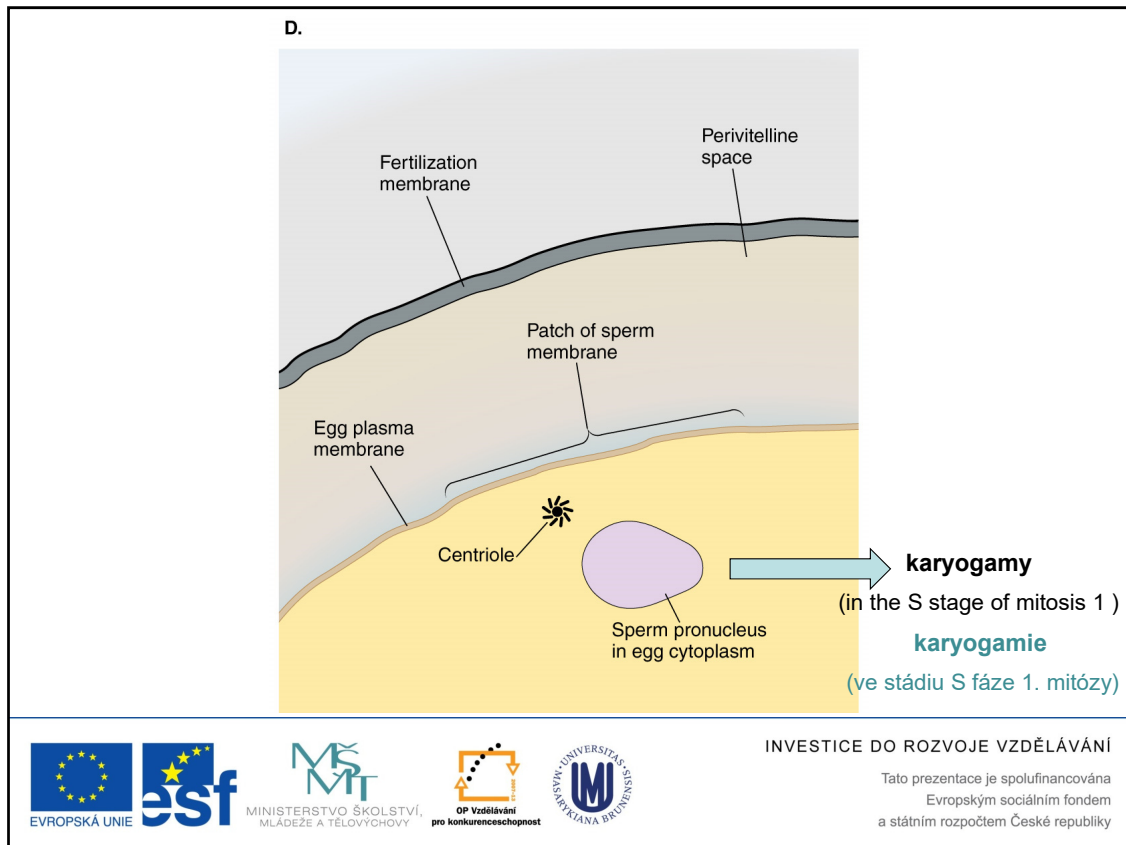


The contents of the granules vary with the species, and are not fully understood. The macromolecules released by the cortical granules lead to the increase of the osmotic pressure and lifting of the vitelline envelope.



