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Morphogenesis of Bovine Ovaries in Prenatal Ontogenesis in Norm and in Pathology of Metabolism in Cows-Mothers

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Abstract: Although, many aspects of embryonic ovarian development have been studied, relatively little is known about the effect of gestation on fetal ovary formation. The objective of this investigation was to study the functional morphology of ovaries in prenatal ontogenesis in norm and in pathology of metabolism in cows-mothers. According to our data, gonads in bull-calves and in cow calves are laid at the beginning of prenatal ontogenesis on the 34th day on the surface of mesonephros. By the 45th day in cow calves they differentiate into ovaries and contain all the basic structures of the organ. Oogenesis in the fetuses of cows is not synchronous. Oocytes can be detected in ovaries at different stages of the first meiotic division prophase and with different chromatin ultrastructure. At the end of the 6th month of prenatal development in the ovaries there appear single cavitary follicles which can be seen with the naked eye. In our opinion, the development and involution of these normal structures play an important role in ovarian morphogenesis. Disappearance of the cavity of the tertiary follicles of the ovaries of the bovine cattle fetuses proceeds in two ways, obliteration and luteinization. In chronic diseases of pregnant cows at the last stage of pregnancy, late fetuses develop hypoplasia of reproductive organs and in acute diseases an increase in the number and size of antral follicles in the ovaries of fetuses subjected to cystic changes (polycystosis of the fetal ovaries) is found.

Key words: Embryonic development, fetal ovary, ovarian morphogenesis, cow, pathology of metabolism, single

INTRODUCTION

The damage from infertility of cattle is very significant. In the United States and in a number of countries in Western Europe, the annual culling rate reaches 34% mainly due to prolonged infertility (Poliantsev, 2015). Replenishment of these losses can be done only be an intensive introduction of fresh cows into the breeder flock. The following factors constrain this process. Firstly, they have problems with fertilization much more often than full-aged cows. Secondly, in the agricultural enterprises of the Russian Federation, the onset of sexual and physiological maturity in cow calves is often delayed for a period of 6-12 months and with the late entry of heifers into productive life, the need for them grows to 50% of the number of the main flock which is a consequence not only of the meager feeding of growing animals but also of inborn infertility which is of a genetic nature or due to the diseases of pregnant cows (Skorikov *et al*., 2017; Poliantsev, 2015).

Among the external factors predisposing to the occurrence of diseases of pregnant cows, first, errors in feeding, including general starvation, chronic deficiency of certain nutrients, poor-quality feed containing poisonous substances, mycotoxins, etc. can be mentioned. Fetoplacental insufficiency developing at the same time manifests itself not only in abortions but also in impaired fetal development (hypotrophy, immunodeficiencies, etc.) and in the subsequent decrease in the ability of heifers to fertilize due to abnormalities of the genitals (Mikhalev *et al*., 2017; Skovorodin, 1997a, b).

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In connection with the foregoing, the objective of this investigation is to study the functional morphology of ovaries in prenatal ontogenesis in norm and in pathology of metabolism in cows-mothers.

The scientific novelty of this research is that for the first time a detailed study of the formation of bovine ovaries in prenatal ontogenesis in norm and in the pathology of pregnancy in cows-mothers was carried out using a complex of macroscopic, morphometric, histological, histochemical and electron microscopic methods. The distribution of anomalies and malformations of heifer's reproductive organs has been studied. For the first time, polycystosis of the ovaries of the bovine cattle fetuses is described as a form of ovarian glandular hyperplasia, the causes and morphological manifestations of fetalovarian hypoplasia associated with abnormalities in the mother-placenta-fetus system have been studied in detail. The presented long-term research makes it possible to clarify these issues and develop concrete practical measures for the prevention of congenital infertility.

It is known from the literature that the normal development of the ovaries during the embryonic period further determines the fertility and reproductive function of the sexually mature animal (Sarraj and Drummond, 2012). For example, anomalies in the prenatal development of cattle may be manifested by a decrease in the number of proper primordial follicles which leads to a decrease in proper embryos (Ireland *et al*., 2007).

Gonads initially possess a labile organization then ovaries or testes are formed (Gilbert, 2013; McGeady *et al*., 2017). Development of reproductive organs is a complex process that is realized during several stages of ontogeny. The elucidation of the mechanisms of organogenesis makes it possible to clearly determine the etiopathogenesis of abnormalities and deformities in the reproductive system and consequently, purposefully prevent these pathological processes.

The first stage of sexual differentiation is the moment of fertilization when the sex chromosome of the sperm determines the genetic sex of the zygote. This stage is the first critical phase of the divergent differentiation of sex when the change in the number of chromosomes may lead to deformities and violations of the reproductive function in the future.

The second stage is the appearance of morphological signs of the somatic sex. Under the influence of testosterone, the mesonephric ducts develop into the vas deferens, the prostate and the vesicular glands form (Kenngott and Sinowatz, 2008). Under the action of the Müller inhibitory factor, paramesonephric ducts degenerate (Monniaux *et al*., 2012). This factor probably inhibits the development of ovaries (Pankhurst *et al*., 2018).

Indifferent gonads contain migrated gonocytes as well as cells of the surface epithelium, connective tissue mesenchyme and mesonephros. The latter has an effect on the further evolution of somatic elements and sex cells in the gonad buds (Kenngott and Sinowatz, 2008).

The formation of gonads in cattle occurs at the end of the first month of development in embryos with a body length of 8-11 mm. The first gonocytes in the gonads of this species of animals are found in embryos 13 mm long that is by the age of 30 days (Kenngott *et al*., 2013). The differentiation toward the ovaries in embryos begins at the end of the embryonic period. It increases in size due to the growth of the epithelium and the mesenchymal base. By the 35th days, germinal epithelium a mesenchyme with the developing epithelial strands and gonocytes are found in the gonad (Kenngott *et al*., 2013). During this period a rete ovary develops from mesonephric cells immigrating to the gonads of the embryo (Kenngott and Sinowatz, 2008). In a 6 week embryo of a cow the gonads are already differentiated by sex. The entire organ is filled with a cortical substance from the surface epithelium, the stroma and to the mesonephros on which the ovary is fixed. By the beginning of the 7th week, the organ separates from the primordial kidney and the vascular pedicle of the ovary (mesovarium) is formed (Kurilo *et al*., 1987).

