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STRUCTURAL AND MORPHOLOGICAL CHANGES OF ENDOMETRIUM RELATED TO OVARY CYCLE AND CONDITION OF GENITAL FUNCTION OF COWS

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The article analyses structural and morphological changes of endometrium related to ovary cycle and condition of genital function of cows. It has been determined that during estrus, we find high functional activity, activation of physiological secretion processes, intensification of protein lipid metabolism. Endometrium of proesrtrus shows activation of regenerative processes accompanied with development of connective endometrium matrix. Such condition characterized by accumulation glycosaminglycans and increase the reactive activity of acid proteinglycans of deep phase of fibrous matrix proteins.

Endometrium of cows during metestrus is charachterized by simultaneous dystrophic degenerative changes caused by hormone-dependent cell desquamation, and cell proliferation initiation.

In postmorbid condition endometrium shows differentiation of stroma cells, vessel territories and uterine glands, reduction of proliferation processes, depression of regenerative ability and neoangenogenesis.

Keywords: *cows, structural and morphological endometrium changes, postmorbid endometrium.*

Introduction

Today, scientists studying the point of infertility, first of all study the processes in preimplant endometrium, because of its important role in nidation and gestation (Sidel'nikova, 2002). Basic changes in endometrium happen during the period of decidual transformations, among them angiogenesis and

transformation of extracellular matrix of functional layer take an important place.

Structural and morphological changes in endometrium of cows dynamically transform during every stage of rut and are controlled by inner and outer irritator factors coming to CNS, determining production of gonadal hormones. Numerous molecular factors have the role of receptor and ligands together, they influence biochemical processes which provide implantation of copula. Pathological and morphological disorders caused by trophic and metabolic disorders of endometrium make it impossible to give adequate trophic aid to implanting copula or to developing chorium (Nikitina et al., 2007, Radzinskij et al., 2010).

However, in order to have reliable data on the mentioned point, we have to discover the consistent pattern of local regulatory interaction between structural components in endometrium during the rut stage, that is why studying of functions and structure of endometrium is important in finding the schemes and methods of genital function correction (Demidova et al., 2006, Nikitina et al., 2007).

Endometrium is a complex, multi-component structure, having basal and functional layers with epithelium and matrix, stroma and glands (Brsikyan, 1990, Kostishin, 1999).

The surface of endometrium is divided into three different morphofunctional areas: caruncular, intercaruncular and gland with open uterine gland ducts. Caruncular area has a distinct complex of decidual tissue (with ability to bacteriolysis, inactivation of their toxins, synthesis of carbohydrates, lipids, proteins, prolactins and prostaglandines). In intercaruncular area, epithelium has terminable deterioration; while gland area has resistant-intensive secrete epithelium and the endometrium stroma does not change significantly.

Literature Review. Special bodies in endometrium – caruncles are the aniages of placenta. In blood they have a form of flatten mushroom, during gestation, embryo part (cotyledon) covers caruncle, rooting by its projections into the crypts. Epithelium covering crypts of caruncle is monolayer, cubic, having giant cells. Connecting tissue of caruncle has a great number of reticuloendothelia system cells and well developed capillary net. From the second month of gestation on the surface of caruncles appear the crypts divided by the connecting tissue septum. Septum stroma has wide capillary. Septum walls are covered with cubic epithelium, containing mono- and bi- nuclear giant cells. At the back of septum appear blood vessels covered by stroma tissues (Vlasov, 2000, Saenko, 2001).

According to the researches of Vlasov (2000), covering epithelium of endometrium undergoes lysis in contact places with the tissues of embryo that is why separate points of epithelium, having a form of waves, appear on the surface of endometrium.

Changes in structural components of endometrium during the stage of rut, create supportive environment for copula implantation. Basal layer does not have significant changes during the period of estrous cycle. However the functional layer - is a dynamic structure having dynamic structural and functional changes under the influence of gonadal hormones.

Stroma cells appear as quaggy spindle fiber and form the important cellular populations that provide aid to endometrium homeostasis. This cellular population, through specific secretion, takes part in growth regulation, reproduction and specific functioning of gland epithelium. Arteries have the spiral form and attain the surface of epithelium during the rut stage (Boltovskaya, 2002).

Uterine glands appear as circular tubes with epithelial cells overhanging in lumens. Gland epithelium is cylindrical, one layered, with big bright cells having the form bladder on basal membrane of glands. In case of their absence, we can determine uterine gland epithelium atrophy. Structural and functional changes in uterine gland cells happen during succeeding stages and phenomena of estrous cycle. On the beginning of the rut stage, sex steroids together with cells proliferation, stimulate the development of secretory apparatus of uterine glands and receptor synthesis to estrogens and progesterone. These changes stipulate the beginning of secretory stage (Boltovskaya, 2002, Ilyina et al., 2006).