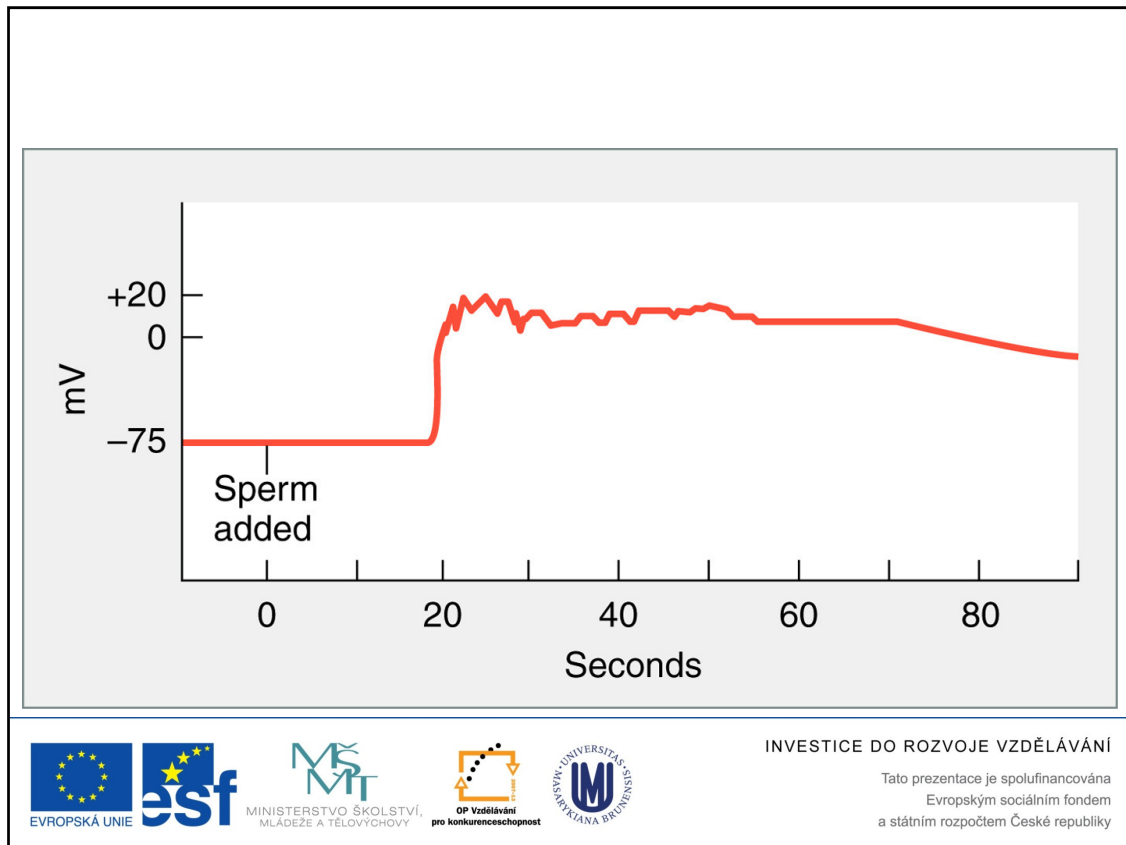
Macromolecules accumulate in the perivitelline space and integrate into the vitelline envelope leading to the thickening of the membrane and forming of what is called **fertilization envelope**.

Material in the cortical granules might contain proteolytical enzymes that remove the egg receptors from the egg surface. Together with changes in the mechanical features and composition of the vitelline envelope and finally changes in the membrane potential (see later) these events prevent **polyspermy** (presence of more sperm cells in the egg).



Finally, the sperm cell fuses with the egg, releasing its content to the egg cytoplasm.

These events initiate finalization of meiosis, followed after **karyogamy** (i.e. fusion of both male and female pronuclei) by the S-phase of the first mitotic division.



There are numeral molecular events after fusion of the egg and sperm cells.