In a month and a half the sex glands are separated from the mesonephros but are connected with it by the orifice of the ovarian gland (Kurilo *et al*., 1987). Later, ovaries differ from testes by histological features.

Very early, long before the formation of follicles, the ovaries start producing steroid hormones. In this case in the bud of the ovaries, cellular strands are formed, consisting of somatic cells, oogonia. According to the histological structure, during this period the buds of gonads of females and males become very similar. The presence of Leydig cells in the bud of the testicle is the only histological criterion that makes it possible to distinguish the sex of the bud of the gonad (Allen *et al*., 2016).

There is a linear increase in the volume of the ovary during prenatal ontogenesis (Skovorodin, 1997a, b). The weight of the ovary in embryos increases faster than body weight. In rats, for example these processes occur after birth in parallel (Burkhart *et al*., 2010).

Researchers noted variability of the macroscopic structure of the ovaries, especially in the last month of gestation. Growth and development of follicles in embryos of cow calves begins very early. A significant portion of the follicles undergoes atresia not even achieving a secondary structure but some of them turn into tertiary follicles reaching 4 mm in diameter. The researcher did not observe ovulation (Fortune *et al*., 2010a, b).

The period of intensive growth of the ovaries is described in 6-8 months old bovine fetuses. The development of follicles is noted until full maturation and ovulation, without the formation of lutein structures.

During this period, the ovaries of the embryos resemble the ovaries of a mature animal. By the 3rd month of embryogenesis, the onset of mass growth of the follicles up to the formation of a cavity visible to the naked eye takes place (Ireland *et al*., 2007).

In 6 months old fetuses, follicles are visible on the surface of the ovaries. In some animals they may be filled with blood. However, it is indicated that in some 6 months old fetuses there are organs in which no developed follicles are found which indicates a great variability. In newborns, the ovaries are already well developed and reach a size of more than 18 mm which gives their surface a vesicular appearance. Follicles reach 5 mm in diameter but most of them are filled with connective tissue elements (obliteration). Probably, it can be considered as a sign of the onset of the endocrine function of the ovarian glands necessary for the formation of the whole organism of the female (Tanaka *et al*., 2001).

Some researchers noted the formation of macroscopically revealed cavitary follicles in the ovaries of 7-8 months old fetguses. By the 9th month, secondary and tertiary follicles underwent obliterative and cystic atresia. Therefore, the parenchyma of the gonadal cortex did not contain growing and ripening follicles (Yang and Fortune, 2008).

A significant number of follicles in late bovine fetuses were in a state of atresia. Only a part of them was luteinized. Luteal atretic bodies in fetuses are formed by hypertrophied cells of theca and granulosa. In their structure these atretic bodies resembled the yellow bodies of sexually mature animals (Fortune *et al*., 2013).

The presented article describes the study of the formation and development of bovine ovary in embryogenesis in cows-mothers in health and under pathology of metabolism.

MATERIALS AND METHODS

Feeding diets and conditions of keeping cows were studied on farms. The clinical condition of the animals was determined by standard methods and the state of metabolism was confirmed with the help of the biochemical blood test. Animals were selected and classified into the following groups (Table 1). The first group consists of cows without metabolic pathology and fetuses and newborn calves secured from them (n = 91). The second group-cows whose feeding was energetically inferior, rations were unbalanced for the main nutrients and they were kept without active exercise with signs of metabolic disorders (ketosis, disturbances in mineral metabolism, hypovitaminosis, confirmed by biochemical blood test, also fetuses and newborns calves (n = 71).

At the meat processing plant, after slaughter a thorough examination of the carcasses and organs of the cows and the fetuses secured from them was carried out.

Table 1:Characteristics of the material used

Age of fetuses	The first group	The second group
Early and middle stage of prenatal ontogenesis		
20-90 days	10	10
Late stage of prenatal ontogenesis (Months)		
3	10	5
4	10	5
5	10	5
6	10	10
7	10	7
8	10	7
9	10	7
10	10	7
Total fetuses	81	56
Newborn calves		
1-14 days after birth	10	15
Total animals	91	71

The age of the fetuses was determined by the date of insemination of the cow, specified by weight and length of the body from the base of the tail to the occipital bone (Crown-Rump Length (CRL)). Newborn calves under the age of 15 days were slaughtered for subsequent organometric, histological, histochemical and electron microscopic studies.

All the experiments were conducted in accordance with the legislation of the Russian Federation (Anonymous, 1977) and taking into account the European Convention for the Protection of Pets (Council of Europe-ETS No. 125-European Convention for the Protection of Pet Animals).

The material was fixed in Carnoy liquid, dehydrated in spirits of increasing concentration and poured into paraffin. On a rotary microtome, slices 5 km thick were obtained and after dewaxing they were stained with hematoxylin-eosin and according to the method of Pappenheim. Collagen fibers were detected according to the methods of van Gieson and Mallory. Deoxyribonucleoproteids and ribonucleoproteids were detected in sections according to the methods of Bruce and Einarson, according to the method of Felgen; glycogen and neutral glycoproteids were detected according to the method of McManus, acid glycosaminoglycans were determined by Stidman's method. After fixation in liquid nitrogen, sections were obtained in the cryostat and the activity of alkaline and acidic phosphatase according to the method of Burston was determined in them histoenzymologically, counting the number of granules in the standard cut area (Mulisch and Welsch, 2010).

For electron microscopy, the material was fixed in chilled 2.5% glutaraldehyde on phosphate buffer with pH 7.3. The material was fixed in a 1% solution of osmic acid on phosphate buffer. Pieces were poured into epon-araldite. Ultrathin sections were mounted, contrasted with lead citrate and examined in the electron microscope EMF-100I at magnification of 6000-22000.

In early embryos, serial sections were obtained from the dorsal-caudal part of the trunk. In older fetuses, linear parameters of ovary were determined. The volume of ovarian fetal glands was calculated by the formula proposed by us: Ovary volume = $(4/3\pi)*(0.5*L)*(0.5*W)*(0.5*T)$, where: L is length, W-Width, T-Thickness of the organ (Skovorodin, 1997a, b).

For the statistical analysis, student's criteria, Whit's T-criterion, Van der Waerden X-criterion were used depending on the distribution.

