GAMETOGENESIS

OBJECTIVES

• Student will understands the processes of gametogenesis in different animals; he will be able to distinguish between oogenesis and spermatogenesis, and is able to describe or compare these processes
• Student will be able to identify, sketch and describe the basic structures and organs of the male and female reproductive systems in selected animals
• Student will differentiate between types of gametes and their functions
• Based on acquired knowledge and skills, the student will complete the laboratory report

FUNDAMENTAL LAW OF NATURE *"Survive and reproduce..."*

GAMETOGENESIS

• Process of producing highly specialized reproductive cells (gametes)
• In lower organisms, gametes are formed from tissue undifferentiated cells that transform into gametes due to external conditions (e. g. unfavorable conditions)
• In most animals, primordial germ cells are established at the beginning of gametogenesis → gametes
• Meiosis, spermatogenesis, oogenesis

OOGENESIS

• Process of developing highly specialized reproductive cells - eggs (*ovum* in Latin)
• It involves several stages, including mitosis, meiosis, and cell differentiation
• Oogonia → 1 egg and 2-3 polar bodies
• Oocytes are often arrested in prophase of the first meiotic division (diplotene phase) until the female reaches sexual maturity

Eggs

• Spherical cells with asymmetrical internal structure containing genetic information and materials for nourishing the embryo (at least in early stages of embryonic development)
• Upon polar body formation, the animal pole (containing the nucleus) is separated from the opposite vegetal pole, with an axis between = animal-vegetal axis
• Eggs have stabilizing shells specific to different species: chorion in insects (a rigid chitinous covering); the jelly envelope in amphibians; leathery shell in reptiles; vitelline membrane (encloses the yolk), albumen, inner and outer shell membranes, and calcareous shell in birds; zona pellucida in mammals
• Eggs of different animal species vary in the amount and distribution of yolk (nutritive material) in the cytoplasm, which significantly influences cleavage and embryonic development

Types of eggs based on the amount of yolk

• Alecithal: without yolk

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• Oligolecithal: with a small amount of yolk that does not hinder cleavage; cleavage is total (holoblastic) with blastomeres completely separating from each other; equal cleavage (blastomeres are of equal size); in mammalian (including human) and echinoderm eggs
• Mesolecithal: larger amount of yolk that does not hinder complete cleavage; cleavage is total but unequal (the first two cleavages divide the egg into four equal blastomeres, the third cleavage divides the embryo into smaller micromeres and larger macromeres richer in yolk); in amphibias
• Polylecithal: large amount of yolk that strongly influences the cleavage; cleavage is referred to as meroblastic (partial), with the yolk mass remaining uncleaved; yolk is concentrated at the vegetal pole (heavier); in fish, reptiles, and birds; yolk accumulates in the center of the insect egg

Types of eggs based on yolk distribution
• Isolecithal: yolk evenly distributed in the cytoplasm; cleavage is total and equal; typical for oligolecithal eggs of mammals (including humans) and some invertebrates
• Telolecithal: yolk is concentrated at the vegetal (heavier) pole of the egg; cleavage is total but unequal in mesolecithal eggs (amphibians) and discoidal in polylecithal eggs (cleavage occurs at the animal pole; in fish, reptiles, and birds)
• Centrolecithal: centrally located yolk surrounded by a layer of yolk-free cytoplasm; superficial cleavage occurs, with blastomeres separating only at the surface; the yolk mass in the center of the egg remains uncleaved; in insects

Mammalian ovary and oogenesis (human, mouse, etc.)
• *Medulla* (blood vessels, nerves, loose fibrous connective tissue) + *cortex* (ovarian follicles, *corpus luteum, corpus albicans*, fibroblasts)
• Oocytes are oligolecithal and isolecithal