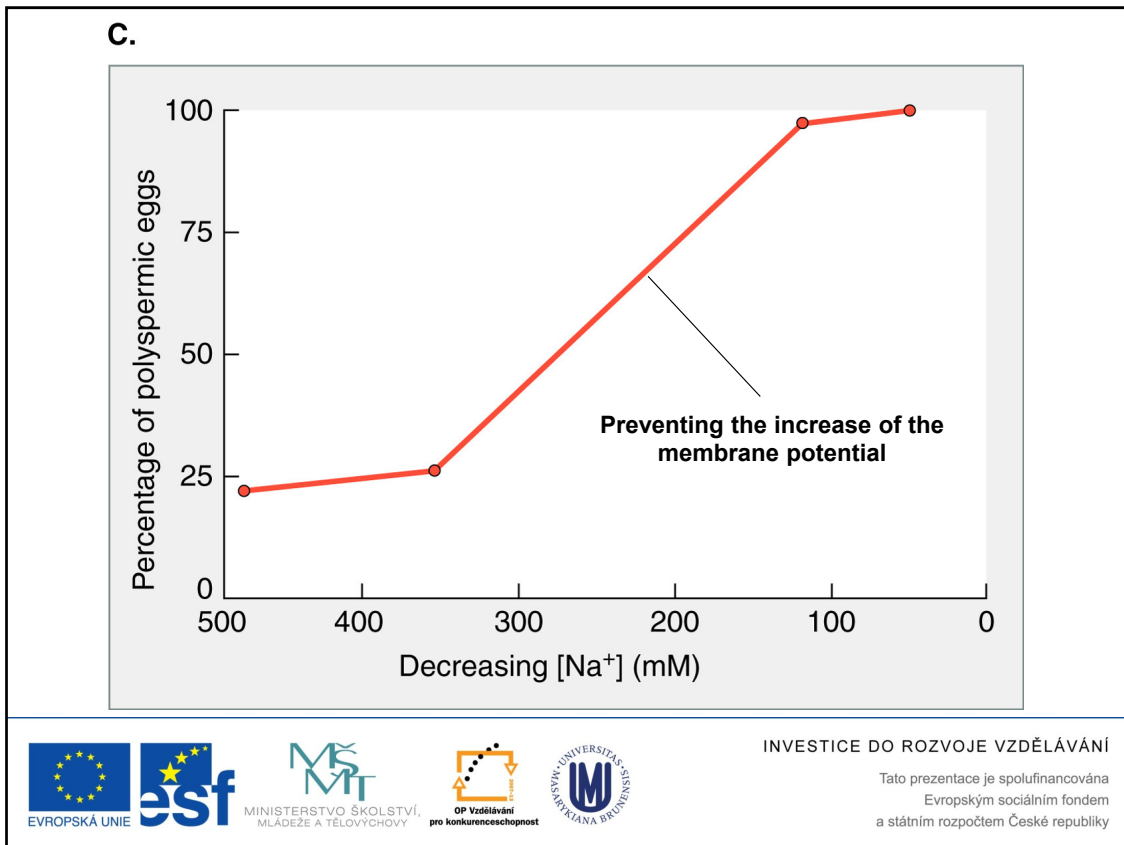
One of the earliest is the change in the egg cell membrane potential (from -70 mV to approx. +20 mV, see the figure above).

How the ion channels are activated it is still unclear. Parameters of these early changes in the mebrane potential differ among animals, but seem to be of general importance.



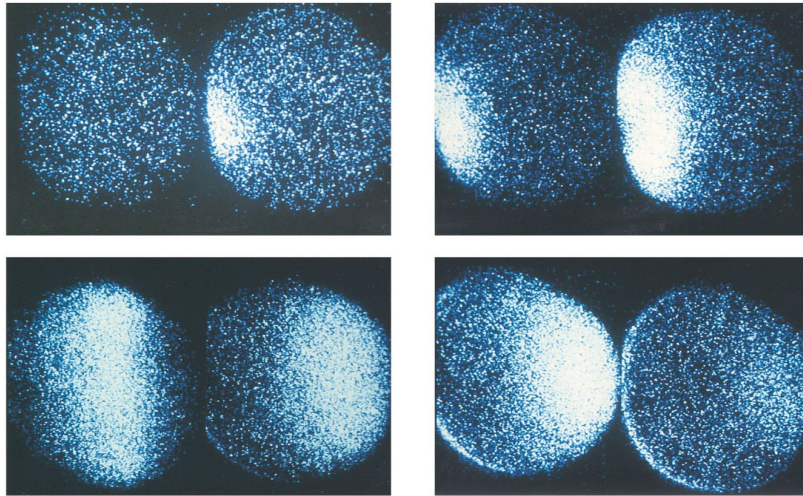
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That immediate increase of the membrane potential prevents polyspermy.