RESULTS AND DISCUSSION

It was established that the indifferent gonads of bull-calves and cow calves are laid at the beginning of the prenatal ontogenesis on the 20-34th day in the form of protrusions of the ventromedial surface of mesonephroses. Coelomic epithelium a mesenchymal base, sinusoidal capillaries and oogonia are distinguished in them. Gonads grow rapidly due to mitotic division of cells. Morphologically, the ovaries can be distinguished in 34-45 days old embryos. They contain the basic structures of the organ: mesovarium, cortical substance with a rete ovary, medullary substance and surface epithelium.

Since, the subsequent formation and development of the ovaries is determined by the intensive mitotic division of the oogonia and their entry into the first meiotic division prophase, the formation of egg nests, the density of the location of the sex cells in the cortex by the 3rd month of the prenatal period is significantly increased. In the deep zones of the cortical and medullary substance of the 4 months old fetuses, follicles are formed. By birth, the follicular stages of the sex cells predominate and the oogonia located in the egg nests disappear.

Electron microscopy and histochemical study of developing follicles attests to asynchronous oogenesis in bovine fetuses. In the ovaries, sex cells are found in several stages of the first meiotic division prophase with different morphofunctional state of the nucleus (Fig. 1-5). Apoptosis of the sex cells begins after 45 days of fetal development and is recorded later throughout life.

The intensity of programmed death is ensured by the gradual disappearance of oogonia and oocytes in the composition of egg nests and by the selection of growing follicles. Apoptosis of the sex cells is manifested by violations of meiotic transformations of the nucleus and cytoplasm and in pathology it is manifested by the presence of anomalous inclusions (Fig. 6 and 7). It should be noted that in pathology, the morphofunctional state of the histone of the follicle plays a major role in the disappearance of the sex cells, directly affecting the development of these structures (Fig. 8-12).

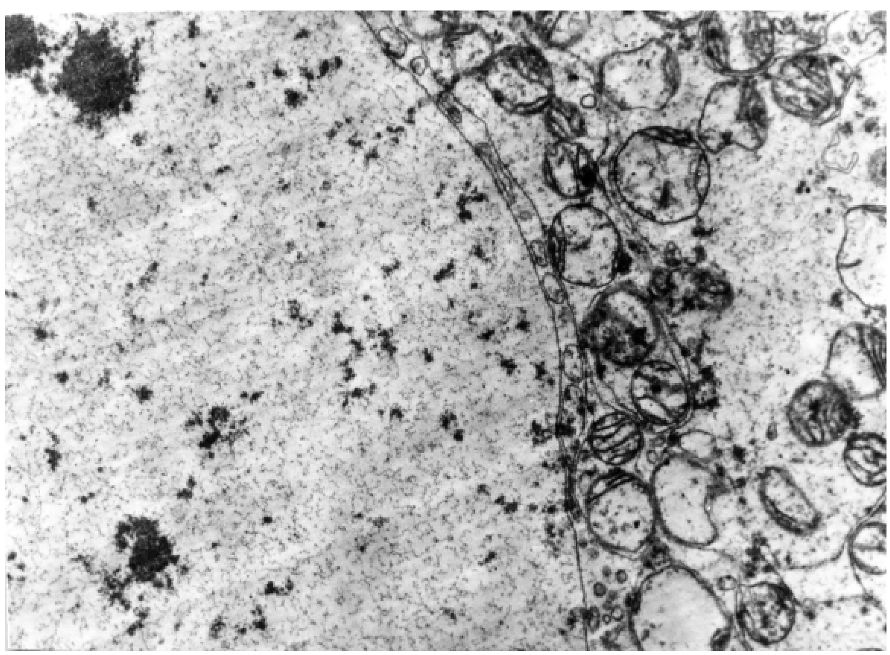

Fig. 1: Electron micrograph of the ovarian cortical substance of the 6 months old fetus. Oocyte in the dictyotene stage of the first meiotic division prophase. The heterochromatin of the nucleus is decondensed. Accumulations of mitochondria and elements of the endoplasmic reticulum in the cytoplasm. ×12000

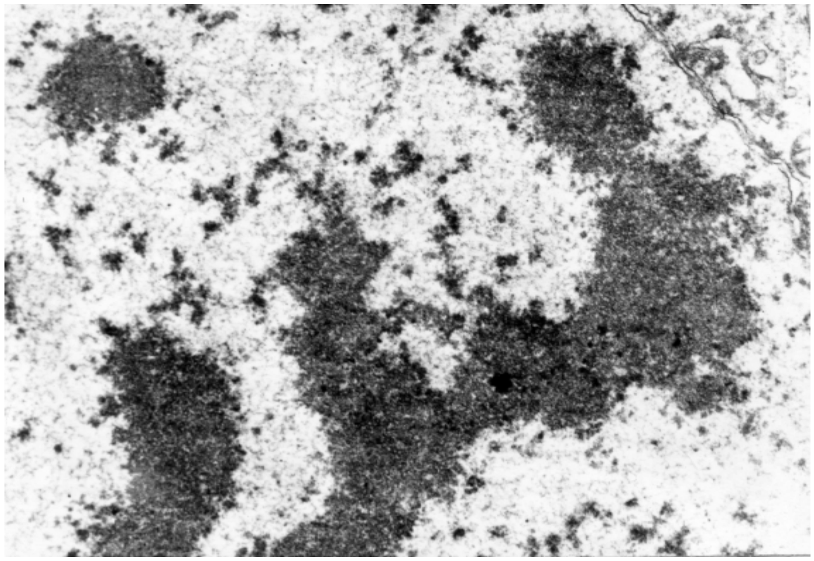

Fig. 2: Electron micrograph of the ovarian cortical substance of the 7 months old fetus. Oocyte in the diplotene stage of the first meiotic division prophase. Condensation of the heterochromatin of the nucleus surrounded by the nuclear membrane with small extensions. ×12000