Gland epithelium proceeds through structural and functional changes during different phenomena and stages of estrous cycle. While rutting uterine glands cells actively reproduce and grow. During the period of heat and ovulation glycogen from basal area of a gland moves to apicalis. Duration of this period is designated by the time needed for growing of the dominant follicle in ovary. In slowdown stage, few hours after ovulation, secretory transformation of endometrium is in appearance of basal vacuoles (accumulation of glycogen) in uterine glands cells. The secretion of uterine glands, containing glycodelin protein, is an immunosuppressive agent and protects chorion and embryo from immune response of the mother's body (Ilyina et al., 2006, Misajlov et al., 2006).

It is known, that functional conditions of epithelial cells in uterine glands and cell elements of endometrium stroma under the conditions of estrous cycle have heterogeneous characteristics. Scientists consider that heterogeneous condition helps the cell adaptation in changeable conditions of mother's organism by actuation of additional structures (Gunin et al., 1998, Boltovskaya, 2002, Misajlov et al., 2006).

Stage of secretion, due to increased level of progesterone, is characterized by presence in endometrium winding spiral arteries having the maximum length and developing swelling of stroma cells. Swelling appears due to increase in vascular penetration and changes in macromolecular structure of the main matter (its transformation from sol into gel). Vessels hug the uterine gland, it regulates the condition of gland epithelium in direct way – through hormones taken from the blood. Fibroblast like cells of stroma influence indirectly influence the uterine gland through the synthetic activity products. Unlike the proliferation stage, where the processes of anaerobic glycolysis are dominant, on the stage of secretion dominate the processes of aerobic glycolysis. The stage of secretion is characterized by the processes of

synthesis in epithelium cells of glands and discharge of complex secretion containing mainly glycogen and glycosaminglicans. Glycogen is accumulated in stroma cell cytoplasm and glycosaminglicans take part in formation of tissues. Carbohydrates synthesis has its maximum during the early stage of secretion and protein synthesis on the beginning of the mid stage (Ilyina et al., 2006, Misajlov et al., 2006).

If the implantation occurred, predecidual cell begin to form around the spiral arteriolas of functional layer. This process is accompanied by formation of compact and spongy layers. On surface of functional layer, the lacunar distension of capillars are found and as a result of fiber structures melting appear areas of division of stroma cells in epithelium of the glands. In gland epithelium, intercellular space and macrophagocyte appear basophilic granules (apoptic bodies), appearing as a result of natural genetically determined dying of cells causing the desquamation of functional layer of endometrium. As the scientists found, only the compact and small part of spongy layer. The largest part of spongy layer cells remains and takes part in endometrium regeneration. Together with rejection of functional layer starts its proliferation. (Gunin and Sharov, 1998, Petitti, 2003).

Acording to scientific literature endometrium regeneration starts during the stage of balancing, because of the low level of sex hormones. Such conditions of female body cause interaction of local grow factors with endometrium cells receptors. Mitosis in cells of gland epithelium are characteristic for endometrium in the early stage of proliferation. During this stag epithelial cells are poorly differentiated, whereas the stroma cells, especially fibroblast like, start collagen production, elastin, proteoglicans and glycoproteins. Collagen and elastin are not only the skeleton for cells they also have informational role during morphogenesis. Proteoglycans and glycoproteins have trophic function, determining cell reproduction, including epithelial cells. Lymphocytes coming from vessels into the endometrium stroma, determine inter tissue regulation for proliferation of different tissues, and probably, can take part in regulation of mitotic activity and differentiation of gland epithelium. Due to mitotic activity of gland component, appears uterine gland winding, especially intense during the late stage of proliferation. Proliferation phase happens with the high rates of nucleic acids and protein metabolism (Misajlov et al., 2006).

At the beginning of slowdown stage, with decreasing of the estrogen and progesterone rates, endometrium undergoes the regressive changes. Nucleus cells of gland epithelium in compact layers have picnosis, stroma coiled arteries – stasis and hypoxia, natural cells-killers appear from numerous lymphocytes. Cells of stroma and epithelium produce relaxant helping in destroying of argyrophilic fibers of stroma (Gunin and Sharov, 1998, Petitti, 2003).

