EMBRYONIC DEVELOPMENT

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RECOMMENDED LITERATURE

- **Mescher AL (2015). Junqueira´s Basic Histology Text and Atlas 14th Edition (in campus library under code: 611.16-MESC**), chapters: 4, 5 ,6 ,7 ,8 ,9 , 10 ,11 ,15 ,16 ,17, 18, 19, 21, 22
- Faculty of Medicine, Masaryk University. Atlas of histology FM MU at https://is.muni.cz/do/rect/el/estud/lf/js18/histologie_atlas/web/index_en.html
- virtual microscope at http://medsci.indiana.edu/junqueira/virtual/junqueira.htm
- or any text-book related to histology and organology

EXAM

- through „ROPOTs and Quizzes" in IS MUNI: 50 test questions, time limit: 60 minutes, each test question has just 1 correct answer, the question set can be opened only once between February 1st and February 12th; to pass the exam, at least 25 correct answers are required
- IMPORTANT: Self-Assessment Questions in "*Mescher AL (2015). Junqueira´s Basic Histology Text and Atlas 14th Edition*" can help you significantly to get ready for the exam (chapters: 4, 5 ,6 ,7 ,8, 9, 10 ,11 ,15 ,16 ,17, 18, 19, 21, 22)

HALL OF FAME *(will not be examined)*

ARISTOTLE (384-22 B. C.)

- theory of male and female semen
- founder of biology as a science
- the first textbook of reproductive biology *De generatione animalium* (On animal reproduction)
- 1st written records of the development: incubated and observed chicken eggs at various stages of development, he described the gradual formation of a shape from an unstructured mass
- his theory of the origin and development of animals is very complex, it includes plants and animals, but also spontaneous births - *generatio spontanea* (lat.): life arising from non-living matter, mice born of mud or butterflies of dewdrops etc.

CLAUDIUS GALENUS (130-200 A. D.): theory of male and female semen

WILLIAM HARVEY (1578-1657):

- the first who recognized the full circulation of the blood in humans, rejected the theory of spontaneous birth formulated by Aristotle
- "***Omne vivum ex ovo***" (all animals develop from an egg, but was unable to prove its existence)
- *De Generatione Animalium* (1651): the beginning of the "embryological revolution", WH believed that sperm after entering the uterus metamorphose to form an embryo

ANTONY VAN LEEUWENHOEK (1632-1723): amateur researcher, used a single-lensed microscopes of his own design and construction (magnification up to 275 times or even more (?)), founder of microscopic anatomy, „father of mikrobiology", discovered sperm, follower of preformism (= (ontogenesis is the unpacking of preformed forms): supposed to observe all parts of man in the embryo and sperm (homunculi), similarly leaves / flowers are present in seeds

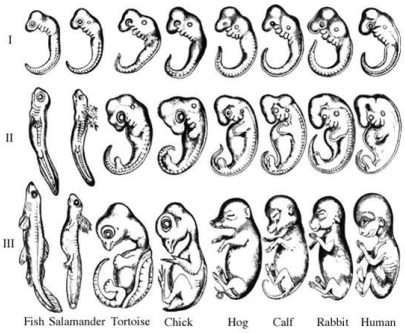
MARCELLO MALPIGHI (1628-1694): observed homunculi in eggs ☺, thought that unfertilized chicken eggs contained miniature chickens

LAZARRO SPALLANZANI (1729-1799): first artificial fertilization (frog) and insemination (dog), proven importance of egg and sperm, refused theory of preformisn and spontaneous birth

CARL LINNÉ (1707-1778): plant pollination experiments (1740)

CARL ERNST VON BAER (1791 – 1876):

- microscopic description of a mammalian egg (1827)
- comparison of embryonic development in mammals → Baer's ontogenetic law: general features common to large groups occur earlier in embryos than specialized features
- embryos of different species differ more and more during development
- the early embryo of „a higher animal" (phylotypic stage) is similar to the early embryo of „a lower animal", not an adult



J. E. PURKYNĚ (1787–1869): description of egg nucleus (*„vescicola germinale"*)

J. G. MENDEL (1822-1884): "father of genetics", discovered the fundamental laws of inheritance (through his work on *Pisum sativum*), he deduced the existence of hereditary units (now known as genes), which are present in pairs and are inherited as distinct units; he tracked the segregation of parental hereditary units and their appearance in the offspring generation as dominant or recessive features; was able to recognize the mathematical patterns of inheritance → 1866 Versuche über Pflanzen-Hybriden (his work and laws of inheritance were not appreciated in his time)

Video: https://www.youtube.com/watch?v=QmSJGhPTB5E

RUDOLF VIRCHOW (1821-1902): cell theory: ***„Omnis cellula ex cellula"***

ERNST HAECKEL (1834-1919): biogenetic law (recapitulation theory): ontogeny recapitulates phylogeny

AUGUST WEISMANN (1834-1914): germ plasm theory: heritable features are transferred to the next generation with the help of germ cells that are present in the ovaries and testicles

OSCAR AND RICHARD HERTWIG (19./20. stol.): study of fertilization (model organism: sea urchin, zygote comes from the union of two different germ cells), mesoblast: middle germ leaf

O. HERTWIG (1875): fertilization ← a single sperm, 1890 description of the stages of meiosis

THEODOR BOVERI (1862-1915**):** chromosome theory: defined nuclear complexes with different effects on different cells

THOMAS HUNT MORGAN (1866 - 1945): model animal *Drosophila*, 1933: Nobel Prize as the first biologist!

HANS SPEMANN (1869-1941): focus on amphibian research, embryonic induction (1924) = the ability of a embryonic tissue to induce differentiation of another tissue, 1935: Nobel Prize

SYDNEY BRENNER (1927): model organism *Caenorhabditis elegans*, 2002: Nobel Prize; *interesting videos about the importance of Caenorhabditis elegans in biology: http://www.jove.com/science-education/5110/c-elegans-development-and-reproduction* and *http://www.jove.com/video/2852/time-lapse-microscopy-of-early-embryogenesis-in-caenorhabditis-elegans*

ROBERT EDWARDS AND PATRICK STEPTOE (1978): Louise Brown (UK) is the first "test tube baby" in the world; R. Edwards (2010): Nobel Prize in Physiology and Medicine (Steptoe died 1988); Luisa's birth raised a number of questions → 1984 Warnock Report (model document for assisted reproduction legislation in the UK and other countries)

Video ☺: https://www.youtube.com/watch?v=tbfA1miS_HU

JAMES THOMPSON AND HIS TEAM

- 1998, for the first time in history, a line of embryonic stem cells from human embryos (blastocysts) was established (1981 in mice - Gail Martin, Martin Evans) - huge potential for research
- Masaryk University, Faculty of Medicine 2003: Aleš Hampl and Petr Dvořák obtained the first lines of human embryonic cells
- pluripotent stem cells can also be obtained from cells of an adult organism, e.g. by transforming skin cells (enthusiasm → care). Ian Wilmut (1996, dolly the sheep): use of skin cells is much more socially acceptable than the use of embryos, we shlould folllow both ways."

MODEL ANIMALS IN EMBRYOLOGY, HISTOLOGY AND ORGANOLOGY

- their research acquires information about these organisms (species), but also enables to study general phenomena and derive laws applicable to other organisms. The study of these species has enabled significant discoveries in many disciplines, including embryology and developmental biology, histology etc. with far-reaching implications for practice (see also "Hall of Fame" in this presentation).

Important model species include:

- worm (*Caenorhabditis elegans, Phylum: Nematode)*
- fruit fly (*Drosophila* ("dew lover") *melanogaster* ("dark gut"), *Phylum: Arthropod)*
- sea urchin (genus *Echinus*, *Phylum: Echinoderm)*
- zebrafish (*Brachydanio rerio*, *Phylum: Chordate*)
- African clawed frog (*Xenopus laevis*, *Phylum: Chordate)*
- chicken (*Gallus gallus domesticus*, *Phylum: Chordate)*
- mouse (*Mus musculus*, *Phylum: Chordate)*
- *Rattus norvegicus* (*Phylum: Chordate*)
- dog (*Canis lupus familiaris*, *Phylum: Chordate*)

Advantages of *Xenopus* as a Model Organism

Category:	C. elegans	Drosophila	Zebrafish	Xenopus	Chicken	Mouse
Broodsize	250-300	80-100	100-200	500-3000+	1	5-8
Cost per embryo	low	low	low	low	medium	high
High-throughput multiwell-format screening	good	good	good	good	poor	poor
Access to embryos	good	good	good	good	poor	poor
Micro-manipulation of embryos	limited	limited	fair	good	good	poor
Genome	known	known	known	known	known	known
Genetics	good	good	good	fair	none	good
Knockdowns (RNAi, morpholinos)	good	good	good	good	limited	limited
Transgenesis	good	good	good	good	poor	good
Evolutionary distance to human	very distant	very distant	distant	intermediate	intermediate	close

Color code: green, best in category; red, worst in category.
Adapted from Wheeler & Brändli 2009 Dev Dyn 238:1287-1308.