Preventing increase of the membrane potential via decrease of the  $Na^+$  concentration in the sea water decreases the polyspermy barrier allowing thus increase of the percentage of polyspermic eggs (see above).



Lectures of Michael Lehmann, Ph.D., University of Arkansas, USA

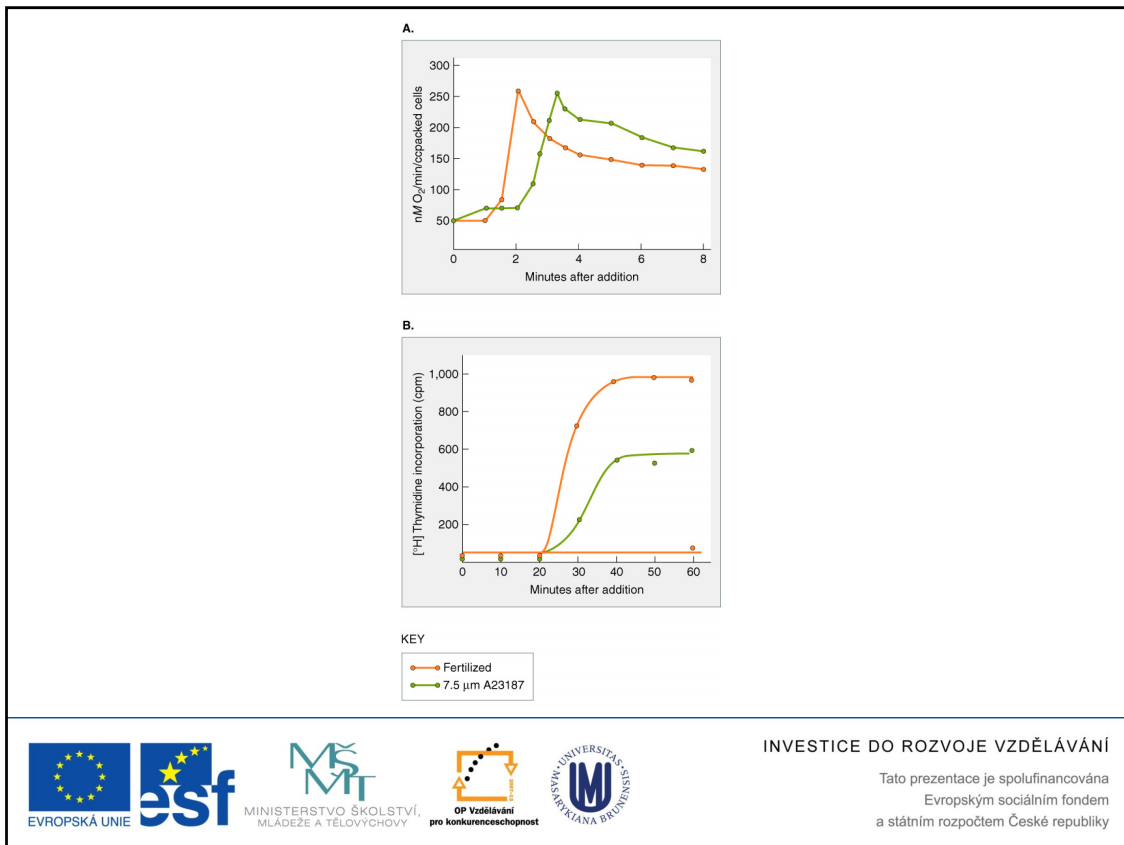


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Further, one of the crucial events early after fusion with sperm and egg cells is a transient wave of  $\text{Ca}^{2+}$ .