Due to a liquid loss, endometrium hydropy and crinkling of functional layer stroma is found. Glands become even more rugose, being closer to each other, gland secretion stops. Endometrium arteriolas are spread, their winding becomes excessive with low blood circulation, with thrombosis and stasis as a result. Before desquamation of functional layer, vascular distension changes into a spasm, it happens because of different protein decay products and other bioactive substances influence and decreasing rates of progesterone.

Epithelium of endometrium (during the slowdown stage) is flat, wrinkled or ribbed. Secretory and cells appear on the surface of epithelium during rutting. Despite the similar cell structure, cycle changes of surface epithelium in endometrium are less prominent than in uterine glands epithelium (Leung, 2004).

So endometrium contains numerous immunocompetent cells, certain amount of which provides optimal conditions for nidation. Endometrium regeneration in post morbid period if compared with morphotype under the conditions of physiologic ovary cycle is retarded and depends on numerous factors - difficulty of the disease, time and effectiveness of treatment, complications etc (Bhutani, 2004).

Different morph-functional changes of post morbid endometrium, cause disorders in adequate receptor's answer of stroma cells to hormonal influence, determining the disorders in adequate proliferation and secretory transformation. The structure of endometrium corresponding to rutting period has a diagnostic importance, because if found during the ovulation or slowdown stage shows hormonal disorders.

The researches of post morbid endometrium fibers found disorders in structural transformation and inadequacy in secretory transformation of endometrium structural components, but the detailed research of recovery processes and functional condition of endometrium able to implantation is needed (Korneeva et al., 2005, Ozturk et al., 2010).

The **aims** of the research was the definition and substantiation for structural and morphological changes of endometrium depending on stages of ovary cycle and conditions of genital function of cattle pedigree stock on study farms in time of visible ovary cycle.

Materials and methods. Material for the research was endometrium fragments taken from the upper third part of uterine horn of cattle on study farms of VAT "Mikhaylivka", Lebedin region, Sumy oblast, FE "Vitaliya", Buryn region, Sumy oblast, LTD "Lan" and LTD "Vladna", Sumy region, Sumy oblast. Endometrium samples were taken from slaughtered animals 3-10 years, on the 1st day of ovary cycle (estrus), on 7-8th day of ovary cycle, and also samples taken from apparently healthy animals, which had endometritis or placenta retention. Fiber materials were preserved in 10% formalin solution. Then they were processed in water, drained and lightened in spirit xylene solution, put into a celloidine blocks then a series of histologic sections of 10 mkm on sliding microtome have been done. For observing microscopy, histic preparation were colored by hematoxylin staining, for studying of connective tissue structure by picro-fuchsin (Selivanov, 2003, Goral's'kij et al., 2011).

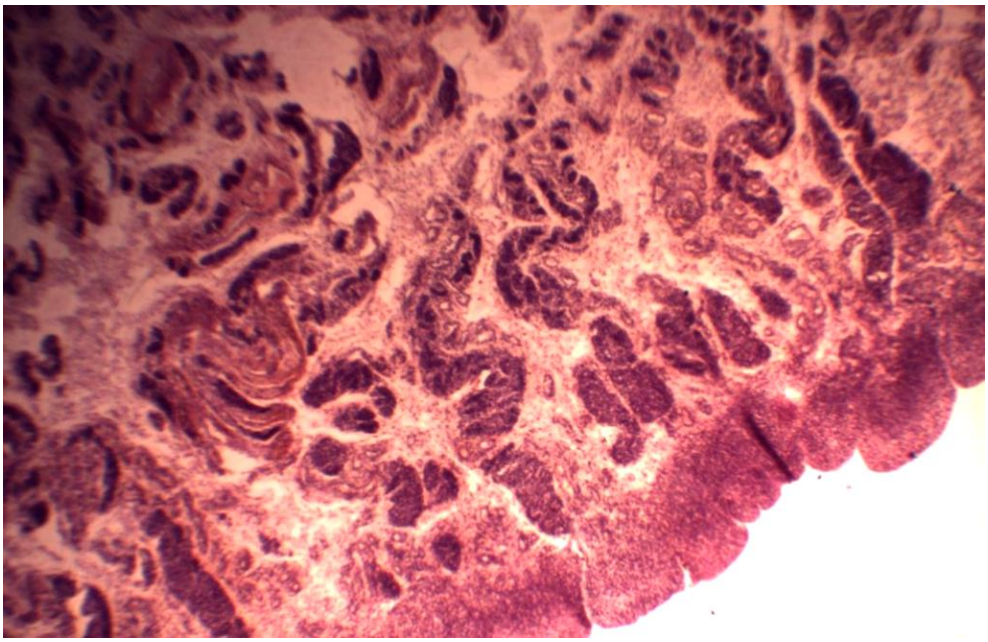
Research results and discussion.