ANIMAL LIFE STAGES

- gametogenesis
- fertilization → embryonic development → postembryonic development → death
- embryonic development: blastogenesis and organogenesis
- postembryonic development: adolescence, adulthood, old age

GAMETOGENESIS

- the process through which male and female gametes (sperms and oocytes) are created in gonads, testes and ovaries
- the nuclei of gametes ≠ the nuclei of somatic cells: gametes have half the number of chromosomes in comparison with somatic cells
- **multiplicative phase**: mitotic division of primordial oogonia and spermatogonia
- **growth phase**: increasing the volume of the cytoplasm, formation of primary spermatocytes and oocytes
- **maturation phase**: formation of haploid secondary spermatocytes and oocytes (← 1st meiotic division), 2nd meiotic division → haploid spermatids and an ovulated secondary oocyte; while spermatids must undergo another process of differentiation, so-called spermateliosis (the course depends on what type of sperm are produced, the most common type are with *flagellum* = tail), the oocyte is a mature egg cell after the second meiotic division
- spermatogenesis → 4 mature spermatozoa
- oogenesis → 1 mature oocyte and 3 polar bodies (or 2 if the first polar body does not undergo cell division (as in humans))

WHEN SOMETING GOES WRONG

a) numerical chromosomal abnormalities - aneuploidies

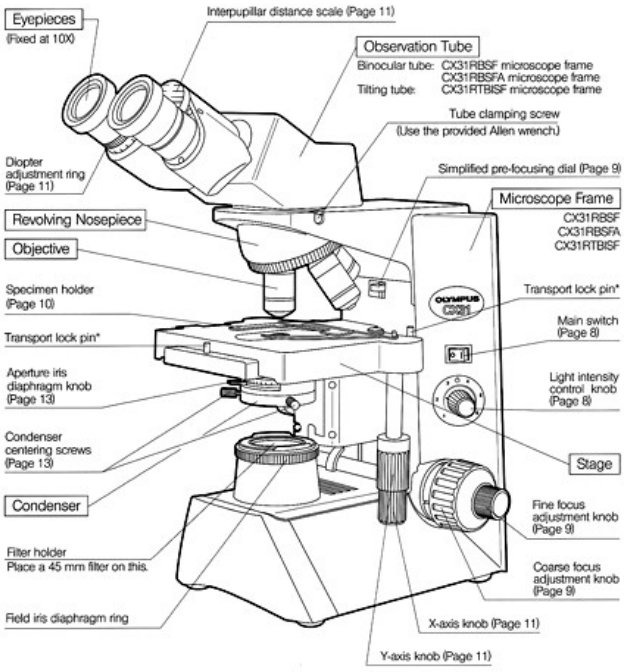
- **Down's syndrome:** trisomy 21 (chromosome 21 in 3 copies), mental retardation, with small mouth, low set ears, heart troubles, ….
- **Patau's syndrome:** trisomy 13, severe physical birth defects and mental retardation
- **Edward's syndrome:** trisomy 18, severe physical birth defects and mental retardation
- **Klinefelter syndrome:** one (or more) extra X chromosome(s) in a male with sexual features of a female, e.g. 44+XXY, 44+XXXY, 44+XXXXY , 44+XXYY
- **Turner syndrome:** the absence of one X-chromosome in a female, 44+X0
- **Super-female**: one extra X-chromosome in a female, 44+XXX
- **Super-male:** one extra Y chromosome in a male, 44+XYY

b) structural chromosomal abnormalities: translocation of a part of a chromosome to another (common for chromosomes 13, 14, 15, 21, 22); loss of a chromosomal part (cri-du-chat syndrome = cat scream syndrome = partial deletion of short arm of chromosome 5), inversion,…

c) gene mutations

- **note**: > 75% of miscarriages during the first 2 weeks of pregnancy and > 60 % of miscarriages in the 1st trimester are caused by chromosomal abnormalities (the most common are Turner syndrome, triploidy, trisomy 16).
- chromosomal abnormalities cause 7 % of congenital malformations and gene mutations another 8 % of congenital malformations

PRACTICAL EXERCISE



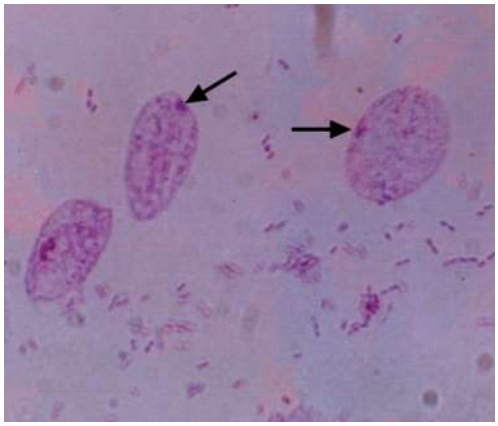
1. place the microscope slide with the specimen on the stage, fix with the clip
2. rotate the objective with the lowest magnification into position.
3. turn the coarse focus knob to move the stage as close as it can get to the objective/lens without touching the lens.
4. always watch from the side whenever you rotate objectives or you move a specimen towards any lens to make sure the lens can not damage your specimen!
5. while looking through the ocular lens, turn the coarse focus knob carefully, and slowly move the stage away from the lens until the specimen comes into focus. Then, use the fine focus knob to bring the specimen into sharp focus.
6. adjust the light source for optimum illumination for each new slide and for each change in magnification.
7. once you have brought the specimen into sharp focus with the first 3 objectives (from low-power to high-power), you can observe your specimen under oil immersion
8. place a drop of oil on the slide directly over the area to be viewed
9. rotate the nosepiece until the oil-immersion objective locks into position. Care should be taken not to allow the high-power objective to touch the drop of oil. The slide is observed from the side as the oil-immersion objective is rotated slowly into position. This will ensure that the objective will be properly immersed in the oil.
10. the fine-adjustment knob is readjusted to bring the image into sharp focus**.**

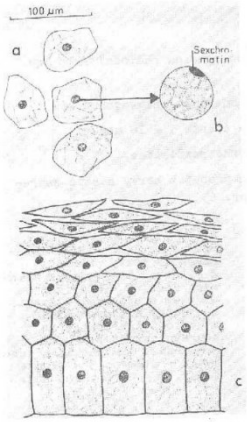
PRACTICAL EXERCISE

OBSERVATION OF BARR BODY

THEORY: Women have 2 copies of X-chromosome, man have only a single copy. To compensate the dosage of X-linked genes in females, one X-chromosome in females is permanently deactivated and is present as darkly stained body (Barr body, condensed) near nuclear membrane in the interphase nucleus of human somatic cells. Barr bodies are only seen in women who have 2 sex chromosomes (XX). Men do not have a Barr body, they have only one X-chromosome. Detection of a Barr body can be used to identify the gender of e.g. athletes. Barr body cannot usually be demonstrated in all cells (in women, it is found in approximately 40 % of the cells of the oral mucosa).

PROCEDURE: Scrape the epithelial cells of the oral mucosa with a spatula, apply the material to the glass slide. Add Lugol's solution, cover with a cover glass and observe.