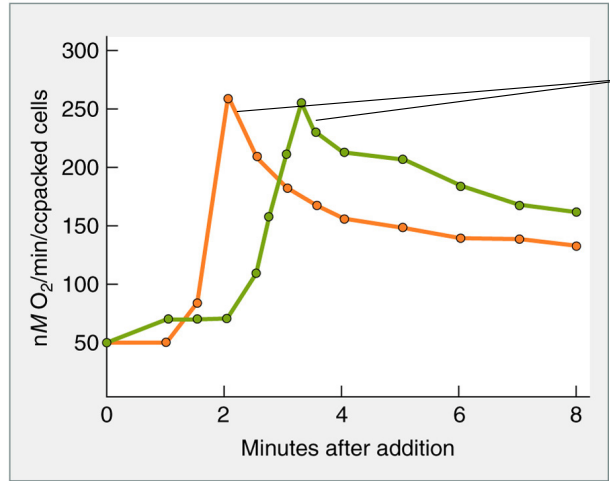
Wave of calcium release (bright signal) across sea urchin eggs during fertilization.



The Ca<sup>2+</sup> is released and again sequestered very fast, leading to the formation of the Ca<sup>2+</sup> wave that spread across the surface of the egg.

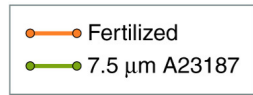
Even more importantly, experimental release of Ca<sup>2+</sup> from internal cell reservoirs via addition of calcium ionophores leads to the activation of fertilization egg responses, e.g. activation of respiration (graph A) or DNA synthesis (graph B).

A.



Activation of respiration

KEY

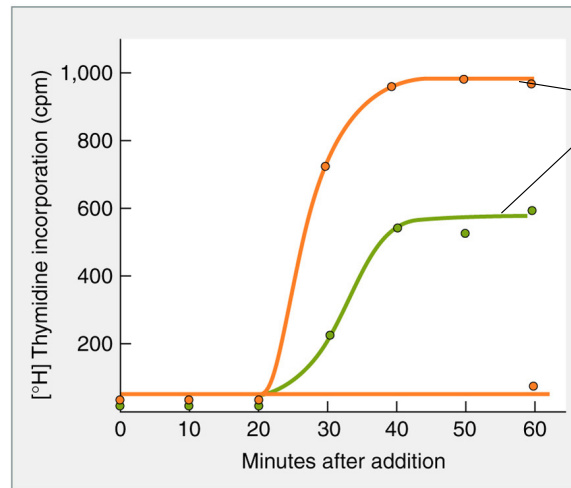


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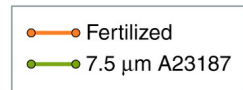


B.



