Ministry of health Republic of Belarus
Establishment of education "Gomel state medical university"

Department of histology, cytology and embryology

MANUAL
for 1-st year students of faculty of foreign students on gynecology

Topic: 14:
HUMAN EMBRYONIC DEVELOPMENT (PROGENESIS, FERTILIZATION, CLEAVAGE, IMPLANTATION, GASTRULATION, HISTOGENESIS, ORGAN-OGENESIS)

Duration 4 hours

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THE MOTIVATIONAL CHARACTERISTIC OF THE THEME

Studying human embryonic developments allows finding out the general features in embryogenesis of the person and at the same time to establish features of development of a germ of the person. The knowledge of processes of fertilization, cleavage, implantation, gastrulation, and also features of development of a placenta, and extra embryonic organs and its medical value. It enables to comprehend and estimate all cycle of the biological phenomena accompanying current of pregnancy and development of a fetus. Rational supervision of pregnant women, conducting sorts, realization of many medical and preventive actions in obstetrics and is impossible for gynecology without a profound knowledge of embryology.

THE PURPOSE

Studying and mastering of essence and features of the consecutive, causally-caused processes embryogenesis the person.

PROBLEMS

The student should know:

1. Features of development and a structure of ovum in the person.
2. Morphology of process of fertilization, cleavage, early and late stages of gastrulation in the person.
3. Features of a differentiation germinal ectoderm, endoderm, mesoderm in the person.
4. To know about the critical periods of development of the person.

The student should be able:

1. To characterize the general and individual features of male's and female sexual cells of the person. To define sexual cells and their ultra microstructures on micro preparations and electronic micro photos.
2. To distinguish germs at early stages of fertilization, cleavage and formation of germinal leaves
3. To define axial bodies – a nervous tube, a chord.
4. To define developing internal organs and systems of a germ of the person, processes of a differentiation of it.

REQUIREMENTS TO THE INITIAL LEVEL OF KNOWLEDGE

For full mastering a theme it is necessary for student to repeat stages of embryonic developments of the person and questions from medical biology and the genetics, connected with process of gastrulation.

CONTROL QUESTIONS FROM RELATED SUBJECTS

1) The Structure and development of sexual cells. Essence meiosis as process of formation of sexual cells and its stages.
2. Types of ovum’s
3. Fertilization, its essence. A zygote
4. Cleavage and its types.
5. Stages of embryonic developments, their interrelation.

CONTROL QUESTIONS ON THE THEME

1. The basic stages of embryonic developments.
2. Process of fertilization. A zygote.
3. Cleavage of a zygote.
4. Concept about gastrulation and germinal leaves.
5. The first phase gastrulation (7-14dayes).
6. The second phase gastrulation (14-21 day).
7. Formation of axial complex
8. Ectoderm and its differentiation and derivatives:
9. Entoderma and its differentiation and derivatives
10. Mesoderm and its differentiation and derivatives
11. Mesenchyme and its differentiation and derivatives.

THE PRACTICAL PART

1 Scheme of a structure of an early stage embryogenesis in the person-enter designations (Exercise №1 in album).

2. The scheme of a cross-section cut of a primary strip-enter designations (Exercise № 2 in album).

3. The scheme of a structure of a germ at a stage neurulation -enter designations (Exercise № 3 in album).

4. The scheme of a structure of cross-section cuts of a germ-enter designations (Exercise № 4 in album).

5 Scheme of a structure of a germ of the person (1 month of development)-enter designations (Exercise № 5 in album).

QUESTIONS FOR SELF-CHECKING KNOWLEDGE

1. The basic human embryogenesis stages
2. Fertilization. Biological essence. Zygote.
3. Cleavage. Blastula.
4. Gastrulation and formation of embryo layers
5. First stage of gastrulation (7-14 days)
6. Second stage of gastrulation (15-21 days)
7. Axial complex
8. Ectoderm
9. Mesoderm
10. Endoderm
11. Mesenchyme
12. Organogenesis

HUMAN EMBRYONIC DEVELOPMENT (PROGENESIS, FERTILIZATION, CLEAVAGE, IMPLANTATION, GASTRULATION, HISTOGENESIS, ORGANOGENESIS)

Embryology is the science about embryonic development of the individual. Medical embryology studies human embryonic development, causes of its failures, influence of exogenic factors on embryogenesis and mechanisms of its regulation.

Embryogenesis as part of ontogenesis, includes the time between fertilization and birth. The process of embryo development involves many processes, namely cell *division, migration, growth, induction and differentiation,* which more or less depend upon each other.

Human embryogenesis is divided into three periods: initial period (first week), embryonic period (2^{nd}-8^{th} week), and fetal period (9^{th} week until birth) [1].