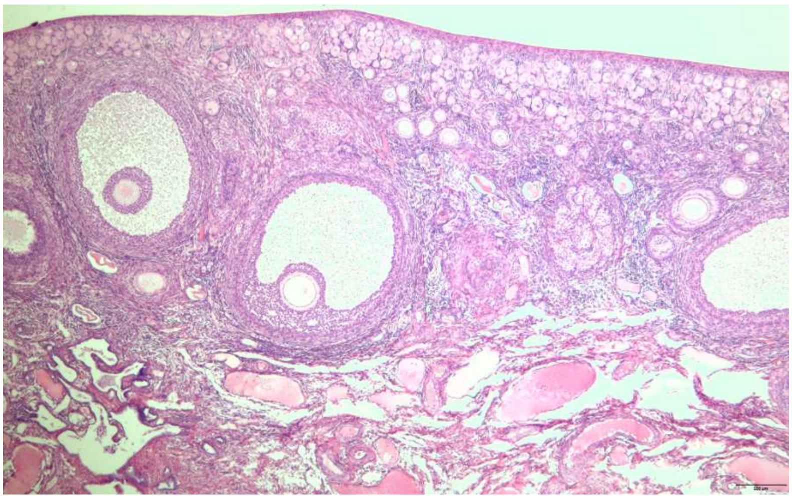

Fig. 1. Cross section of a mammalian ovary (*cortex* and *medulla*) with different stages of follicle development (Nejezchlebová, 2023).

a) Primordial ovarian follicles: the most numerous during the prenatal period, located in the peripheral cortex, beneath *tunica albuginea*. The primary oocyte is surrounded by a single layer of flat follicular cells connected by desmosomes. The follicular cells rest on the basement membrane, and the

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adjacent stroma is rich in capillaries. In humans, the diameter of the oocyte is 25 µm, and the diameter of the follicle is 40 µm

b) Growing follicles: They include single-layered primary follicles and multilayered/vesicular follicles

- **Single-layered primary follicle**: growth of the follicular cells (which are cuboidal) and the oocyte. The nucleus also enlarges → the germinal vesicle (described by J. E. Purkinje). This stage of oocyte development is known as the GV (germinal vesicle) stage. In humans, the diameter of the follicle is cca 100 µm

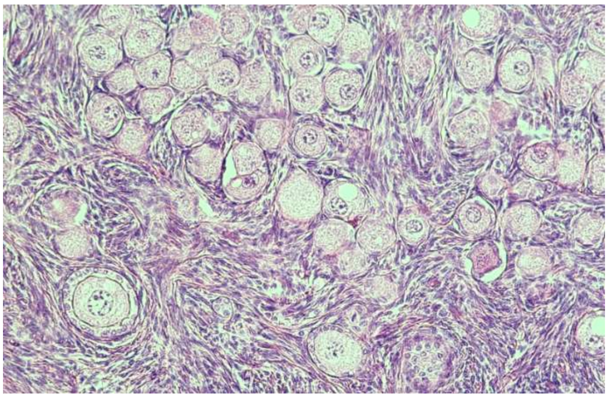

Fig. 2. Detail of the ovarian cortex: numerous primordial follicles in the upper part of the picture and primary follicles in the lower part. Compare their sizes (Nejezchlebová, 2023).

- **Multilayered/vesicular follicle**: proliferation of layers of follicular cells, forming gap junctions and the *zona pellucida* (ZP, produced by both the oocyte and follicular cells), follicular fluid (transudate of plasma and follicular cell secretions) accumulates in cavities between the follicular cells
- **Antral/mature Graafian follicle**: a "vesicle" protruding above the surface of the ovary, with a cavity called *antrum folliculi* containing follicular fluid rich in hyaluronan and proteoglycans. The surrounding ovarian tissue forms *theca folliculi*, which consists of *theca folliculi interna* (containing blood vessels and multiple layers of epithelioid cells producing androgens) and *theca folliculi externa* (dense connective tissue with concentrically arranged myofibroblasts). The granulosa cells cooperate with *theca interna* in the conversion of androgens to estrogens. The oocyte is surrounded by *cumulus oophorus*, which extends into the cavity. The cells in the first layer around the oocyte, in contact with the ZP, are called *corona radiata* (which persists during transport in the oviduct). In humans, the diameter of the follicle is approximately 2.5 cm