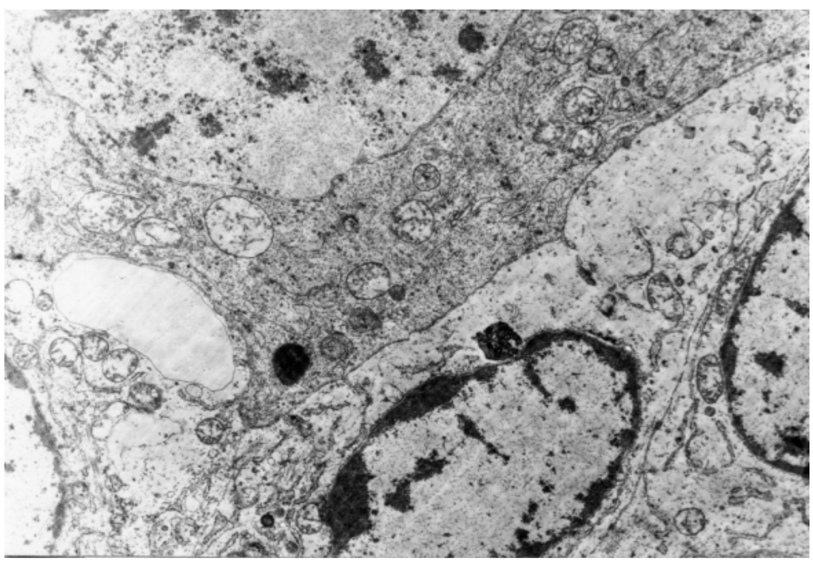

Fig. 3: Electron micrograph of the ovarian cortical substance of the 7 months old fetus. Primordial follicle with oocyte in the diplotene stage of the first meiotic division prophase surrounded by follicular cells. ×12000

We found that at the end of the 6th months of prenatal development of the fetus, single cavitary follicles could be seen with the naked eye in the ovaries. We believe that these are normal structures, the development and involution of which plays an important role in the formation of the cortical and medullary substance of the ovary.

The presence of asymmetry in the development of ovaries was investigated. To do this, we determined the mass and volume of the right and left ovaries starting with the 3 months old fetus. The right ovary is heavier and larger than the left one. The difference is significant at $p<0.05$. This is especially, pronounced, since, the intensive development of the cavitary follicles (7 months of prenatal development).

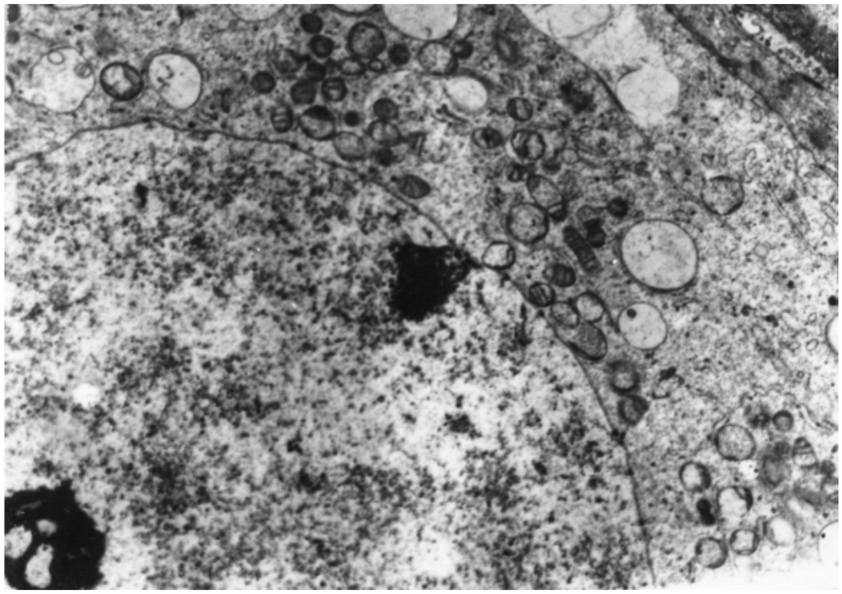

Fig. 4: Electron micrograph of the ovarian cortex of a 9 months old fetus. Oocyte in the diplotene stage of the first meiotic division prophase. Heterochromatin is collected by groups, having the average electron density. The nucleolus is of compressed fibrillar type. ×12000

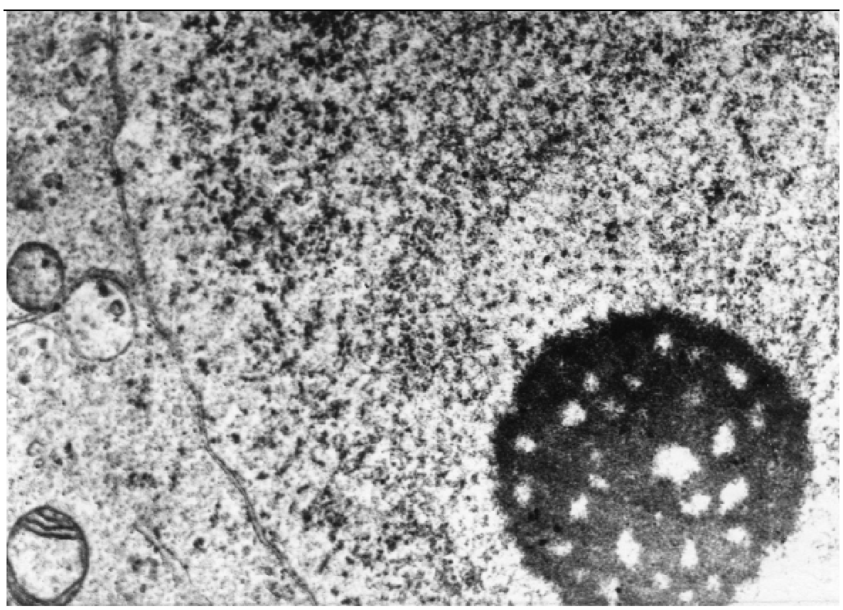

Fig. 5: Electron micrograph of the ovarian cortical substance of the 9 months old fetus. Oocyte in the diplotene stage of the first meiotic division prophase. Oocyte nucleus with characteristic large reticular nucleolus and unformed decondensed heterochromatin. ×22000

Multilayered and cavitary follicles undergo various forms of atresia. It was established that the filling of the cavity of ovarian follicles of bovine fetuses could proceed in two ways. The first type the most common is obliteration. It is characterized by dystrophy and atrophy of folliculocytes and filling the cavity with the connective tissue. The second type luteinization is characterized by hyperplasia of cellular elements of granulosa and internal theca. Cellular elements of these structures fill the cavity of the vesicle.