The Histological examination of endometrium has shown that in during estrus a strict differentiation of functional layer on compact and spongy was present. Endometrium arterioles are on maximum spread level, filled with blood, form

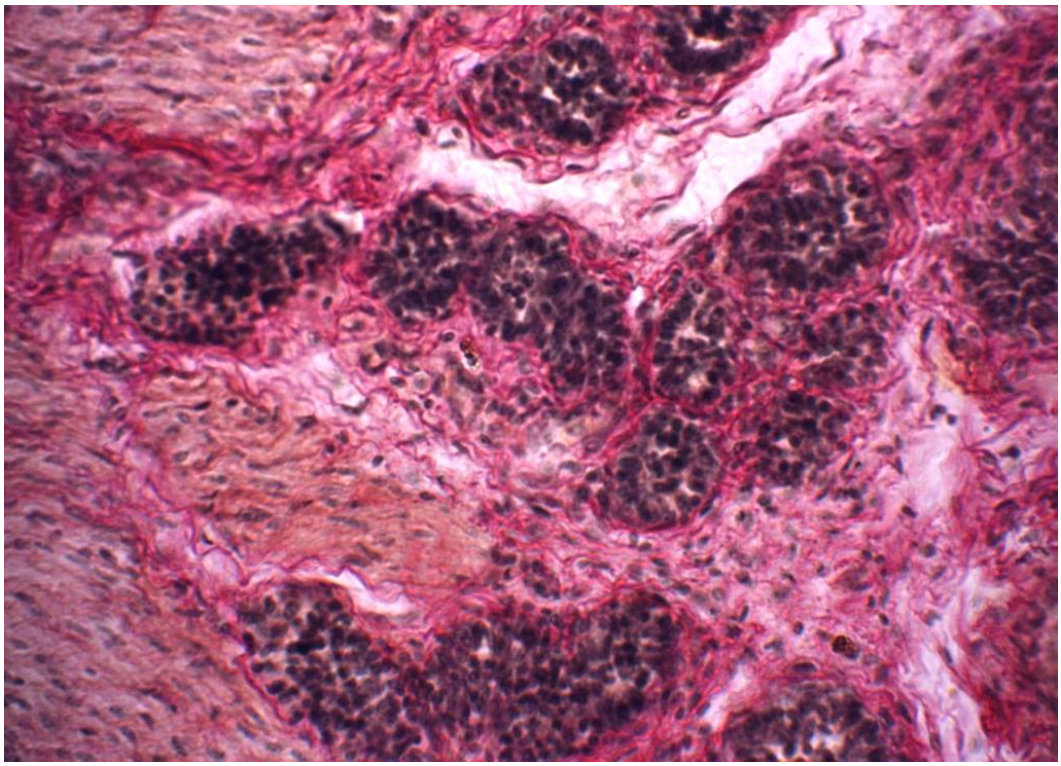
gloms, their walls are edematic, (pic.1,5).

In endometrium tissues mitotic activity of is present, number of stroma cells increases, they are decidual like (future cells of mother's part of placenta) and substantial edema is found. Numerous uterine glands, due to increased secretory activity of cells, have the form of bunches. Uterine has has extension of uterine glands cavity filled with secretion having high concentration of carbohydrate protein combinations, (pic.2-4). Epithelial cells of uterine glands, have irregular cubik forms, polynuclear, with unbalanced basal vacuolization, probably because of the glycogen accumulation, (pic.4).