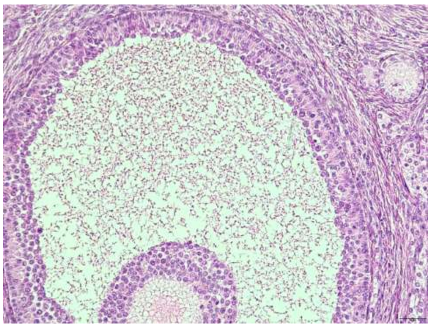

Fig. 3. An antral follicle surrounded by thecal layers compared to a primary follicle (top right) (Nejezchlebová, 2023).

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d) Follicular atresia: follicular cells and oocytes undergo apoptosis and are phagocytosed. The process begins even before birth and can occur at any stage of follicle development. The granulosa cell stop their mitotic activity, detachment of these cells from the basement membrane occurs. Atresia is particularly intense after birth (influenced by maternal hormones), during puberty, and pregnancy
e) Ovulation: The rupture of the follicle and release of the oocyte due to LH (luteinizing hormone) influence. The oocyte is captured by the fimbriae of the fallopian tube. A sign indicating ovulation is the stigma, a site on the surface of the follicle with low vascularity
f) Post-ovulation stage: The granulosa cells and *theca interna* form *corpus luteum* (a temporary endocrine gland) → progesterone (prevents further ovulation) and estrogens. The granulosa cells of the corpus luteum transform into granulosa-lutein cells, which produce steroids, while *theca interna* transforms into theca-lutein cells
g) If pregnancy occurs, *corpus luteum* is stimulated by hCG (human chorionic gonadotropin, ← blastocyst) → *c. luteum graviditatis* → progesterone and relaxin (softens *symphysis pubis*)
h) If pregnancy does not occur, corpus luteum undergoes regression → *c. luteum menstruationis*
i) Both types of *corpus luteum* degenerate through autolysis and are phagocytosed by macrophages. A fibrous scar called *corpus albicans* is formed

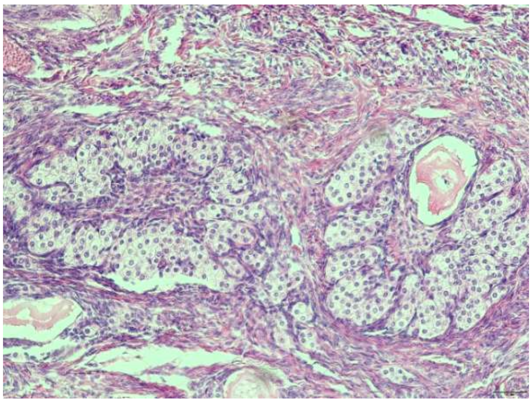

Fig. 4. Postovulatory follicular development associated with the formation of *corpus luteum* (Nejezchlebová, 2023).

Oogenesis in birds (*Gallus gallus domesticus*)
• Avian ovary: usually developed only on the left side
• Eggs are polylecithal and telolecithal, containing a large amount of yolk and albumen. The albumen surrounds the vitelline membrane externally and is surrounded by papery membranes and the shell. Partial (incomplete, discoidal) cleavage occurs, with cleavage only occurring in the cytoplasm with the nucleus at the animal pole. The yolk mass does not undergo cleavage

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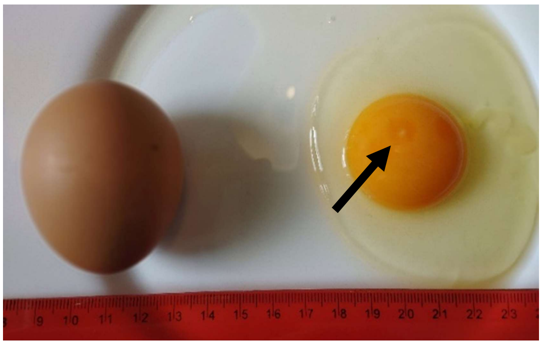

Fig. 5. Example of cells visible by a naked eye - macroscopic eggs of the domestic chicken (*Gallus gallus domesticus*). The arrow indicates a visible blastodisc (cytoplasm with a nucleus) at the animal pole, which lies just beneath the vitelline membrane (enclosing the yolk) (Nejezchlebová, 2023).