Fig. 6: Electron micrograph of the ovarian cortical substance of the 7 months old fetus. Cytoplasmic inclusions in the nucleus of the oocyte. ×22000

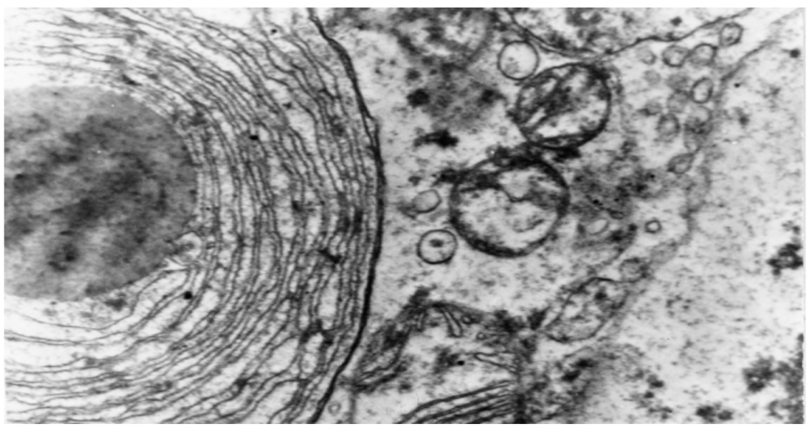

Fig. 7: Electron micrograph of the cortical substance of the ovary of the 9 months old fetus. Myelin-like inclusions in the cytoplasm of the oocyte. ×22000

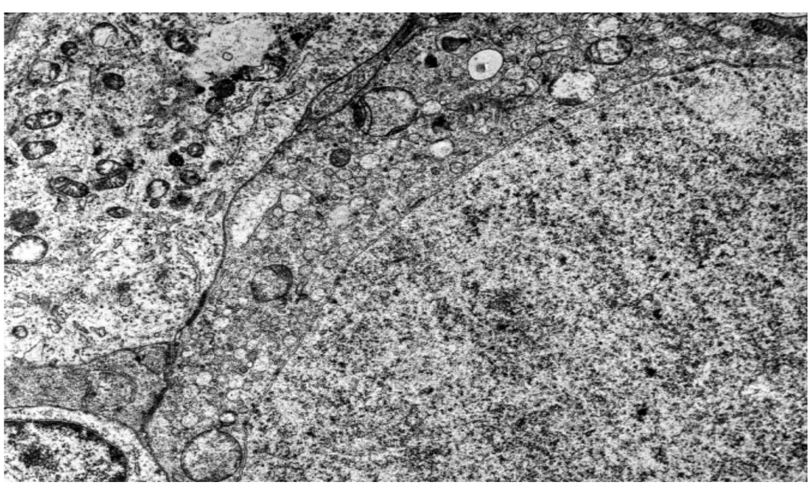

Fig. 8: Electron micrograph of the cortical substance of the ovary of a newborn heifer. The nucleus and cytoplasm of the primary follicle. Nuclear pores are weakly expressed. The cytoplasm contains few mitochondria. ×12000

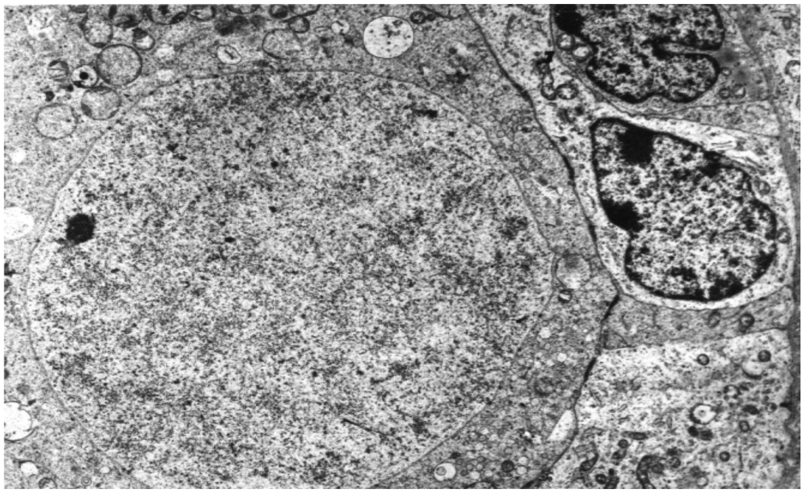

Fig. 9: Electron micrograph of the cortical substance of the ovary of a newborn heifer. The vascular-tissue region of the follicle. Oocyte, follicular cells with different electron density of the cytoplasm, basal membrane and surrounding connective tissue. ×8000

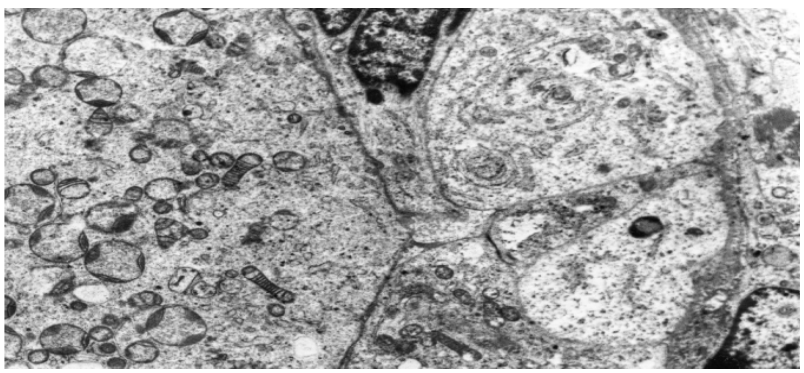

Fig. 10: Electron micrograph of the cortical substance of the ovary of a newborn heifer. Intercellular contacts in the primary follicle. Desmos-like contacts, extensions and annular nexus. ×12000