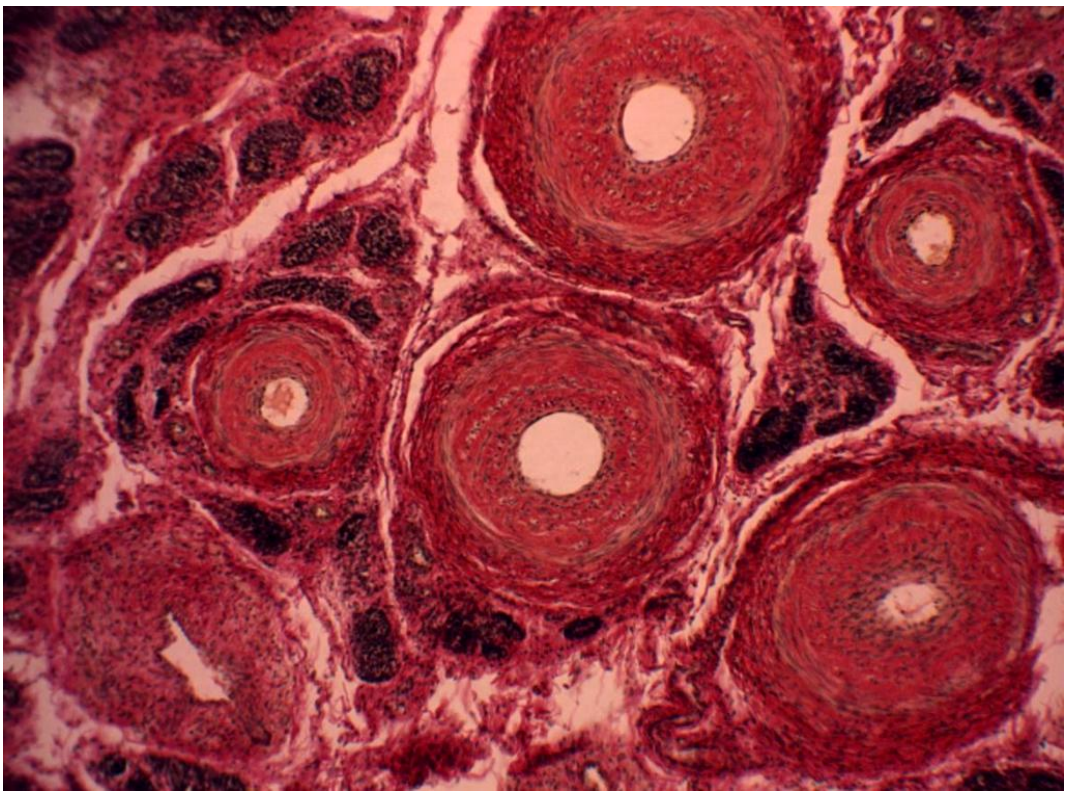
Coloring of endometrium with picro-fuchsin, during estrus (pic.2,3,5.), shows that stroma is filled with protein-carbohydrate combinations, that, according to data proves high functional activity of tissue, and activation of secretory physiological processes. (Tomitova, 2011).



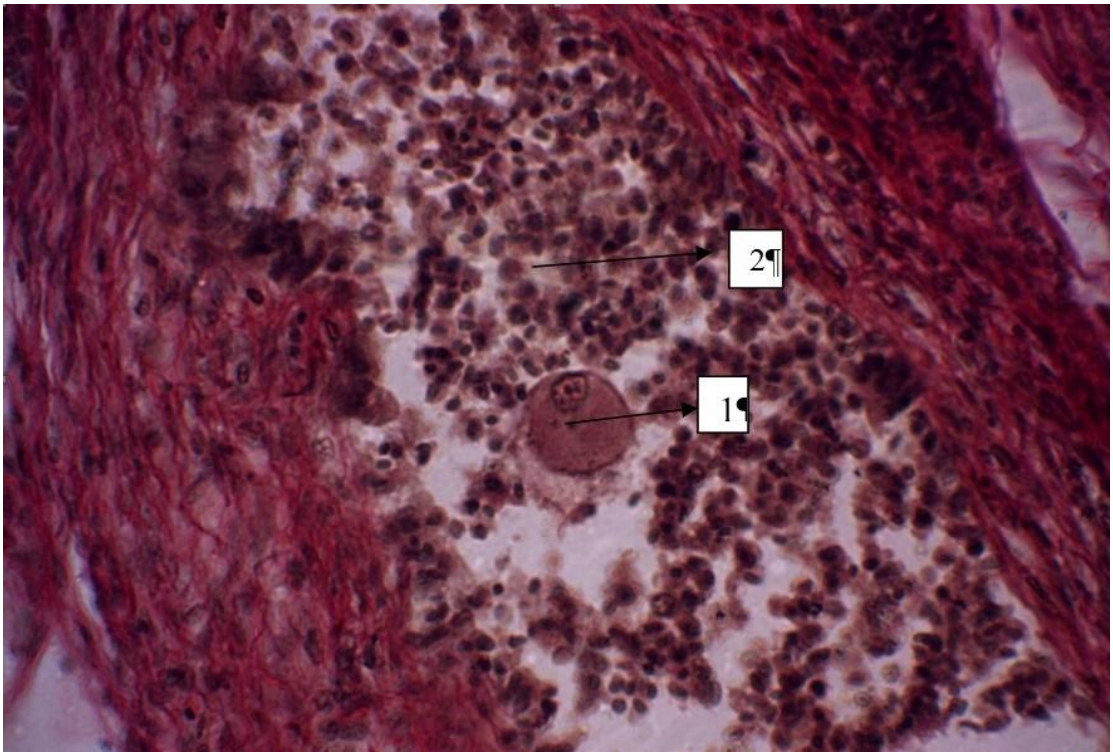
Pic. 1 – Compact and spongy layers of endometrium, estrus (colored by hematoxylin, 10x20).