Basic Human Embryogenesis stages are similar to another vertebrates.

Early:

1. Fertilization, which results in zygote formation
2. Cleavage, which results in blastula formation.
3. Gastrulation, which results in formation of the germ layers.
4. Notogenesis, which results in formation axial buds complex

Late:

5 Histogenesis and organogenesis. During this stage the tissues, organs and organ systems are formed.

Many embryologists refer the formation of reproductive cells – progenesis -- to embryogenesis [1, 2].

Gametogenesis is a process of sex cell formation. There are spermatogenesis (spermatozoa formation) and ovogenesis (ovicell formation). The development of sex cells in embryogenesis happens early in development. They appear at the end of *3′* week of embryogenesis in extraembryonic yolk entoderm and later migrate to the gonads. These cell are called gonoblasts.

Spermatogenesis

There are 4 phases of the spermatogenesis: mitotic phase, growth period, meiotic phase and spermiogenesis. Spermatogonia are the first cell of the spermatogenesis. They arise from gonoblast. During 1 phase diploid spermatogonia divide some times and are transformed into spermatocyte I. Then spermatocyte I enlarge their size up to 4 times (growth period).

Spermatocytes I enter meiotic phase, which consist of two following divisions (meiosis I and meiosis II),

Meiosis J is called reduction division, because it leads to chromosome number reduction and formation of haploid chromosome set. Spermatocytes II form.

Meiosis II is also called equation division. It is resemble ordinary mitosis. It results in spermatids formation. Spermatids, as spermatocytes II, have haploid chromosome set, each chromosome is presented by one chromatid.

Spermiogenesis is longest phase. It results in spermatozoa formation from spermatids. It lasts for about 50 days. The process begins from formation of acrosome, which contain enzymes to dissolve coats of the ovum. The centrosome moves on the opposite pole. The proximal centriole lies close to the nucleus, whereas a distal centriole divides on two parts. From one of them the flagella is formed. The second plays a role of basal body. The sperm cytoplasm is subject to reduction. The nucleus elongates and become more compact [2].

Ovogenesis

In general it similar spermatogenesis, but it has its particular features. Mitotic phase lasts

during early ontogenesis in the ovarium. At the end of embryonic development the number of oogonia is around 7 millions. After birth the division is terminated and all oogonia are transformed to oocytes I. These oocytes I are blocked on a stage of diplotene of first meiotic division.

Then oocytes I enter the long growth period. It is subdivided into period of small growth (from birth to sexual maturation) and period of large or rapid growth (it happens regulary during each month). Thus the growth period may last 12-50 years. The meiotic phase starts just before ovulation. The first meiotic division results in formation of oocyte II and reduction body. In it turn reduction body may split on two. Oocyte II is blocked in metaphase of meiosis II. Further maturation is induced by fertilization. Oocyte II splits on ovicell and reduction body. As result of maturation we have three reduction body and one ovicell. Ovicell loses its centrioles. The reduction bodies are phagocyted by other cells [2].

Spermatozoon (sperm).

The mature human sperm is a highly specialized cell. It consists of a head, a neck, a middle piece and a tail. This is a very small cell about 60 micrometers long. There is a big dense nucleus. The nucleus coat hasn't pores. The genetic material is haploid and contains 22 autosomes and 1 sex chromosome (X or Y). The cytoplasm is reduced.

The head is capped by the acrosomal cap. Acrosome is a derivate of coplex Golgi and has similar structure as lysosome.

The neck contains a proximal centriole. The acsonemm passes through middle piece and the tail. It consist of two central microtubules and nine outer doublet microtubules. The middle piece also contains mitochondrial sheath.

With help of the tail movements the spermatozoa are able to move with the speed 1-5 mm per second [1 – 3].

Ovum (egg, oocyte !!) is a big round haploid cell about 130 micrometers in diameter. It is surrounded by noncellular covering zona pellucida and by layer of follicular cells (corona radiate).

The cell has all organells but only one centriole. The cell gets another centriole from the sperm during fertilization.

There are many inclusions (pigment and yolk or lecithin). Type of the human ovum with the small amount of yolk inclusions distributed throughout the cytoplasm is called secondary *isolecithal* and *oligolecithal* type of an ovum.

Complex of ovicell membrane and cytoplasm layer just under it is called *cortical layer*. This layer contains granules with several enzymes, which can change properties of zona pellucida after fertilization .The ovicells have very good developed cytoskeleton [3].

Fertilization

The development of organism begins from one cell, called **zygote.** The zygote appears as a result of the fusion of the mature gametes. This process is *called fertilization.*

The fertilization happens in the uterine tube, where the ovum enters from the ovary, and consists of two phases – distant phase and contact phase.