Obr. 63
Sexchromatin v epitelových buňkách ústní sliznice ženy. a - epitelové buňky s jádrem;v nich b - lze detekovat sexchromatin, c - schéma stavby plochého vrstevnatého epitelu ústní sliznice

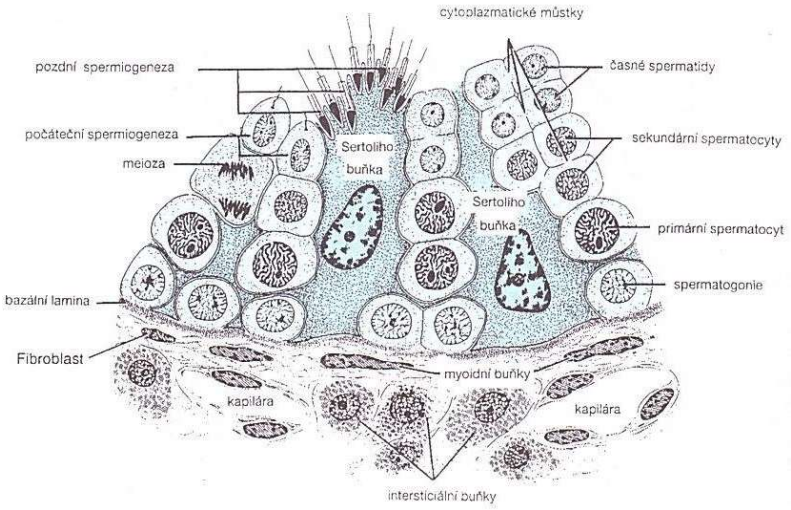
GAMETOGENESIS

- includes development of germ cells (extraembryonic origin) and their migration into the gonads
- further development according to different schemes for ♂ and ♀:
  a) mitotic germ cells proliferation in the germinal epithelium
  b) in humans, oogonia multiplie in ♀ by the end of the 5th month of pregnancy, up to 7 million germ cells are formed, then their loss (atresia) until menopause
  c) spermatogonia retain the ability to reproduce throughout life

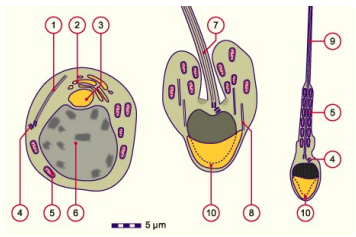
SPERMATOGENESIS

Video: HISTOLOGY OF MALE REPRODUCTIVE TRACT
https://www.youtube.com/watch?v=1WOcWthZjrE

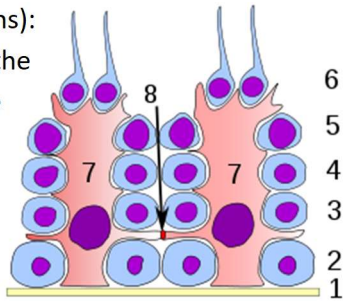
- spermatogonia: on the basement membrane of the seminiferous tubules → type A, IM and B.
- type B leaves the basal compartment and enters the adluminal compartment between Sertoli cells → mitosis → 2 spermatocytes I. Thus, spermatogonia repeatedly divide (reproductive zone), enriched with nutrients and mitotically divided into spermatocytes I. We call this stage the growth stage.



- when the cell enters the maturation stage, the first meiotic division occurs. Spermatocytes II are formed (prespermatids, relatively shortest period of existence within spermatogenesis). The second meiotic division produces spherical spermatids with haploid sets of chromosomes →
- **spermiohistogenesis (spermateliosis)**: reduction of the nucleus and chromatin condensation, cytoplasm reduction: → residual bodies (phagocytosis by Sertoli cells), condensation of the Golgi apparatus → acrosome: hyaluronidase, acrosin, proacrosin, collagenase, flagellum development: mitochondria spirally arranged in the proximal part in the tale



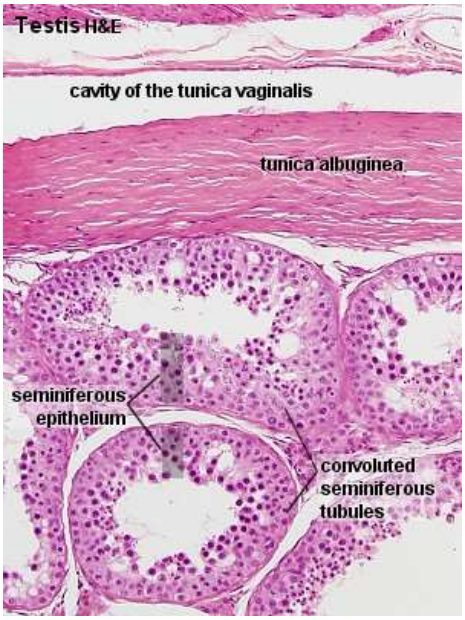
1 Axonemal structure,first flagellar primordium
2 Golgi complex
3 Acrosomal vesicle
4 Pair of centrioles (distal and proximal)
5 Mitochondrion
6 Nucleus
7 Flagellar primordium
8 Microtubules
9 Sperm cells tail
10 Acrosomal cap



- hematotesticular barrier (blood-testis barrier, tight junctions): prevents the prevents the contact of the immune system with the differentiating sperm and thus prevents the autoimmune response.
- mammalian sperm cells in the seminiferous tubules: only morphological maturity, immobile, incapable fertilization, in ♀ reproductive tract: capacitacion - changes in cytoplasmatic membrane, acquisition of fertilization ability
- human sperm: movement 1 to 4 mm/min., few reserve substances, diffusion nutrition, limited life, calcium fundamentally affects functionality

PRACTICAL EXERCISE

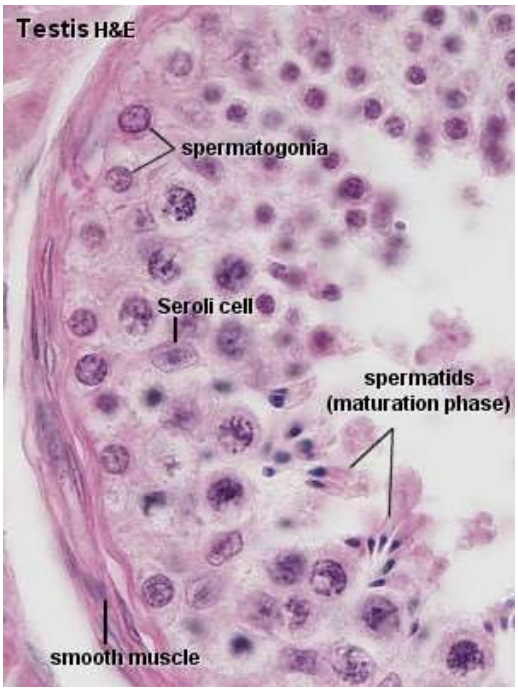
TESTES, SPERMATOGENESIS

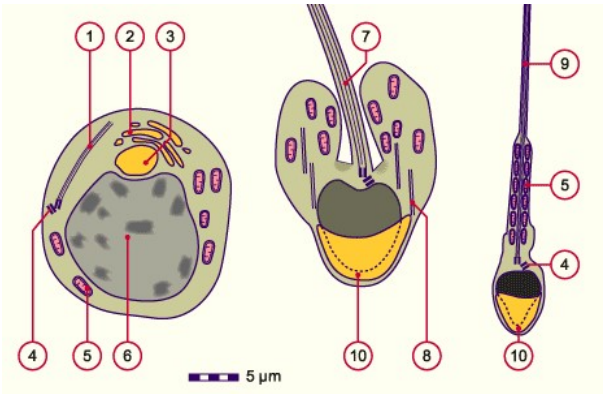


- **seminiferous tubules:** surrounded by a thick basal lamina, externally covered by cca 3 layers of smooth muscle cells (or myoid cells). The insides of the tubules are lined; seminiferous epithelium = spermatogenic cells + Sertoli cells
- **Sertoli cells:** nutritive function, mechanical support for the spermatogenic cells, far less numerous than the spermatogenic cells and are evenly distributed between them, irregular/columnar shape, they extend from the basement membrane to the luminal space, lightly stained nucleus with a large nucleolus; folded nuclear membrane is characteristic for Sertoli cells but not always visible in the LM, Sertoli cells are a laterally connected by tight junctions= basis for the blood-testis barrier
- **spermatogonia:** dormant until puberty, always in contact with the basal lamina of the tubule;
- **type A spermatogonia:** stem cells → new generations of type A and type B spermatogonia; rounded nucleus with fine chromatin grains, 1-2 nucleoli.
  b) type B spermatogonia: rounded nuclei with chromatin granules of variable size, 1 nucleolus; they do not function as stem cells → final mitosis →
- **primary spermatocytes:** in the cell layer luminal to the spermatogonia, larger than spermatogonia. They immediately enter the prophase of the first meiotic division, which is extremely prolonged (about 22 days!). A large number of primary spermatocytes is always visible in cross-sections through seminiferous tubules. Cell divisions, from the formation of primary spermatocytes

and onwards, to the production of the spermatocytes, are incomplete - the cells remain connected by bridges of cytoplasm. The completion of the first meiotic division →

- **secondary spermatocytes:** smaler, seldom seen in histological slides (rapidly enter and complete the second meiotic division) →
- **spermatids:** in the luminal part of the seminiferous tubules, small (10 µm in diameter), initially very light (eccentric) nukleus; the chromatin condenses during the maturation into spermatozoa → the smaller and darker nucleus; →
- **sperm cells:** head, neck, tail
- head: flattened, the nucleus with condensed chromatin; cca 2/3 of the nucleus covered by the acrosome (enzymes important tor fertilization)
- neck: short (cca 1 µm)
- flagellum = middle piece (axonema = arrangement of microtubules, a sheath of mitochondria) + principal piece (axonema) + end piece;