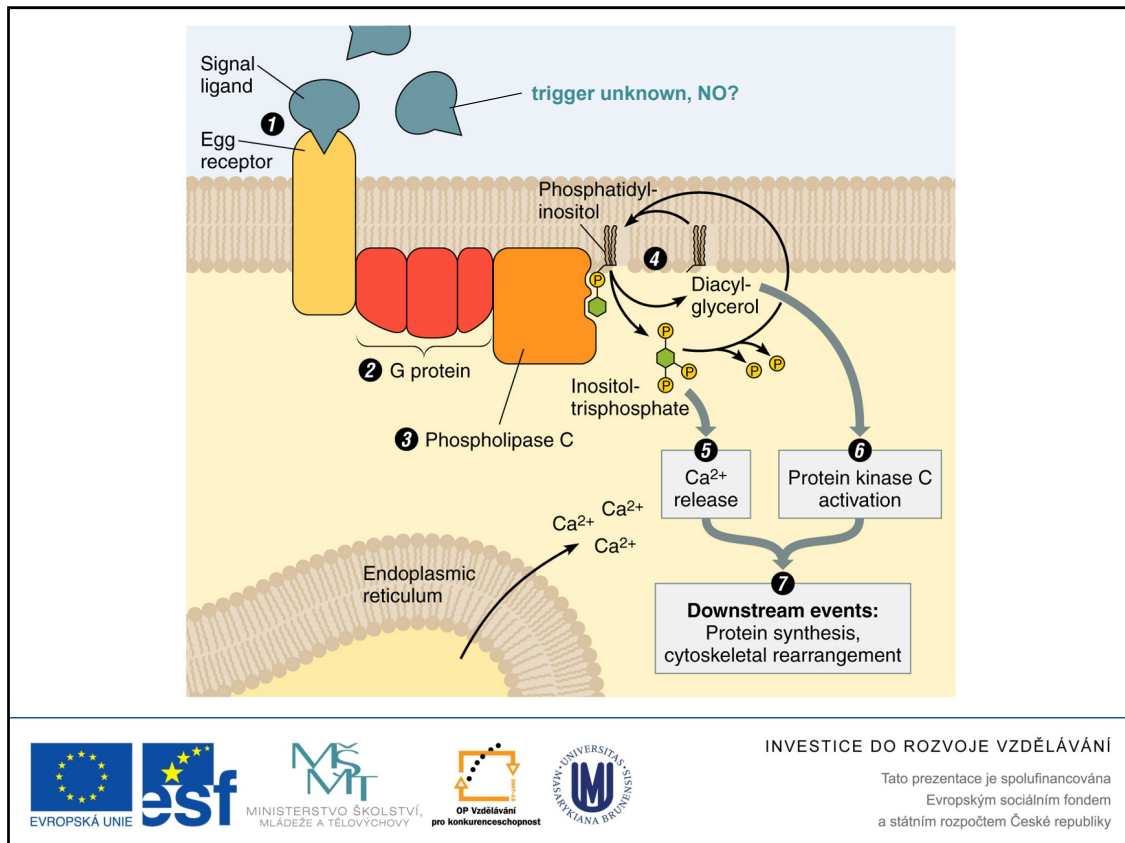
Activation of  
DNA  
synthesis

KEY



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In spite of the molecular mechanisms leading to the activation of Ca<sup>2+</sup> release are largely unknown, the G-protein mediated signalling might be involved in those processes.

The release of Ca<sup>2+</sup> might then serve as a signal for further cell processes.

Based on recent results, more signalling pathways seem to be involved in the regulation of the fertilization egg response; *phospholipase C* is probably involved in Ca<sup>2+</sup> release, but not the changes in the membrane potential.

The identification of single receptor, responsible for the activation of the downstream fertilization cascade is still not successful (it is not the receptor allowing the egg cell and sperm initial interaction that is bindin in sea urchin, see previous slides).

Alternatively, the signalling molecule might be delivered via the sperm fusion directly to the egg cell cytoplasm. One of the examples is import of NO-producing enzymes (nitric oxide synthase) by the sperm cell. Microinjection of NO effects a fertilization reaction in the egg cell. However, the role of NO synthesis in the Ca<sup>2+</sup> release is unclear.

Also the role of Ca<sup>2+</sup> in the regulation of the downstream events, e.g. activation of proton pumps leading to extrusion of protons and increase in the intracellular pH, is unclear.