Pic. 2 – Compact and spongy layers of endometrium (crypt – numerous uterine glands), estrus (colored with picro-fuchsin, 20x40).

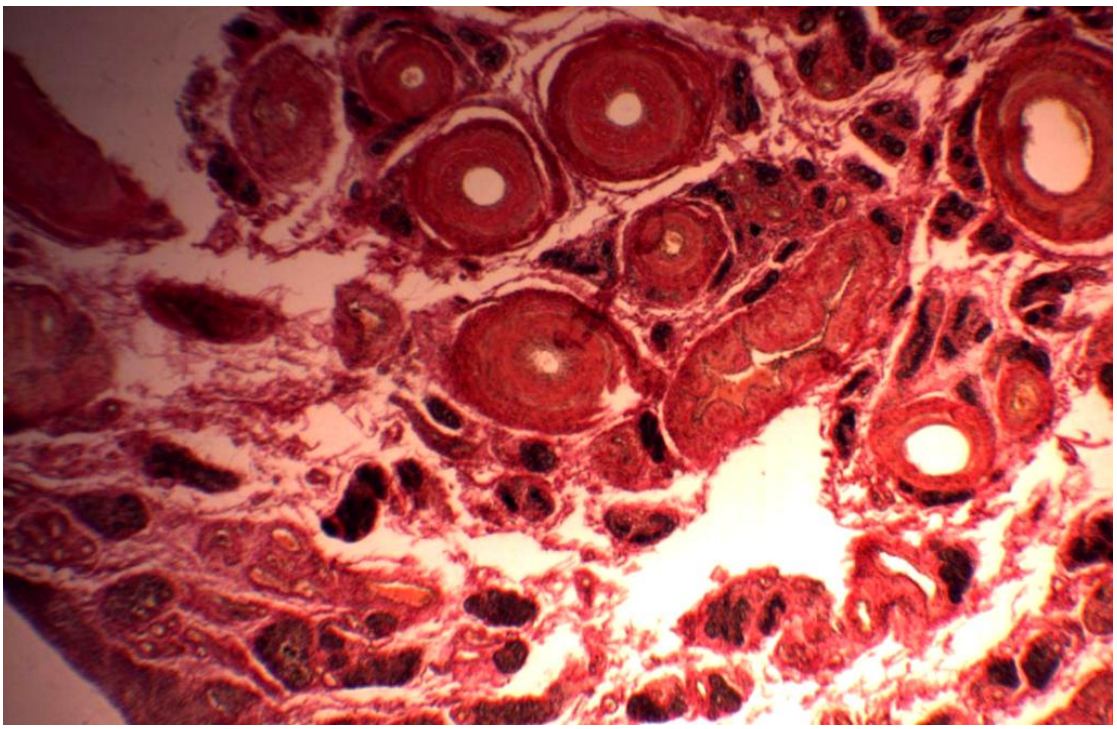


Pic. 3 – Arteriolar edema of endometrium compact layer, estrus, (colored with picro-fuchsin 15x10).



Pic. 4– Uterine gland of compact layer, estrus, (colored with picro-fuchsin 32x40).

1- round grain like cells (glycogen accumulations). 2- uterine glands cavity filled with secretion



Pic. 5 – Surface epithelium, compact and spongy layers of endometrium (septum – a great number of blood vessels and uterine glands among them), (colored with picro-fuchsin 10x10).