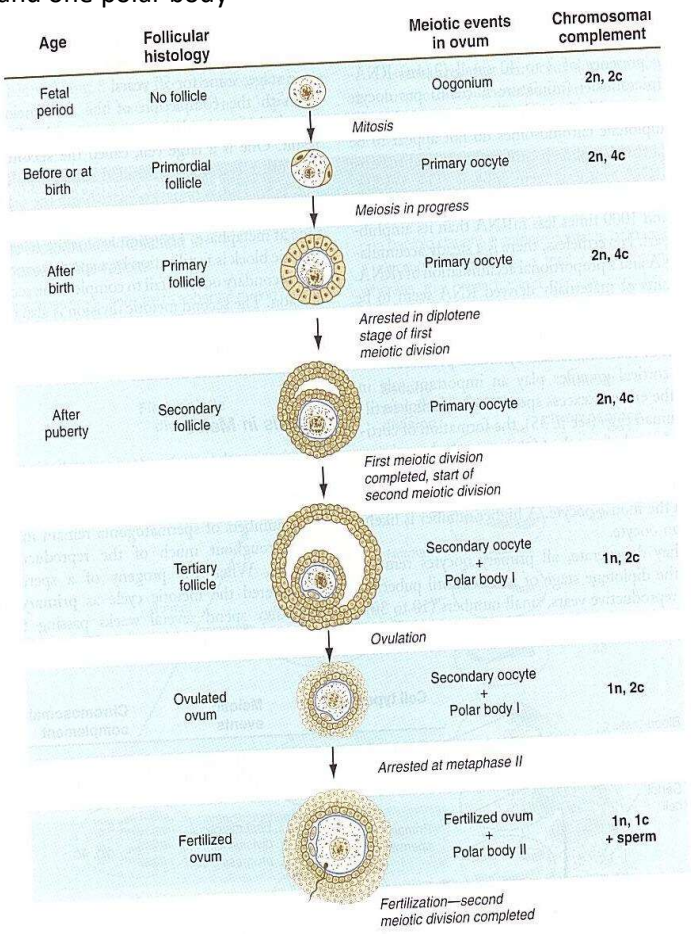
1 Axonemal structure,first flagellar primordium
2 Golgi complex
3 Acrosomal vesicle
4 Pair of centrioles (distal and proximal)
5 Mitochondrion
6 Nucleus
7 Flagellar primordium
8 Microtubules
9 Sperm cells tail
10 Acrosomal cap

OOGENESIS

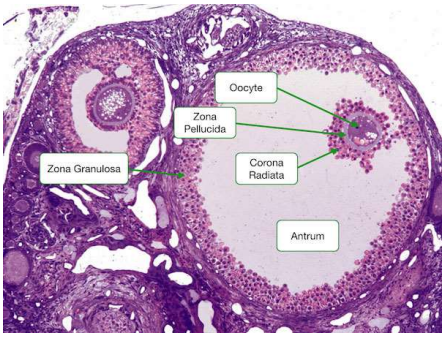
Video: HISTOLOGY OF FEMALE REPRODUCTIVE TRACT:
https://www.youtube.com/watch?v=APUkKB5FAR8
https://www.youtube.com/watch?v=6EC4DFP78k0

- **oocytes:** *ova (plural, lat.), ovum* (singular, lat.), unicellular formations that contain genetic information and nutrition material in the early stages of embryo development
- spherical, with an asymmetric internal structure
- the process by which female germ cells develop into mature eggs (ova)
- humans: oligolecital, isolecital oocytes, total and equal cleavage
- maturation of the follicles continues only in and after puberty due to hormones

- meiosis I → two haploid cells: one oocyte II and one polar body
- meiosis II is initiated at the time of ovulation (in most mammals) and is completed only after the sperm penetrates the egg: oocyte II. → one egg and the second pole body are formed
- at the same time, the first polar body undergoes mitosis, it is divided into two, all 3 pole bodies are resorbed (this is generally the case, in humans the first polar body does not undergo division)
- ovulation in uniparous animals: ovulation of a single egg (human, apes, elephants,…) ; ovulation is caused by LH peak → ovulated oocyte (human): limited lifespan (!), no reserve substances, diffuse nutrition, energy source: pyruvate, nucleic acid synthesis does not take place, but the oocyte contains them
- in humans: at the beginning of the cycle a group of about 20 early antral follicles begins to evolve, but only about 3 reach Ø 8 mm → one of the growing follicles (most sensitive to gonadotropins) reaches ovulation as the so-called dominant follicle, the others undergo atresia (apoptosis))



- AMH ("anti-Müllerian hormone"): important role in oocyte development - produced by granulosa cells of preantral and early antral follicles, its concentration correlates with ovarian reserve (diagnostic significance)
- after ovulation: luteinization of granulosa cells → *corpus luteum* → progesterone (affects the differentiation of tissues of the reproductive system)
- absence of fertilization: luteolysis (apoptosis) and collagenous degeneration of *corpus luteum* → corpus albicans
- fertilization: hCG (human chorionic gonadotropin, produced by the syncytiotrophoblast blastocyst), hCG supports the activity of the *corpus luteum graviditatis* (produces progesterone especially in the first 2 months of pregnancy)



- ovulation in multiparous animals: ovulation of more eggs (rodents, carnivores,…)
- ovulation is caused by LH peak → ovulated oocyte (human): limited lifespan (!), no reserve substances, diffuse nutrition, energy source: pyruvate, nucleic acid synthesis does not take place, but the oocyte contains them

	Oogenesis	Spermatogenesis
Cell division	Begin with mitosis and later on involve meiosis	
Growth	Involve cell enlargement before meiosis	
Product	Haploid cells (gametes)	
Differentiation	Produce specialised gametes	
Location	Eggs/ova produced in the ovaries	Sperm produced in the testes
Initiated	During development of fetus	During puberty
Pauses	During prophase I and between prophase II and metaphase II	None
cytokinesis	Unequal, producing polar bodies	Equal
Number of gametes	One ovum, polar bodies degenerate	Four sperm
Release	14th day, midpoint of the menstrual cycle	Continuous production, released during sexual intercourse
Ceases	At the menopause	Continuous until death

PRACTICAL EXERCISE

MAMMALIAN OVARIES, OOGENESIS

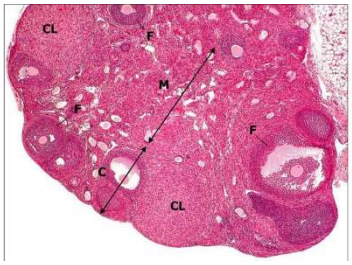
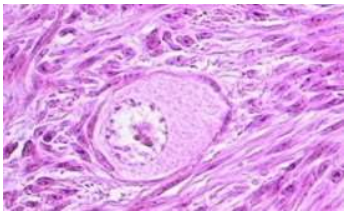


Figure 1.2 – Subgross anatomy of the normal rodent ovary (mouse, H&E x4). The cortex (C) contains numerous follicles at various stages of maturation. The medulla (M), which is not always present in histological sections, contains lymphatics, nerves and numerous blood vessels.

C	cortex
M	medulla
CL	corpus luteum
F	developing follicles

- almond shaped organs, covered by ovarian surface epithelium and a thick connective tissue = tunica albuginea
- an outer cortex: the follicles with oocytes
- inner medulla: fibrous tissue, rich blood supply, lymphatic ducts, nerves

a) primordial follicles



- the earliest stage of follicular development
- they form during early fetal development
- located within the peripheral cortex (beneath the tunica albuginea)
- a large oocyte + a layer of flattened follicular cells
- arrested in prophase

b) primary follicles

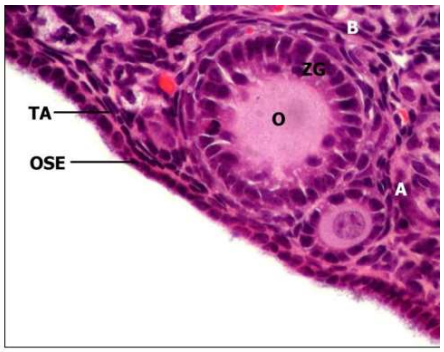


Figure 1.4 – Primary (A) and early secondary follicle (B) (rat, H&E x40).