Oogenesis in amphibians (*Xenopus laevis*)

• Large oocytes (>1 mm in diameter): they have a large nucleus (100,000 times larger than the nucleus in somatic cells) and account for approximately one-third of the oocyte volume

• Oocytes undergo synchronous cell cycles, allowing for the easy collection of a large number of fertilized eggs

• The eggs have a vitelline membrane and a jelly coat (tertiary coat produced by accessory glands of the female reproductive system)

DID YOU KNOW THAT...

... the claws on the hind limbs gave to name to this frog - African clawed frogs (Xenopus laevis)?

Fig. 6: Hind limbs of the African clawed frog with claws (Nejezchlebová, 2023).

Oogenesis in fish (*Danio rerio*)

• Polylecithal and telolecithal eggs

• Partial (incomplete) cleavage occurs, with cleavage only occurring in the cytoplasm with the nucleus at the animal pole. The yolk accumulated at the vegetative pole does not undergo cleavage, resulting in discoidal cleavage

Oogenesis in insects (*Drosophila melanogaster*)

• Centrolecithal eggs, fertilization occurs only in the region of the oocyte, which becomes the anterior part of the embryo

• Outer shell - chorion (secondary shell formed by follicular cells)

SPERMATOGENESIS

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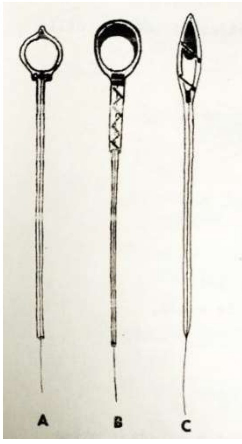
• Process of developing highly specialized reproductive cells (gametes) - sperm
• Spermatogonia → 4 spermatozoa (equivalent cells)
• It involves several phases, including mitosis, meiosis, and cell differentiation
• Includes spermatocytogenesis (from spermatogonia to spermatids) + spermiohistogenesis (spermiogenesis, the transformation where spermatids mature into spermatozoa).
• Ejaculate = semen = seminal fluid = secretory product of the male reproductive system

Spermatozoa

• Flagellated: most common type
• Without flagellum: e.g., found in mites, spermatozoa of crustaceans with adhesive structures for attachment to the egg surface



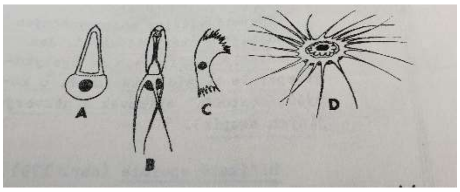


Fig. 7 (on the left). Flagellated spermatozoa. A - echinoderms, some mollusks; B - mammals; C - insects, some mollusks (Knoz, 1979).
Fig. 8 (on the top right). Non-flagellated spermatozoa. A - spermatozoa of *Ascaris* sp.; B, C, D - spermatozoa of different crustacean species (Knoz, 1979).
Fig. 9 (on the bottom right). Human spermatozoa (smear preparation), 1000 x (Nejezchlebová, 2023).