  
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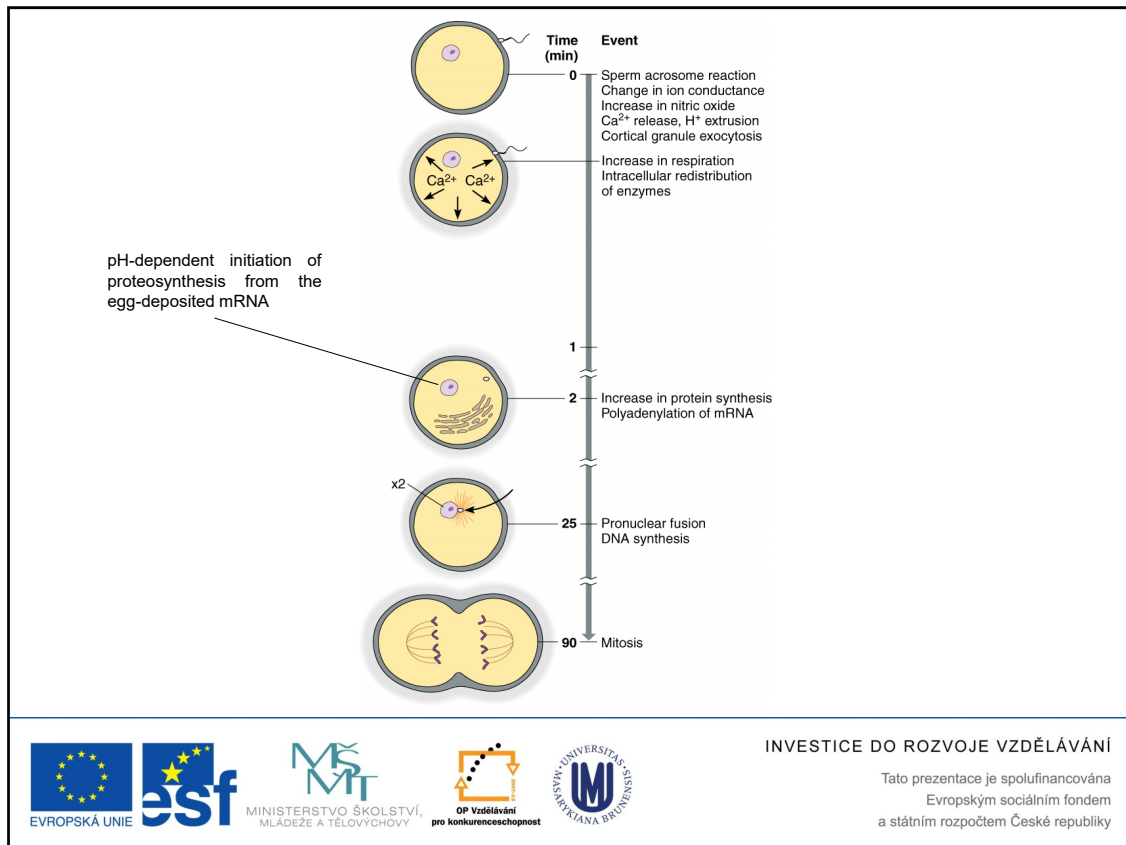
  
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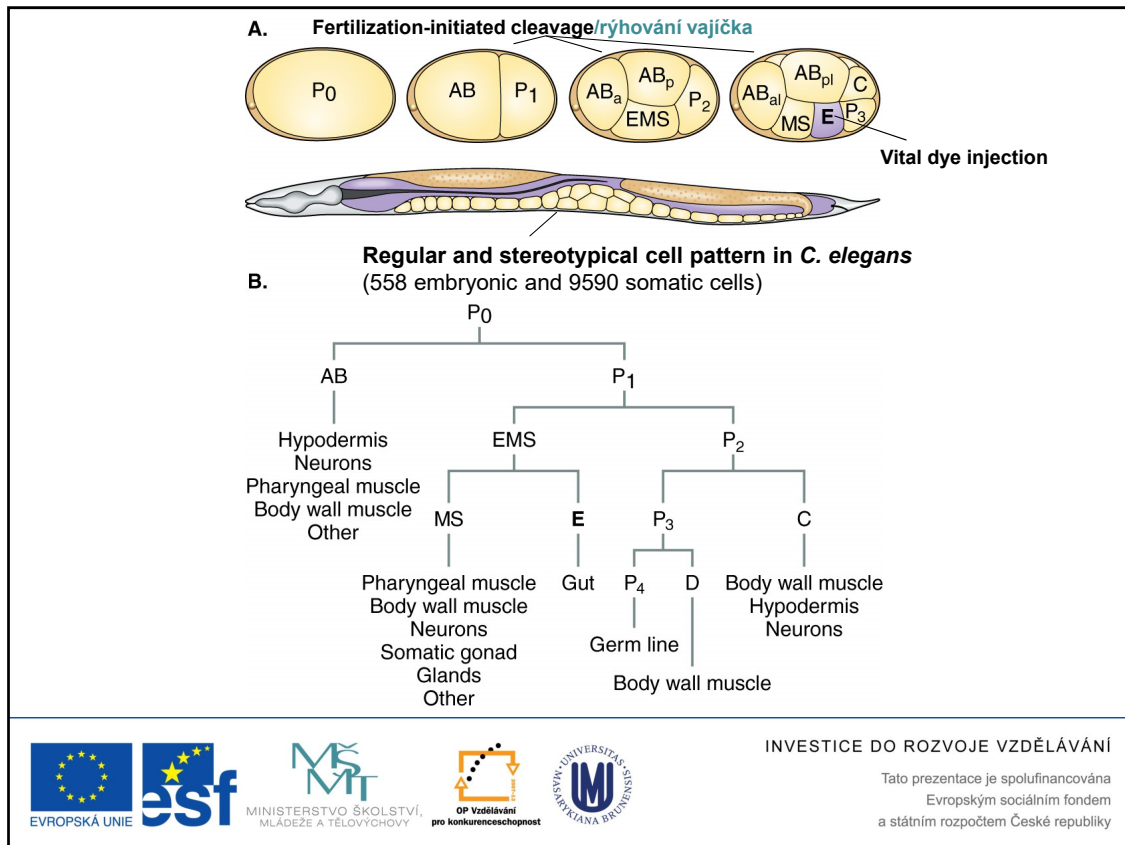
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See the video for the molecular mechanism of PLC signaling; SOCs: store-operated channels (activated by ER-released  $\text{Ca}^{2+}$ )