In time of *distant phase* sperms and ovum produce physiological active substances, which are necessary for distant interaction, for stimulation of the sperms moving and gametes meeting.

Note the following: the sperms get the ability to fertilize the ovum only after they

have been in the female genital tract. This final step in their maturation is called *capacitation,*

The second phase of fertilization is *contact phase* when the sperm and the ovum fuse. To perform fertilization process it is nessesary to have at least 200 millions spermatozoa. If there are less than this amount ferilization can not be performed because of lack of proteolytic enzyme activity.

A few hundreds of the sperms from millions reach the ovum, but only one, which has maximum activity, goes across corona radiate, surrounding the ovum. Then sperm's acrosomal cap is shed and the enzymes are released from the acrosome. This process is called the *acrosomal reaction.* The enzymes make a local lysis in the follicular cells layer and zona pellucida, after that the sperm's head penetrates the ovum membrane and sinks into the cytoplasm. Only the head with nucleus and proximal centriole sink. The middle piece and the tail don't penetrate into ovum [2].

The membrane of the ovum overlying the region of the sperm head fuses. After that another sperms cannot penetrate the ovum. There are several mechanism of polyspermia blocking.

l.The cortical reaction in the ovicell that lead to liberation of cortical layer enzymes, which change zona pellucida making it unpermeable to spermatozoa and transform to *cover of fertilization.*

2. The enzymes also degrade ZP2 and ZP3 receptors and block acrosome reaction of spermatozoa.

3. Also after fertilization the charge of cytoplasm of ovicell is changed to negative. And negative charged spermatozoa are repulsed from it.

The condition of the fertilized ovum, when it has two pronuclei (nucleus of a sperm and nucleus of an ovum) is called *syncaryon.* Pronuclei draw together and fuse.

So, in summary, fertilization properly consists of the entry of the sperm's head into the ovum. This is followed by the fusion of the male and female pronuclei to restore the diploid number of chromosomes.

The process of fertilization leads to formation of the *zygote* and ends with the initiation of its cleavage [3].

Cleavage

Cleavage is the quickly mitotic segmentation of the zygote, resulting to formation of multicellular unilayer embryo.

The cell are divided very quickly because G_1 period absence and do not grow. So the total size of embryo is the same during cleavage (C*overor* membrane *of fertilization prevents it also)*

, but the cells become smaller and smaller. It change relationship between the volume of nuclear and cytoplasmic material. The correlation between the nucleus and cytoplasm of an ovum is one to ten, while each somatic cell has correlation one to three.

The type of cleavage depends upon amount of the yolk. That's why the human cleavage is complete (all zigote material is subject to division) , unequal (cells have different size) and asynchronic (cells divide at the different time).

The cells which appear during the cleavage are called *blastomeres.*

Embryo passes down the uterine tube. The stage of 12- 16 cells is called a *morula which* is similar to a mulberry. Soon morula appears in the uterus. Then the cavity forms between blastomers [1].

At 7-th day the morula is transformed into *blastula.* The human blastula is termed a *blastocyst.*

Blastocyst consist of:

1) outer cells – *trophoblast,* which further differentiates to chorion and placenta, so provides the nutrition to the embryo;

2) aggregation of the bigger, ducker blastomers at one pole – *embryoblast,* which serves for embryo body formation and the last extra embryonic organs;

30 *blastocoele*- cavity of the blastocyst [3].

Eventually the membrane of fertilization disappears and the blastocyst becomes attached to the uterine wall. The trophoblast begins produce the enzymes to lysis of inner uterine membrane. In the result the embryo sinks into the substance of the uterine wall. This process is called the implantation and happened on the 6-7 days of embryogenesis.

Gastrulation

The gastrulation is a next stage of embryo development. It is the process by which three germinal layers are formed – ectoderm, endoderm and mesoderm and a complex of axial organs.

It has two phases. The first phase – early gastrulation – occurs at 7-14 th days at the same time when implantation begins.

At 7 day the process of delamination begins, which splits embryoblast into two layers: primary ectoderm (epibiast) and primary entoderm (hypoblast). The proliferation and cells moving lead to the formation of two sacs – upper – amniotic sac and lower – yolk sac.

The cells of the floor of the amniotic sac become tall columnar and form the primary *ectoderm.*

The roof of the yolk sac is *entoderm.*

This process results in the establishment of an *embryonic disc,* which is composed of the two layers of ectodermal and the endodermal cells. Then the extraembryonic mesoderm appears in the way of migration from the embryonic disk. It surrounds both sacs. After that upper bubble will be called *amnion* and its wall consists of two layers – ectoderm and extraembryonic mesoderm and the lower bilayered bubble will be called *yolk sac.* The embryo is suspended from the trophoblast by the connecting stalk, which is formed of the extraembryonic mesoderm.