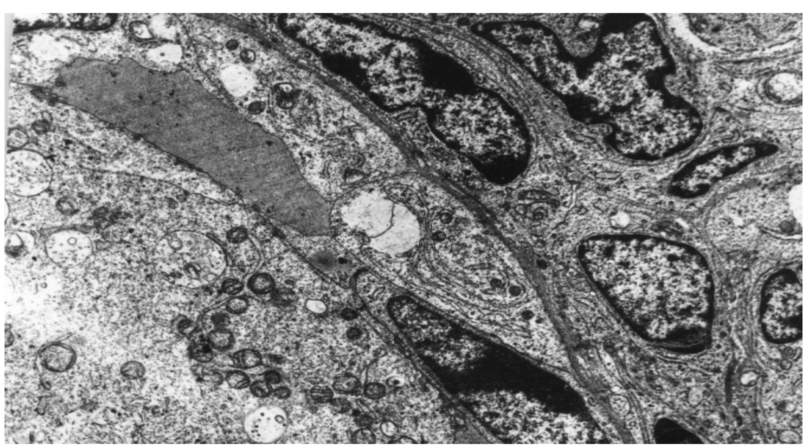

Fig. 11: Electron micrograph of the cortical substance of the ovary of a newborn heifer. Large primary follicle. The beginning of the formation of the transparent zone, separating the oocyte and follicular cells. Formation of microvilli and invaginations penetrating into the transparent zone. ×10000

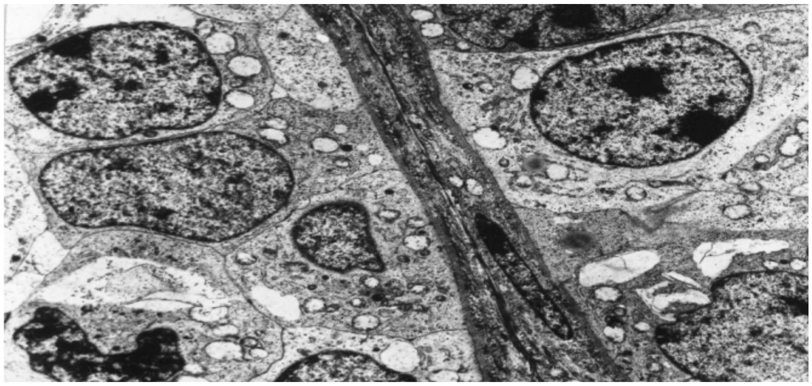

Fig. 12: Electron micrograph of the cortical substance of the newborn heifer's ovary. Follicular cells surrounding closely located oocytes and separated by basal membranes and connective tissue. ×10000

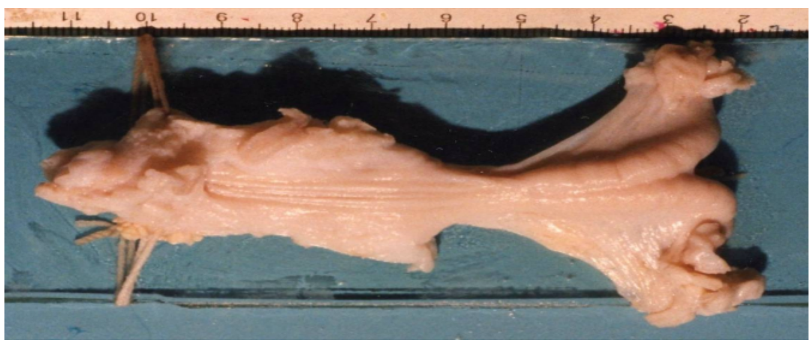

Fig. 13: Vagina, uterus and ovaries of a 9 months old fetus. Hypoplasia of the ovaries

Fig. 14: Vagina, uterus and ovaries of a 9 months old fetus. Polycystosis of the fetal ovaries

This type of atresia was detected in the last month of gestation when the fetal ovary follicles became rather big.

The so-called cystic atresia, described by many authors was not detected by us although the formation of cysts in pathology is possible.

According to our data, the surface epithelium does not participate in the formation of structures of the cortical and medullary substance of the ovaries.

Epitheliocytes perform a secretory function. The intensity of secretion is determined by the level of hormones secreted by the ovaries which is clearly manifested in pathology. In this case, the epithelium and the underlying tunica albuginea form structures resembling depressions, canals, inclusive cysts or papilloma-like structures protruding above the surface of the formation.

The rete ovary (a derivative of mesonephros) is formed at the earliest stages of prenatal ontogenesis. In 2 months of prenatal development, the following parts can be defined in it: intra-ovarian, connective and extra-ovarian. In further periods of ontogenesis they are preserved and develop in proportion to the size of the organ and perform, above all, the excretory function Activation of the intra-ovarian part of the rete ovary during the formation of follicles may indirectly indicate its involvement in the formation of the basic structures of the ovary.

In the pathology of metabolism in cows-mothers, late fetuses develop ovarian gland hypoplasia (Fig. 13). Ovaries are characterized by the small size and mass, the absence of antral follicles in them the mass extinction of generative elements and gametopathies.

Three cows with acute intoxications were found to have an increase in the number and size of antral follicles in the fetal ovaries accompanied by their cystic changes (Fig. 14) and hemorrhage into the cavity of follicles. In our opinion these changes can be considered as a form of hyperplasia of interstitial tissue of ovarian glands or in other words as fetal polycystic ovary associated with impaired hormonal relationship between mother and fetus (fetoplacental insufficiency).

In our research, we investigated the formation of the ovary as a holistic organ. For this purpose a set of organometric, histological, histochemical and electron microscopic methods was used, taking into account the gestation course in cows-mothers. This allowed us to integrate the results obtained at different levels of the study and to present the formation of the ovarian gland as a whole taking into account complex intra-organic connections.

To this day it is not entirely clear from literature how the reproductive organs of females are formed in the early stages of ontogenesis. According to our data, the appearance of primary undifferentiated gonads in cows occurs in embryogenesis rather early. Proliferation of coelomic epithelium on the surface of mesonephros can be detected even in 20 days old fetuses. In the gonads of embryos without sex there is already a mesenchymal base, single oogonia and coelomic epithelium; sinusoidal capillaries are being formed. This is confirmed by the studies of other researchers who found these formations in embryos at slightly different periods of embryogenesis (Kenngott *et al*., 2013). It is very important to reject the idea that gonads are formed later than other organs since, sexual differentiation of the body and organs begins quite early and this process is influenced by different hormonal profiles of females and males.

Later, in 45 days old embryos, the gonads intensively grow and differentiate into the ovaries or testes which occurs against the background of the regression of the primary kidney. The main structures of the body: mesovarium, cortical and medullary substance with the rete ovary, the surface epithelium appear in the ovaries. On this basis, according to histological features they can be distinguished from the testes (Kurilo *et al*., 1987).