Fertilization activates translation of mRNAs that are included in the egg cell cytoplasm. Fertilization also induces poly A polymerase that leads to the elongation of the poly A tale of mRNAs. The functional meaning of the process is still not clear.

The kinetics of the poly-A end ellongation is different from the kinetics of the protein accumulation. One of the probable explanation of the poly-A end elongation might be an increase of the mRNA stability.



Fertilization leads to the initiation of mitosis.

First several rounds of mitosis are not associated with growth, i.e. increase in the biomass of the egg. It is simple several rounds of subsequent divisions of the egg into smaller cells. This process is called **cleavage**.

Several types of cleavage could be classified as based on the yolk type (its amount) and position in the egg (central or lateral). If the yolk amount is large, it might lead to lateral allocation of the cytoplasm. Then, the cleavage proceeds only in this small island of cytoplasm and is called **meroblastic cleavage**.

In animals, the pattern of cellular divisions during embryogenesis is more less stable. That allows, especially in some models, e.g. *Coenorhabditis*, to follow the developmental fate of individual cells.

The cells in the *Coenorhabditis* embryo undergo completely regular and stereotype cell pattern generating 558 cells and after molting they contain exact number of somatic cells (9590 ) and large amount of germ cells.

This stereotypical cell pattern allows tracing of individual cell lines using e.g. injection of cell vital dies into **blastomere**, allowing generation of the so called **lineage diagram** (see the figure above).

In case of *Coenorhabditis*, but also other organisms, including plants, the cell lineages might be manipulated by laser ablation or isolation. Thus, as apparent from the figure, destroying the cell E that normally develops into gut, leads into formation of gutless embryos. Similarly, isolated and cultured E cells differentiate into gut-like cells.

In contrast, this is not true, if the cells are isolated at the early four-cell blastomere. However, presence of P2 cells in the cultivation media might overcome this defect, suggesting that the contact with P2 cells is important for the formation of competent E cells from EMS mother cell and thus proper development of gut cell lineage.



# Key Concepts

- *Asymmetric cell division* and underlying molecular mechanisms are the *core problem* of the developmental biology
- *Genetic integrity is maintained* during cell (de-)differentiation
- *Gametes* arise from a population of stem cells via *differentiation and reduction of the genome* to the haploid level
- *Formation of the oocyte* places a huge demands on the organism and *stocked material* allows the early development
- *Fertilization restores the chromosome number* and activates the egg. The activated gametes *reenter the cell cycle* and trigger the further development
- During animal development, *cell lineages* with *predetermined developmental fate* are *established in the early development*, which is not the case in the development of plants.



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