O	primary oocyte
ZG	developing zona granulosa
TA	tunica albuginea
OSE	ovarian surface epithelium (cuboidal)

- a oocyte + a layer of cuboidal follicular cells
- the zona pellucida (clear zone around the oocyte in the photo) covers the oocyte and separates it from the cuboidal follicular cells

c) secondary follicles

- the stratified granulosa cells there are large with lacunae (later → follicular anthrum - *antrum folliculi*)
- the stromal cells around the follicle → an inner layer (*theca interna*, cells → steroids + an outer layer (*theca externa*, concentrically arranged stromal cells = a support for the developing follicle)

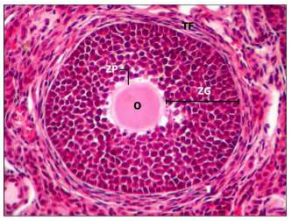
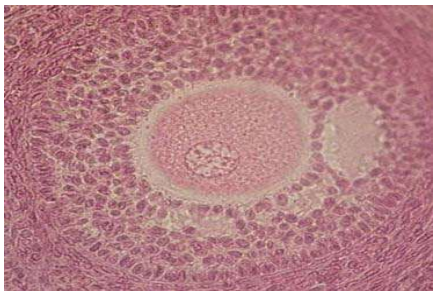


Figure 1.5 – Secondary follicle (mouse, H&E x20).

O	primary oocyte	ZG	zona granulosa
ZP	zona pellucida	TF	theca folliculi
VS	vesicular spaces		



d) Graafian /ovulatory /mature follicle

- large *anthrum folliculi* surrounded by many layers of cuboidal granulosa (follicular) cells
- the oocyte is situated eccentricaly within the follicle in a small hillock (*cumulus oophorus* which protrudes into the antrum)
- the oocyte is of its full size, ready for ovulation
- *zona pellucida* is covered by a layer of follicular cells (*corona radiata*)
- *theca folliculi* is separated from the follicle by a basement membrane and has a rich vascular supply (= *theca interna*; surrounded by spindle-shaped cells in *theca externa*)

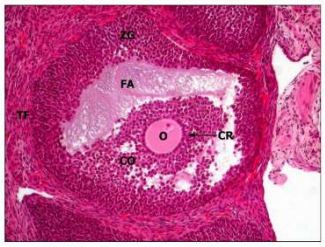
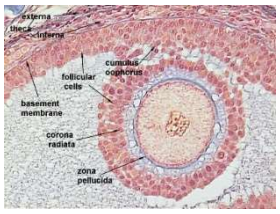


Figure 1.7 – Tertiary follicle (rat, H&E x20). The large follicular antrum (FA) and eccentrically positioned primary oocyte (O) characterise this stage.

O	primary oocyte	CO	cumulus oophorus
CR	corona radiata	FA	follicular antrum
ZG	zona granulosa	TF	theca folliculi

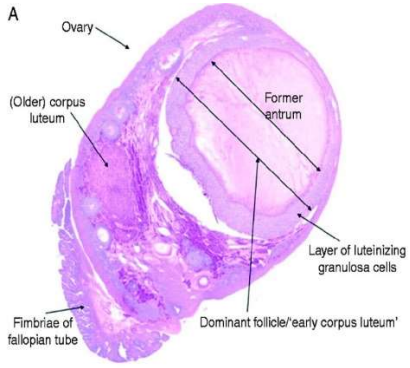


e) atresia

- may begin at any stage in follicular development
- shrinkage and lysis of the cytoplasm of the oocyte and granulosa/follicular cells

f) after ovulation

- after ovulation, the Graffian follicle collapses, becomes infolded and invaded by blood vessels → *corpus luteum*
- during the luteinisation, the granulosa and thecal cells undergo hypertrophy, this is accompanied by degeneration of the basement membrane separating *theca interna* and granulosa cells, and infiltration of the postovulatory follicle by blood vessels from *theca interna*).
- if the egg is fertilized: *corpus luteum* → *corpus luteum* of pregnancy (supported by hCG)
- if the egg is not fertilized: *corpus luteum* degenerates, is infiltrated with collagen (and a few fibroblasts) → *corpus albicans* (white body, fibrous structure)



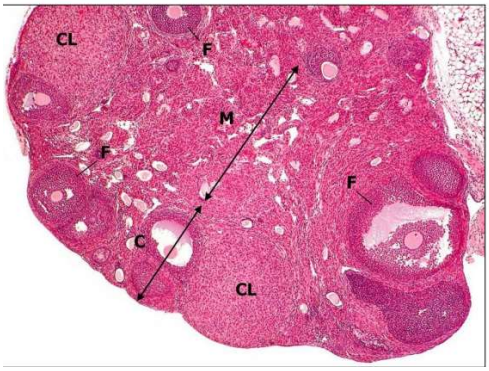


Figure 1.2 – Subgross anatomy of the normal rodent ovary (mouse, H&E x4). *The cortex (**C**) contains numerous follicles at various stages of maturation. The medulla (**M**), which is not always present in histological sections, contains lymphatics, nerves and numerous blood vessels.*

C	cortex
M	medulla
CL	corpus luteum
F	developing follicles

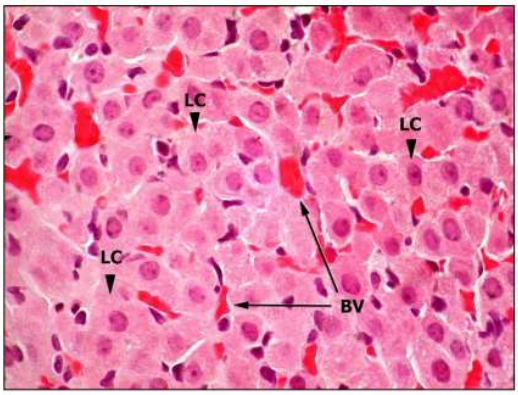
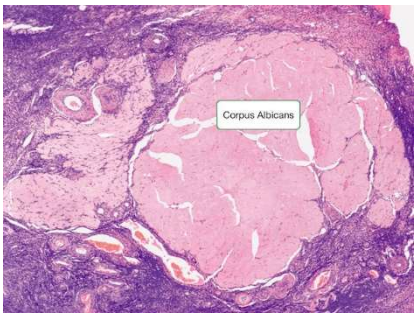


Figure 1.9 – Corpus luteum (rat, H&E x40). The luteal cells (LC) comprising the corpus luteum are plump and polygonal; they contain large nuclei and moderate amounts of eosinophilic cytoplasm. Cytoplasmic vacuoles form within luteal cells as the corpus luteum matures and subsequently degenerates. Numerous blood vessels (BV) are present, consistent with its function as a temporary endocrine gland.



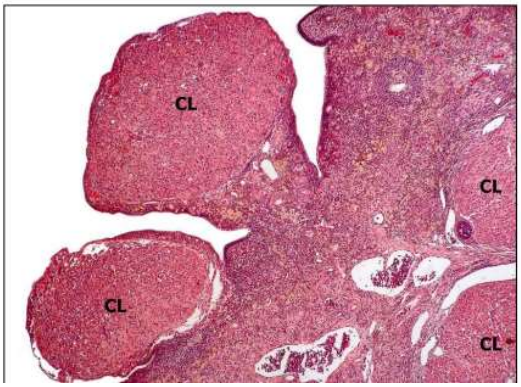


Figure 1.10 – Corpora lutea (CL) (rat, H&E x10). Note the marked protrusion of these large postovulatory follicles beyond the surface of the ovary. Another pair of corpora lutea are present within the body of the ovary.

GAMETOGENESIS - SUMMARY

Sperm (← spermatogenesis):

- extremely small cells which lack most of their cytoplasm
- ability to travel through an (internal or external) aquatic medium to reach the egg cell
- flagellum to move toward the egg
- all sperm consist of three basic pieces: the head (contains the genetic material and is capped by the acrosome (cap) (with enzymes needed for the sperm to penetrate the egg); the middle piece (with mitochondria that fuel the movements of the tail piece); the flagellum (tail piece)

Eggs (← oogenesis):

- designed for providing nutrients to the developing embryo
- contains yolk (lipids, proteins, glycogen for embryo nutrition)
- classified by the amount and the distribution of yolk in the egg
- quantity of yolk and its distribution in the eggs → different types of cleavage after fertilization
- the amounts of yolk are minimal in mammalian eggs, placenthal development is necessary for further embryo development
- large eggs (birds, reptiles) have large amount of yolk to support embryonic development to advanced stages

REPRODUCTION

- the basic property of living organisms
- the ability to form the basis of a system, which is the same as the founding system