We were the first to determine at this age the volume of the ovary with the help of volumetric reconstruction of serial sections, absolute and relative volumes of cortical and medullary matter, density of the location of the sex cells. By the second month, the mass and volume of the ovaries increase markedly. The volume of the medullary substance increases with respect to the cortex which is connected with the fulfillment of the trophic function. In the cortex, the oogonia divide intensively; their number significantly increases (by 6 times).

Some reseachers consider the surface epithelium as "Embryonic" which forms "Epithelial cords" and even generative elements. Histochemical and electron microscopic examination suggests that even in the early stages of fetal development it is mesothelial in nature and has a pronounced secretory activity (Kenngott *et al*., 2013). We did not find any signs of growth of the surface epithelium deep into the ovary between mesenchymal cells or its participation in gametogenesis. The surface zone where the mitotic reproduction of the sex cells occurs and the medullary vascular region are clearly distinguished in the organ.

In mammals, throughout the reproductive life, the vast majority of sex cells die. In the studies on the dynamics of this process, waves of degeneration have been revealed in the ovaries of mammalian fetuses (Tilly, 1996; Vaskivuo and Tapanainen, 2003). However, the description of mechanisms for the elimination of sex cells is contradictory (Fair *et al*., 1997). We found that about 2% of the oogonia have signs of destruction of the nucleus and cytoplasm by the second month of intra-uterine development. The intensity and morphology of this process have pronounced features in each age period.

The middle stage of prenatal ontogenesis is characterized by the formation of the basic structures of ovary. There is already the endocrine function of the ovarian glands which we have established with the help of histochemical and histoenzymological methods of investigation. Biochemical studies of other resaechers indicate that during this period they are capable of producing steroid hormones.

A small amount of testosterone is formed, a moderate amount of androstenediol, estrone and a significant level of estradiol are synthesized (Burkhart *et al*., 2010; Allen *et al*., 2016).

Embryologists and pathologists tend to consider certain moments of embryo development as “critical”. During these phases, the structure and function of biological systems changes qualitatively and the body becomes extremely sensitive to unfavorable factors disrupting homeostasis and gestation (Gilbert, 2013; McGeady *et al*., 2017).

We distinguish three critical phases of the development of the reproductive system from fertilization to the 2 months age of the fetus. The first critical stage is fertilization. If there is a violation in the number of chromosomes or if gametes are inferior, not only the death of the zygote may occur but XY chromosomes chimerism, Turner monomia, gonosomal aneuploidy, X-trisomy, etc. may proceed.

The extra-gonadal origin of sex cells has long been proven (Zayed *et al*., 2007). Throughout the studied period of ontogenesis, we did not observe gametogenesis from stromal ovarian structures, surface epithelium of other components of buds. Primary sex cells migrate to the gonads from the yolk sac of the endoderm (Fortune *et al*., 2010a, b; 2013). This occurs in cattle in the first month of prenatal development. We consider this stage as the second critical phase, since, at this time there may be interruptions in the migration of gonocytes and gametogenesis. The third critical phase is the establishment of the gonadal sex, at the age of 45 days when hermaphroditism and freemartinism may develop.

During the late, final stage of embryonic development, no less significant events occur. First of all it is expressed in the formation of primordial, primary, secondary and even tertiary follicles. After colonization of the gonad buds with primary sex cells their division and entry into the first meiotic division prophase, oocytes pass to the diplotene stage. The process of follicle formation begins (Smitz and Cortvrindt, 2002; Yang and Fortune, 2008).

The data obtained by us testify that by the 3rd month of prenatal development, the sex cells in the ovarian cortex are located very closely, since, oogonia and oocytes are in the egg nests. A careful morphological study of serial sections of the ovary does not allow us to state that in this period follicles are formed which is in line with the results obtained by other researchers (Tanaka *et al*., 2001; Ireland *et al*., 2007; Aerts and Bols, 2010).

Primordial follicles, according to our data, appear in 4 months old fetuses, although, oogonia and oocytes predominate in the egg nests. Later, asynchronous entry of oocytes into the follicular stage occurs.

Their transition to the diplotene stage of the first meiotic division prophase and the formation of the follicular membrane are expressed by vivid structural changes. The central position is occupied by the nucleus. Due to significant transcription activity, DNA accumulation, chromosome decondensation occurs. Nucleoli which are characterized by a nucleoneomic structure, function actively. The number and differentiation of organelles in the cytoplasm increases. The most characteristic structure of primordial oocytes paranuclear complex develops. The activity of oxidation-reduction and hydrolytic enzymes is noticeably increased. The volume of the nucleus and cytoplasm increases.

Most sex cells by the age of 7 months are surrounded by follicular epithelium their ultrastructure is differentiated. The degree of condensation of chromosomes increases. The nucleoli decrease nucleonemic structure and become more osmiophilic. A complex of dilatations in the nuclear membrane appears. Probably, the inclusions observed in the dilatation, may be a preliminary stage in the production of mitochondrial precursor bodies (Fair *et al*., 1997).

The presence of the so-called “hooded-like” mitochondrial cristae, according to our data is a specific feature of the oocytes of the bovine fetuses. Such peculiar mitochondria and a small number of cristae in them indicate that they perform a large number of side functions (Cibas and Ducatman, 2009).

Changes in nuclear-cytoplasmic ratios in the oocytes of primordial follicles are accompanied by a change in the relationship with follicular cells. The latter are located in close connection with the cells they do not have special adjoining structures and separate the sex cells from the surrounding connective tissue of the cortex. Follicular cells are not rich in organelles and their ultrastructure and histochemistry indicate low functional activity.

Folliculogenesis is accompanied by the restructuring of the stromal-vascular component of the organ. By the age of 4 months, the cortex takes up 2/3 of the volume of the ovary which occurs against a background of moderate growth in the mass and volume of the organ. Further, uneven accelerated growth of the medullary substance of the organ is noted. And closer to birth, the increase in the volume of cortex again goes before the growth of the medullary substance.