Further the extraembryonic mesoderm underlyes the trophoblast. After that the last is called *chorion.*

So, at early gastrulation extraembryonic organs and bilayer embryo form.

The second phase of gastrulation begins at 14 – 15th day of development. It is performed by cell migration and partial invagination. The cell of epiblast intensivly reproduce itself. They move to medial axis of the disk and form the *primitive streak.* Then these cells migarate through the primary strip. Third layer – embryonic mesoderm – appears between two layers.

There is the small node – *primitive knot* in front of the primitive streak A cord of cells from this primitive knot sinks inside and grows forward in the axis of the embryonic disc between the ectoderm and the endoderm. This is the *notochordal process.*

Some cells of primary knot migrate to entodermal layer and form prehordal plate. As result of gastrulation a 3 layers embryo is created [4].

So, the types of human gastrulation are *delamination* (splitting) and *migration.*

Stages of Human embryo gastrulation:

Early (7-14 days)

1. Formation of ectoderm and endoderm
2. Formation of extraembryonic organs – amnion, yolk sac and chorion

Late (15-17 days)

1. Formation of embryonic mesoderm
2. Formation of axial organs complex

At 3[d week of embryogenesis the differentiation of germ layers is started.

Differentiation of ECTODERM.

P*rimary ectoderm includes* skin ectoderm, neuroectoderm and prechordal plate.

At the end of 3rd week, central region of ectoderm form the *nervous plate.* Invagination of the neural plate leads to formation of the neural groove and then- *neural tube.*

The process of nervous tube formation is called neurulation.

The neural crests appears as groups of cells, lying along the dorsolateral sides of the neural tube.

Nervous tube is a source for formation of a brain, spinal cord, pituitary gland, motor spinal nerves, cranial nerves, retina. The derivates of the neural crest are: spinal, cranial and autonomic ganglions, and adrenal medulla….

As it was pointed above, some ectoderm cell travel to primary entoderm cells and settle among them. These is prechordal plate. Respiratory epithelium, lining of the esophagus and thymus stroma arise from it.

After that the primary ectoderm become secondary entoderm or skin ectoderm. It is a source for formation of striated eithelia: skin epidermis and its derivates (hairs, nails, glands), oral cavity epithelium, anus epithelium, vagina epithelium, teeth enamel, adenohypophysis, cornea, lens, olfactory epithelium and so on.

Differentiation of MESODERM. It begins from 20's day of embryogenesis. Originally it is loose irregular cell aggregation (presomit mesoderm). Then, it is divided into dorsal and ventral mesoderm. Growing dorsal mesoderm becomes segmented and forms 43 – 44 paired round *somites* along the notochord.

Each somit consist of three parts: external – dermatom, intermediate – myotom, internal – sclerotom. Derma of the skin arises from dermatom. Myotom gives rise for striated muscular tissue. Sclerotom is source of bone and cartilage tissues.

Between ventral and dorsal mesoderm there is intermediate mesoderm – nephrotom. It is subject to segmentation in anterior end of the body and it is not segmented in caudal end. It gives rise for kidney and reproductive organs.

Ventral mesoderm (splanchnotom) is not subject to segmentation. It is subdivided into two layers; visceral and parietal. They enclose secondary cavity of a body – coelom. The layers of splanchotom give rise for mesothelium, striated cardiac tissue, adrenal cortex, gonad epithelium.

Except the dense mesoderm there is a rather loose its portion, which lies everywhere between germ layers and axial organs and called *mesenchyme* or embryonic connective tissue. Small mesenchymal cells with their processes form meshwork and participate in the distribution of the nutrition to all embryonic structures [1] .

The cells of visceral splanchnotom layer form splanchnotom mesenhyme, which forms connective tissue and smooth muscular tissue of internal organs and vessels.

ENTODERM

The very important process is separation of embryo from extraembryonic organs. It is started on 20's day of development. The embryo body is lifted above extraembrionic organs with help *of body's folds.* So the embryo body acquires the tubular shape. This leads to formation of intestinal tube (primitive *gut),* which links with yolk sac by yolk stack. The communication becomes narrower and narrower and eventually disappears.

Primitive *gut* is a source for formation of gastric epithelium, intestinal epithelium, liver, gall bladder and pancreas.

NOT0GENESIS. It is formation of complex of the axial embryonic organs. The notogenesis includes three main processes: neurulation, differentiation of germinal layers and formation of body's folds. Axial complex includes following structures:

I. Skin ectoderm.
2. Nervous tube and neural crest.
3. Somites
4. Nephrotom.
5. Splanchnotom.
6. Notochord.
7. Intestine tube.
8. Mesenhyme [2].

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