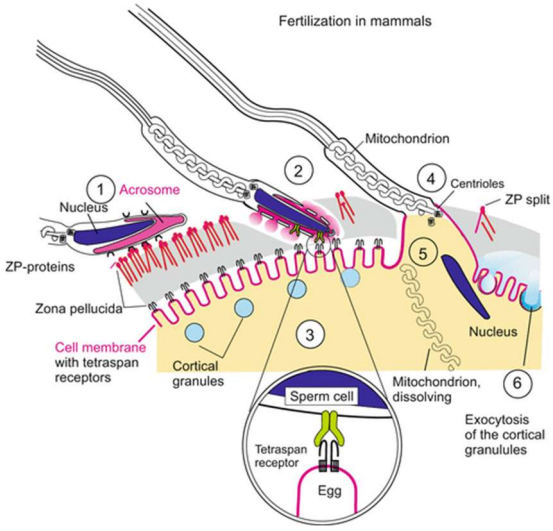
- allows to preserve the species and time continuity of life, to develop, increase the number of individuals, ensure the survival of the genetic lineages
- sexual and asexual, or their alternation (metagenesis)
- the level of reproduction is an indicator of the well-being of an organism in a given environment
- an indicator of the balance of conditions in the external and internal environment of the organism
- alternative definition (MD Aleš Bourek, Ph.D.):

he + she +  **=** 

Insemination: sperm reaches the egg, in the external environment (e. g. in the water in fish) or inside the female's body

Fertilization:

- "navigation miracle", complex set of events involving the fusion of two gametes a new individual, sperm penetrates egg envelopes - in mammals *zona pellucida* (ZP)
- sperm nucleus (1 n) → male pronucleus + egg nucleus (1 n) → female pronucleus → prezygote (2 PN) → zygote
  - extracellular envelope of mammalian oocytes = ZP. This envelope surrounds mammalian oocytes, ovulated eggs and preimplantation embryos. Significance of ZP: keeps the cleaving embryo together during transport through the fallopian tube and prevents it from sticking to the walls of the fallopian tube
  - interaction of sperm with ZP: ZP glycoproteins have specific sperm binding sites, binding of sperm to these sites triggers an acrosomal reaction
  - the gradual development of an acrosomal reaction
  - development of cortical reaction (right, definitive blockade of polyspermia): fusion of cortical granules with the cytoplasmic membrane of the egg, their contents spill (exocytosis) into the space above the cytoplasmic membrane, zona pellucida becomes impermeable to other spermatozoa
  - after the penetration of sperm, the oocyte completes the second maturation division, the maternal and paternal pronuclei are formed, they approach each other → a diploid zygote - see also the following scheme (slide 8)



EMBRYO

„The amazing thing about development is not that it sometimes goes wrong, but that it is ever succeed.“

Veronika van Heyningen (2000)

- zygote: a fertilized activated oocyte, with functional mitochondria of the egg
- genome of the zygote (and other cells) contains complete genetic information, which is, however, used differently in different periods of development (differentiation)!

- an oocyte brings to the embryo: 1 set of autochromosomes, cytoplasm with cellular structures, mitochondria, ribosomes and other RNA, protection against polyspermia, gonosome
- sperm brings embryos: 1 set of autochromosomes, centriole, from millions of sperms only one fertilizing egg - principle of natural selection, gonosome
- embryo: the individual developing from a fertilized egg cell, formed by the first mitosis of the zygote, diploidy is not enough for successful development, the presence of the maternal and paternal genomes is necessary (!)

Humans:

- up to the 8th week = embryo development, then fetus
- 0 - 3 weeks after fertilization: early embryonic development (cleavage, gastrulation)
- 4 - 8 weeks: organogenesis
- 9-38 weeks: fetal period

EMBRYOGENESIS

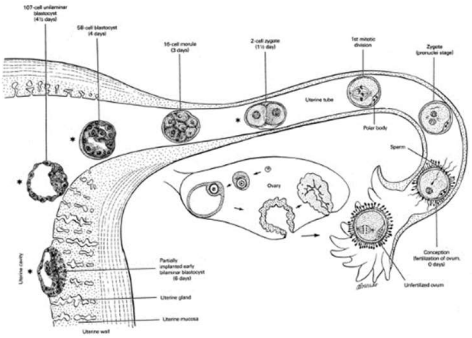
1) blastogenesis: cell division and germ layers differentiation, the embryo does not grows

- cleavage: division of the zygote into blastomers → the restoration of normal cell size (typical for a given species
- gastrulation: formation of germ layers
- formation of organ bases

2) organogenesis: cell differentiation and proliferation, intensive growth, formation of intercellular mass and embryonic cavities.

CLEAVAGE:

- a number of mitoses → blastomeres, dependance on the amount and distribution of (yolk) in the cytoplasm, the yolk is deposited in the oocyte during the so-called vitelogenesis; quantity of yolk and its distribution in the eggs → different types of cleavage after fertilization:



→ holoblastic (total) cleavage (sea urchins, amphibians,…) equal or inequal

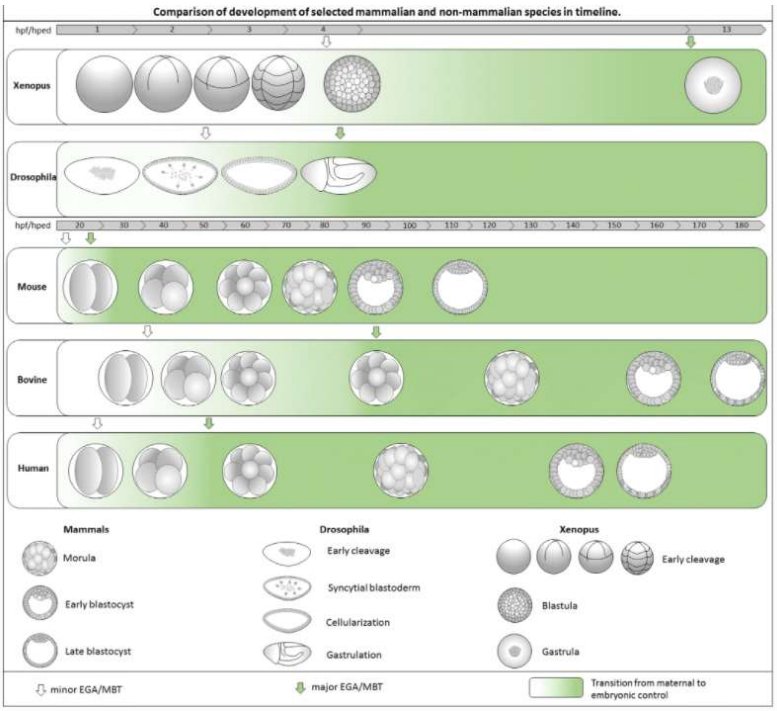
→ meroblastic (partial) cleavage (superficial in insects; disc-shaped in fish, reptiles and birds)

- **in humans/mammals:** takes place during the transport of the embryo through the fallopian tube, in mammals this transport takes 2-4 days
- time data refer to humans: **prezygote** (2 pronuclei visible, 2PN stage) → **zygote** → first mitosis within 30 hours → **2 cell embryos**: 24 - 42 hours → **4 cell embryo**: 39 - 60 hours, embryonic genome switched on: at the 4-8 cell stage, until then, the oocyte´s genes and their products are used → **8 cell embryo**: 54 - 75 hours, totipotent cells → spherical **morula** (*morus* (lat.) = mulberry): approx. 16 cells , day 4, compaction takes place: formation of "tight junctions" in the surface layer, "gap junctions" in the inner layer, morula acquires a compact appearance, still in the ZP → **blastocyst**: day 5, leaves ZP (= hatching)

- blastocyst = trophoblast (cells under the zona pellucida, → chorionic gonadotropin) + embryoblast (ICM, embryonic pole) + inner cavity (*blastocoel*, filled with fluid, blastocoel formation process = cavitation), still in the zona pellucida (ZP), which changes by the influence of both blastomers and maternal tissues, ZP is „digested“ by enzymes produced trophoblast cells → "hatching", increasing the volume of fluid in the cavity also contributes to successful hatching
- blastocyst implantation in the human uterus: 6-7 days after fertilization (day 4 in mice)

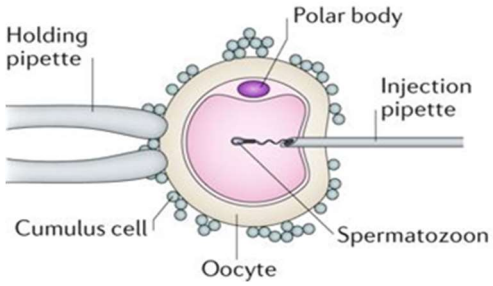
Video: https://www.youtube.com/watch?v=W4pVICcRtxQ