The structure of the connective tissue of the stroma changes. In the presence of egg nests, the intercellular substance is poorly developed. When follicles, especially, antral ones, appear, the fibrous component of the connective tissue develops the growth of the medulla, vessels and nerves of the organ accelerates. In the 4 months old fetuses, the groups of sex cells are surrounded by a common connective tissue membrane and by the end of the prenatal period each follicle has its own granulosa and a thecal membrane. The development of follicles stimulates the growth of the stromal-vascular component of the organ. It in turn, fulfilling the supporting and trophic function, ensures the differentiation and preservation of the pool of the sex cells (Carou *et al*., 2015; Kranc *et al*., 2016). The development of the medullary substance is also, trophic function targeted. The formation of primordial follicles and then of antral follicles is impossible without a qualitative reorganization of the connective tissue that performs mechanical, trophic, protective and structural functions (Rehfeld *et al*., 2017).

The complex multilevel morphological investigation of the connective tissue, surrounding the follicles, carried out by us indicates that it differs from the usual loose connective tissue of other organs. It is characterized primarily by the presence of low differentiated connective tissue cells. The latter have a pronounced process shape, a rounded, euchromatin-rich nucleus and at the same time contain few ribonucleoproteids in the cytoplasm. Researchers view such cells as blast-like or more precisely fibroblast-like cells that can develop into interstitial cells (Kenngott and Sinowatz, 2008). Apparently, the tissue structures surrounding the oocyte are to be considered as a kind of hematofollicular barrier, alterative changes of which can lead to mass death of follicles (Carou *et al*., 2015). This is what we observed in late fetuses from cows with metabolic pathologies. Feeding of these animals was energetically inadequate and unbalanced as regards the basic nutrients.

The underdevelopment of the reproductive organs of the fetuses and especially, of the ovaries was diagnosed. These changes can be considered as hypoplasia. The structures which can be found only in early embryonic development (oogonia, egg nests) are preserved in the organs and at the same time, the development of follicles slows down. Hypoplasia of the digestive organs and central organs of immunity is described in detail by researchers in hypotrophic calves. Hypoplasia of the reproductive organs is described in detail for the first time, although the genetically determined ovarian hypoplasia is known for Swedish cattle.

In pathology, primary changes were found in granulosa. They were characterized by dystrophic changes, disappearance of cell junctions distinctive for these cells. Follicle cells cease to divide, the transparent zone thickens and the oocyte undergoes pycnosis. In the connective tissue of the theca membrane, the phenomena of hemostasis, edema, mucoid and fibrinoid swelling were noted.

In fetuses obtained from cows that were kept on a balanced diet and in optimal conditions of management without metabolic pathology and disorders of gestation, the disappearance of the non-cavitary follicles was not massive. It is caused, first by the violation of meiotic transformations of the nucleus. Earlier we showed that in heifers which were born from cows that received an excessive amount of silage in the dry period and with chronic fusariotoxicosis and hypodynamia, underdevelopment of the reproductive system and delay in puberty could be observed (Skovorodin, 1997a, b).

In this regard it is important to know the regularities of the formation of antral follicles in cows. The data of the literature do not clarify the question when the cavitary follicles form develop and their cavity disappears (Skovorodin, 1997a, b; Leung and Adashi, 2003; Hummitzsch *et al*., 2013; Lukovic *et al*., 2017). According to our data, the cavity in the follicles begins to form already in the 6 months old fetuses but usually they are not found macroscopically. Then this process grows and in 9 months old fetuses, up to three tertiary follicles are detected with the naked eye. Their size can reach 4 mm in diameter.

On the basis of the detection of cavitary follicles with a developed internal theca in the late fetuses, the determination of the histochemical characteristics of the hormone-producing structures (the presence of the sudanophilic and Schick-positive inclusions, the activity of phosphatases) we can conclude that the hypothalamic pituitary ovarian fetal system is active in the last third of gestation, the sex glands actively synthesize hormones at the beginning of their formation and differentiation (Allen *et al*., 2016; Amin, 2016).

Three factors determine the development of antral follicles in fetuses: gonadotropins of the mother's adenohypophysis, chorionic gonadotropin, own gonadotropic hormones of the embryo. A different level of these hormones is indicated by the pronounced variability in the structure of the fetal ovary with respect to the formation of vesicles. The extreme degree of hyperstimulation of the growth of ovarian follicles is the polycystosis of the fetal ovaries described by us for the first time in cattle which is a form of hyperplasia of the follicular apparatus. Similar changes have been reported in other mammals (horses, giraffes) and humans (Barreto *et al*., 2018).

But in late fetuses obtained from cows with chronic forms of metabolic pathology, we found the absence of tertiary follicles, the delay in the involution of embryonic structures, the massive degeneration of the primary follicles. Early signs of atresia appear in consequence of which the tertiary follicles do not have time to form up to the size of macroscopically visible ones. Histochemical study indicates a hypoplasia of hormonal-active theca tissue and a pronounced decrease in its endocrine function.

CONCLUSION

It was established that indifferent gonads of cattle are laid at the end of the embryonic stage (20-34 days of prenatal development). In 34-45 days old embryos they differentiate into the ovaries and contain the basic definitive structures of the organ. Further development of the ovaries is determined by the active mitotic division of oogonia and by the formation of egg nests. In 4 months old fetuses, follicles begin to form on the border of the cortical and medullary substance. In 6-9 months old heifers, oocytes and the surrounding structures reach the final state.

Apoptosis of sex cells begins in 45 days old embryos already; it is recorded throughout the course of prenatal ontogenesis and is determined by changes in the structure of the nucleus. With pathology, the main factor in the disappearance of generative changes is the violation of the structure of the vascular-tissue environment.

Normally, after 6 months of prenatal development, single cavitary follicles which are determined macroscopically, appear in the ovaries. In acute forms of the pathology of gestation, polycystosis of the ovaries is observed and in the case of metabolic disorders in cows-mothers, ovarian gland hypoplasia develops in late fetuses. In newborn calves who have survived gastrointestinal diseases the latter progresses and may be irreversible.

Thus, when developing biotechnological methods of breeding cattle, bioactive and chemotherapeutic drugs, diets, feeding technology and pregnant cow's management we must take into account the fact that certain stages of gestation are "critical" for the formation of fetal ovaries. This is the time of fertilization and the formation of a zygote, formation of indifferent gonads and migration of gonocytes (20-34th day), the divergent sex differentiation (34-45th day), beginning of the formation of primordial follicles (4 months), the formation of antral follicles (6-7 months).

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