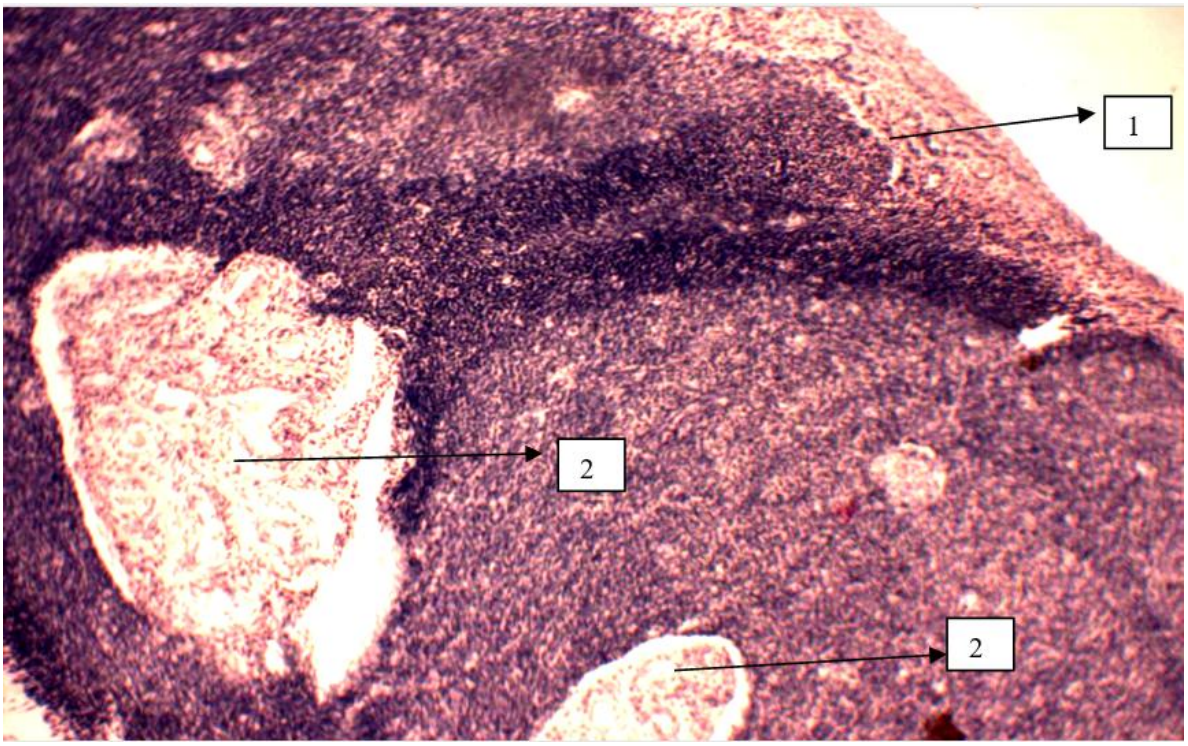
The histological examination of endometrium has shown that in during proestrus (17-18 day of ovary cycle), shows a strict visual differentiation of functional layer on compact and spongy.

Surface epithelium is flat, rugate or wave shaped and contains a great number of uterine glands in stroma cells layer, (pic.6,7,8).

Deep layer of endometrium contains a great number of uterine glands together with low number of stroma cells, but the surface layer, on the contrary, contains individual unformed glands in the layer of stroma cells. Uterine glands do not contain secretion, they are rugate, appear close to each other. There are visible dissection areas of stroma cells and gland epithelium, (pic.7, 9).

Endometrium arterioles are on maximum spread level. There are changes in vessel territories, with intravascular stasis charecteristics and perivascular edema with opening of paravasal fibrillose binding elements, luminal occlusion appearing due to intimal hyperplasia. In some vessels thrombotic masses and their obliterations has been found, together with formation of paravasal fuchsinophil connective tissue fibers, indicating the distress of vessel territories and development of connective tissue endometrium matrix remodulation through their replacement by new developed fibrous elements.