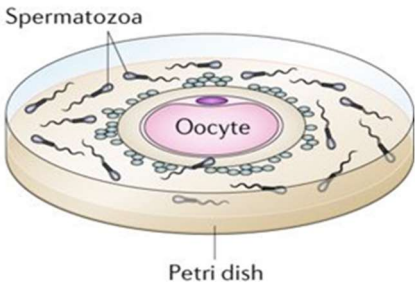
COMPARISON OF EMBRYONAL DEVELOPMENT IN DIFFERENT MODEL ANIMALS



LINKING SCIENCE AND PRACTICE

- fertilization of oocytes, taken by oocyte pick up (OPU) directly from the ovary. Video: **https://www.youtube.com/watch?v=tmy3Z-TfZ5I**
- fertilization outside the woman's body (IVF, in vitro fertilization)
- in order to increase the probability of successful fertilization and obtain more eggs for the needs of this method, hormonal stimulation is performed in order to induce so-called superovulation and obtain more eggs.
- fertilization itself is carried out either by adding sperm to the egg (classical fertilization, picture on the right) followed by cultivation, or by injecting sperm into the oocyte (ICSI method, picture on the left). **Video: https://www.youtube.com/watch?v=GTiKFCkPaUE.**
- IVF is one of the options for the treatment of sterility, intended for women with ovulation disorders, fallopian tube obstructions or endometriosis. Another indication is male sterility factor and idiopathic conditions. Depending on the diagnosis, donated eggs or sperms or embryos can be used. The procedures are linked with the cultivation of embryos in vitro and the subsequent transfer of the selected embryo to the uterus (5th day of cultivation)





Semen parameter	WHO 1980	WHO 1987	WHO 1992	WHO 1999	WHO 2010[1]	WHO 2021
Volume (mL)	ND	≥2	≥2	≥2	1.5	1.4
Sperm concentration ($x10^6$/mL)	20-200	≥20	≥20	≥20	15	16
Total sperm number ($x10^6$)	ND	≥40	≥40	≥40	39	39
Total motility (%)	≥60	≥50	≥50	≥50	40	42
Progressive motility (%)[2]	≥2[3]	≥25	≥25 (grade a)	≥25 (grade a)	32 (a+b)	30
Vitality (%)	ND	≥50	≥75	≥75	58	54
Normal morphology (%)	80.5	≥50	≥30	(14)	4	4

SUMMARY

- MD Aleš Bourek, Ph.D.: there was only one way to "make babies" for ages: sex, however now you have so many possibilities (combine the items in the left and right columns):

intrauterine insemination	parental gametes
“classical” IVF	donated sperm
ICSI	donated oocytes
frozen and thawed embryos	donated embryos
+ surrogacy, PGD, mitochondrial donation etc.	

GASTRULATION

"*It is not birth, marriage, or death, but gastrulation, which is truly the most important time of your life*."

Lewis Wolpert

- the dramatic rearrangement and movement of cells → a massive reorganization of the embryo
- these movements lead to the formation of a new shape to the embryo ↔ morphogenetic movements (*morpho*=form; genetic = production)
- an embryo → a multi-layered organism
- = changes in cell motility, shape and adhesion
- → the primary germ layers (endoderm, mesoderm, and ectoderm)
- endoderm (the most internal germ layer) → the lining of the gastrointestinal tract
- ectoderm (the most exterior germ layer) → epidermis, the nervous system and other external tissues
- mesoderm (the the middle germ layer) → the musculo-skeletal system,the circulatory system

Ectoderm

- Epidermis of skin and its derivatives (including sweat glands, hair follicles)
- Epithelial lining of mouth and anus
- Cornea and lens of eye
- Nervous system
- Sensory receptors in epidermis
- Adrenal medulla
- Tooth enamel
- Epithelium of pineal and pituitary glands

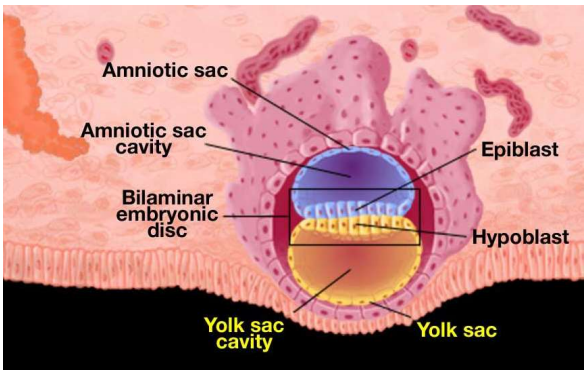
Mesoderm

- Notochord
- Skeletal system
- Muscular layer of stomach and intestine
- Excretory system
- Circulatory and lymphatic systems
- Reproductive system (except germ cells)
- Dermis of skin
- Lining of body cavity
- Adrenal cortex

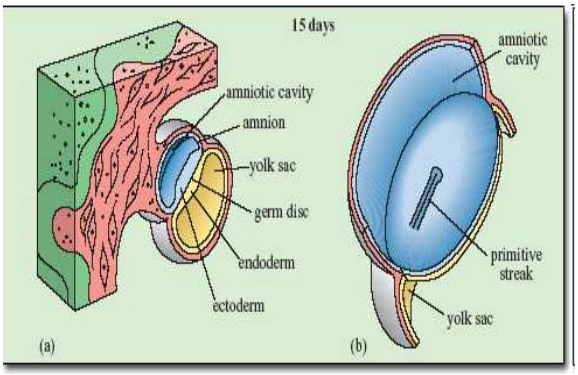
Endoderm

- Epithelial lining of digestive tract
- Epithelial lining of respiratory system
- Lining of urethra, urinary bladder, and reproductive system
- Liver
- Pancreas
- Thymus
- Thyroid and parathyroid glands

- birds and mammals are descendants of reptilian species → mammalian development has parallels that of reptiles and birds
- the mammalian embryo: nutrients directly from the mother, does not rely on yolk ↔ restructuring of the maternal body: formation of the uterus, the development of a fetal organ absorbing maternal nutrients = the chorion: derived from the trophoblast, supplemented with mesodermal cells derived from the ICM; the chorion = the fetal portion of the placenta and will induce the uterine cells → the maternal portion of the placenta (= the decidua: rich in the blood vessels providing oxygen and nutrients to the embryo)



- **the ICM → the hypoblast layer** (the primitive endoderm): lines the blastocoel cavity → the extraembryonic endoderm: forms the yolk sac; these cells do not produce any part of the newborn (as in avian embryos).
- **the remaining ICM → epiblast**: lines the amnionic cavity with amnionic (amniotic) fluid (a shock absorber for the embryo, prevention of desiccation); the embryonic epiblast contains all the cells that will generate the actual embryo (similarly as in the avian epiblast) **→ bilaminar disc**
- **bilaminar disc →** formation of the primitive streak ≈ important event of gastrulation: cells from the epiblast migrate into the interior of the embryo (ingression, a cellular epithelial-to-mesenchymal transition is involved), via the primitive streak



- the initial wave of migrating cells goes through the primitive streak, the cells displace the hypoblast cells and become definitive endoderm, which ultimately produces the future gut derivatives and gut linings
- the following wave of migrating cells populates a layer between the epiblast and the definitive endoderm → mesoderm layer.
- at the posterior end of the embryo: the node forms, the cells migrating through the node → the notochord – the cells converge medially and fold off in a dorsal direction

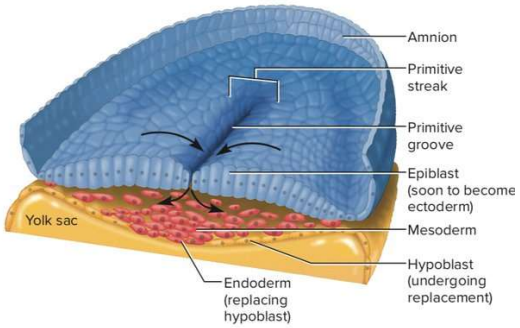
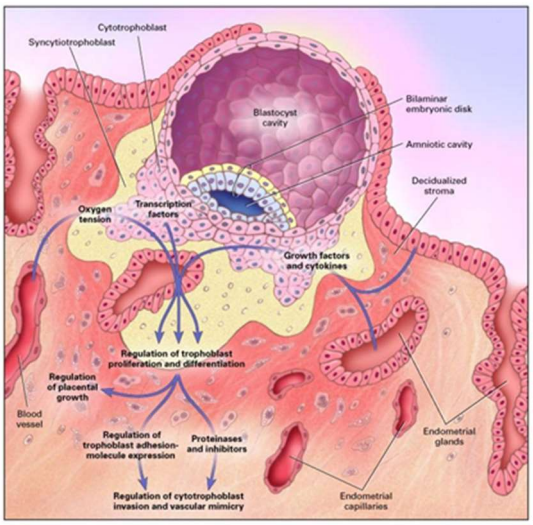


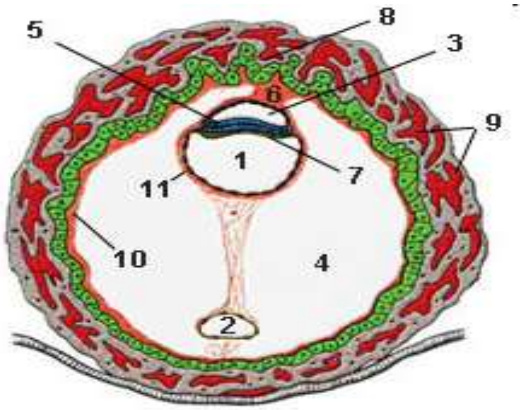
FIGURE 29.5 Formation of the Primary Germ Layers (Gastrulation). Composite view of the embryonic disc at 15 to 16 days. Epiblast cells migrate over the surface and down into the primitive groove, first replacing the hypoblast cells with endoderm, then filling the space with mesoderm. Upon completion of this process, the uppermost layer is considered ectoderm.