Mammalian testes and spermatogenesis

• Paired reproductive organs with secretory function (→ sperm) and endocrine function (→ hormones, such as androgens)
• Testicular parenchyma consists of tubular glands
• Serous membrane: *tunica vaginalis propria - epiorchium*
• Tough connective tissue capsule: *tunica albuginea*
• Coiled seminiferous tubules: *tubuli/canaliculi seminiferi contorti*
• Terminal portions of the seminiferous tubules (*tubuli recti*) open into the *rete testis* region (in the mediastinum)
• From the mediastinum (a fibrous band where the testis meets the epididymis, formed by *tunica albuginea*), connective tissue septa called *septula testis* divide the testis into lobules, each containing 2-3 seminiferous tubules
• Efferent ducts: *tubuli/canaliculi efferentes*, their number varies among species and individuals

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• Seminiferous tubules: the tubular wall consists of a basement membrane, myoid cells, and lymphatic endothelium, forming *lamina propria*. The germinal epithelium of Sertoli cells and developing stages of germ cells attach to this lamina

• Interstitial tissue (located between the tubules): collagen, abundant blood vessels and nerves, fibrocytes, Leydig cells that produce testosterone

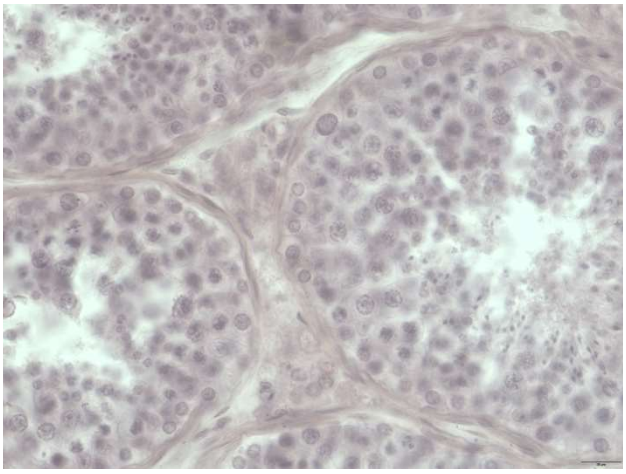

Fig. 10. Cross-section of the human (mammalian) testis showing seminiferous tubules with germinal epithelium and interstitial tissue in the space between the tubules (Nejezchlebová, 2023).

SOME FUNNY FACTS ☺
Did you know, that the first testitular guard in sport was used in 1874, however the first helmet protecting head in 1974. It took a century to realize that brain is important too. ☺ ☺ ☺

Seminiferous tubules:

- **Sertoli cells:** a bright ovoid nucleus with a large nucleolus and a wavy nuclear membrane (not always visible under microscope). They are supportive cells with various functions
- **Spermatogonia:** small cells with pale nuclei, located near the basement membrane
- **Primary spermatocytes**: larger cells located closer to the lumen, with 46 chromosomes and 4N DNA. Most cells in the histological section are in this phase since the preleptotene to the end of the pachytene phase takes about 22 days (in humans). They are the largest cells in the spermatogenic lineage, and their nuclei contain chromosomes in various stages of spiralization, appearing as dark stripes of heterochromatin
- **Secondary spermatocytes:** smaller cells with 23 chromosomes and 2N DNA. These cells exist only shortly and are rarely seen in histological sections
- **Spermatids:** small cells with 23 chromosomes and 1N DNA. They are located close to the lumen and have condensed chromatin in the nucleus
- **Spermatozoa:** head, neck and tail

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TIP…
… let's compare the parameters of domestic pig and human spermatozoa:

	Sus (µm)	*Homo **(µm)***
head		
length	8,7	4,0-5,0
width	4,6	2,5-3,5
flagellum		
midpiece	10	5,0-6,5
principal piece	30	38,5-40
total length	48,2	47,5-51,5

Spermatogenesis in birds (*Gallus gallus domesticus*)

- Sperm with flagellum, morphology: large inter-species variability
- Internal fertilization

Spermatogenesis in amphibians (*Xenopus laevis*)

- Spermatozoa with flagellum, 30 µm long, external fertilization

Fig. 11. Sperm cell, African clawed frog (*Xenopus laevis*) (Nejezchlebová, 2023).

Spermatogenesis in fish (*Danio rerio*)

- Sperm with flagellum, external fertilization

Spermatogenesis in insects (*Drosophila melanogaster*)

- Round spematozoa, 12 µm in diameter → 1.8 mm long!

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Figure sources

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