Formation of extraembryonic membranes in mammals

- the epiblast: cell movements similar to those in reptilian or avian gastrulation, the extraembryonic cells: the distinctly mammalian tissues that enable the embryo/fetus to survive in the uterus
- the division of the trophoblast cells (cytotrophoblast) → cells where a nuclear division occurs in the absence of cytokinesis (= syncytiotrophoblast)
- the cytotrophoblast adheres to the endometrium through adhesion molecules + these cells contain proteolytic enzymes that enable them to enter the uterine wall and remodel the uterine blood vessels so that the maternal blood bathes fetal blood vessels
- the syncytiotrophoblast → further contact of the embryo with the uterine wall by digesting uterine tissue; the uterus, in turn, sends blood vessels into this area, where they contact the syncytiotrophoblast. The yolk sac and the hypoblast are the source of the extraembryonic mesoderm, which extends outward from the gastrulating embryo, joins the trophoblastic extensions and gives rise to the blood vessels nourishing the embryo



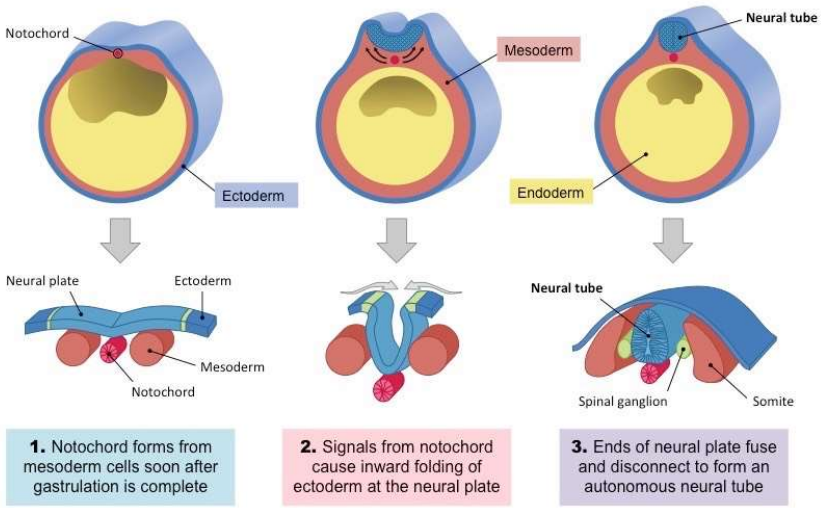
- the connecting stalk of extraembryonic mesoderm links the embryo to the trophoblast → umbilical cord
- the trophoblast + the mesoderm with blood vessels = the chorion - fuses with the uterine wall → the placenta
- the placenta = a maternal component (the endometrium, modified during pregnancy) + a fetal component (the chorion).
- the chorion may be very closely apposed to maternal tissues while still being readily separable from them (the contact placenta of the pig), or it may be very intimately integrated with maternal tissues that the two cannot be separated without damage to both the mother and the developing fetus (the deciduous placenta of most mammals, including humans).



Day 13 (*Homo*):
1 sec. yolk sac
2. resudual primary yolk sac
3. amnion cavity
4. extraembryonal coelom
5. epiblast
6. connecting stalk
7. hypoblast
8. primary villi
9. trophoblastic lacunae
10+11. extraembryonal mesoderm

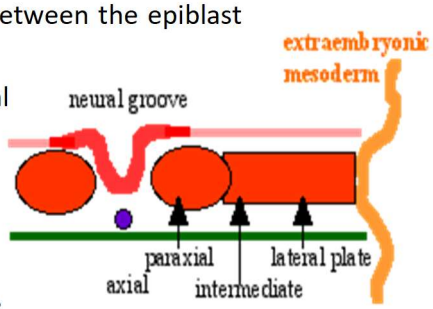
NEURALUTION

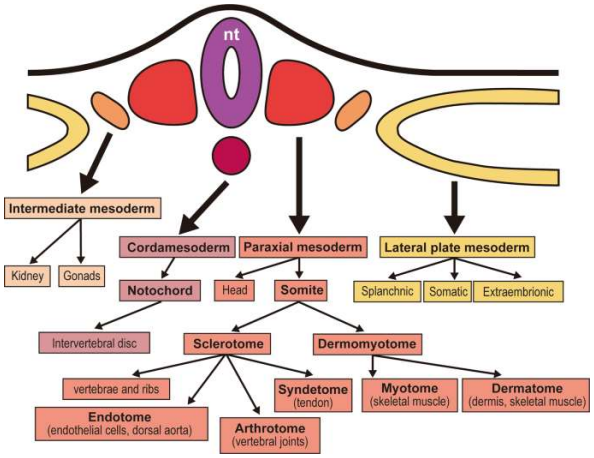
- Hans Spemann: The Nobel Prize in Physiology or Medicine 1935, for the discovery of the organizer effect in embryonic development
- the formation of a neural tube in chordates
- notochord = flexible rod that stimulates neurulation cells in the ectoderm → a neural plate
- the neural plate → a groove + neural crest
- the infolded groove closes off and separates from the neural crest to form the neural tube
- the neural tube will elongate as the embryo develops → brain and spinal cord
- the cells of the neural crest → the components of the peripheral nervous system

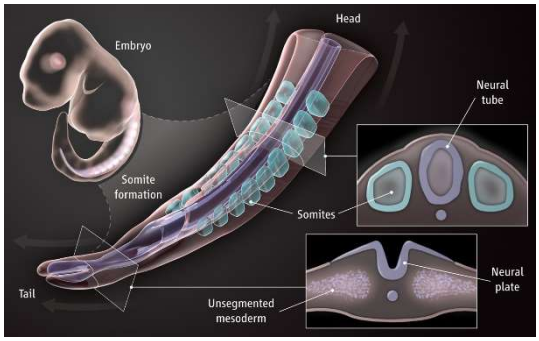


EMBRYONIC MESODERM

- the migrating cells from the epiblast populate the space between the epiblast and the definitive endoderm → the mesoderm layer
- the intraembryonic mesoderm cells → paraxial and intermediate m esoderm, lateral plate mesoderm and a population that forms a midline tube → the notochord
- the mesoderm → the blood, endothelium, heart, kidney, reproductive system, bones, skeletal, smooth muscle and connective tissues, tendons, ligaments, dermis and cartilage

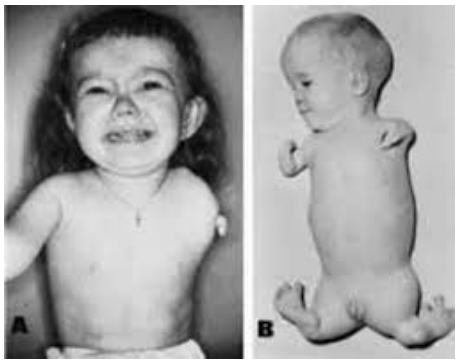






TERATOLOGY

- teratogens = mutagens causing developmental defects , drugs, addictive substances, infections, radiation, mycotoxins (fungal products)…
- biological teratogens = infections: CMV, Toxoplasma, Listeria, Parvovirus,…
- chemical teratogens: cytostatics (cyclophosphamide, colchicine), thalidomide (sedative, nausea relief), synt. retinoids, progestins, sedatives, barbiturates, salicylates (aspirin), antibiotics, alcohol, cocaine - damages the GIT and CNS, smoking, radiation
- within the first 15 days of embryonic development: double monsters, other malformations (anomalies, defects, monsters) arise later, when organ systems form
- teratogens have the strongest influence in the so-called critical period (time of organ formation and differentiation, in humans 4-8 weeks of embryonic development), in this period a large number of congenital malformations may occur
- 1956 - thalidomide (Contergan) - medicine for morning sickness of pregnant women, sedative - a single dose of 50-100 mg was sufficient in the critical period (21st-36th day after fertilization) → congenital malformations (missing limbs), affected approx. 15,000 children (in humans the teratogenic dose is only 0.1 mg / kg; in most animals 20-300 mg / kg!)



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