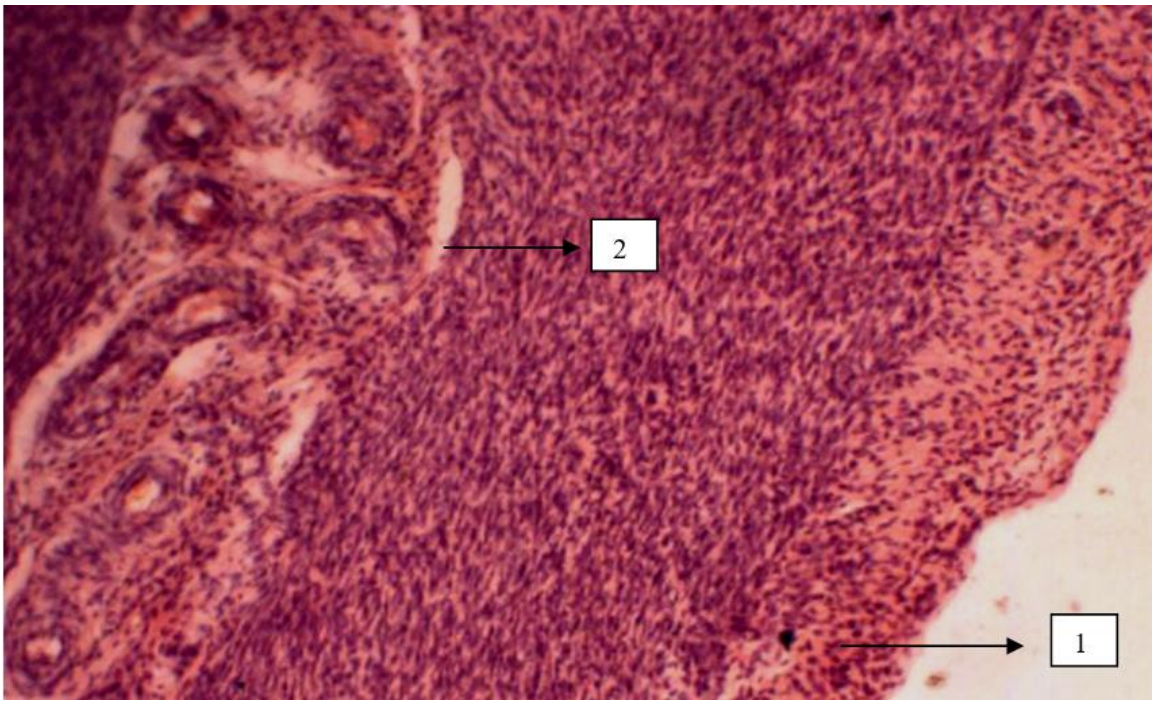
Endometrium coloring with picro-fuchsin during the predicted proestrus (17-18 day of ovary cycle) shows far less (comparing with estrus) concentration of protein-carbohydrate complexes in spongy area of functional layer (pic.7-9), that means, on our opinion, activation of regenerative process.



Pic. 6 – Endometrium surface epithelium and functional layer, proestrus (colored by hematoxylin, 10x10).

1. High cylindrical epithelium is mostly flat;

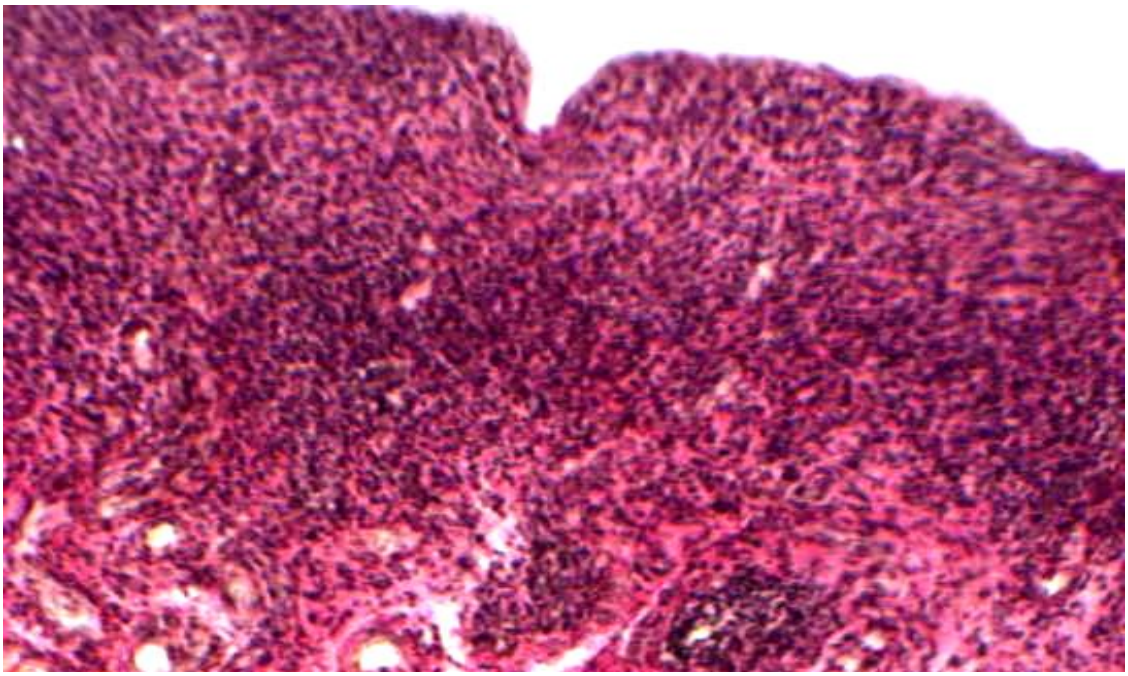
2. Uterine gland without secretion of a very rugete form.



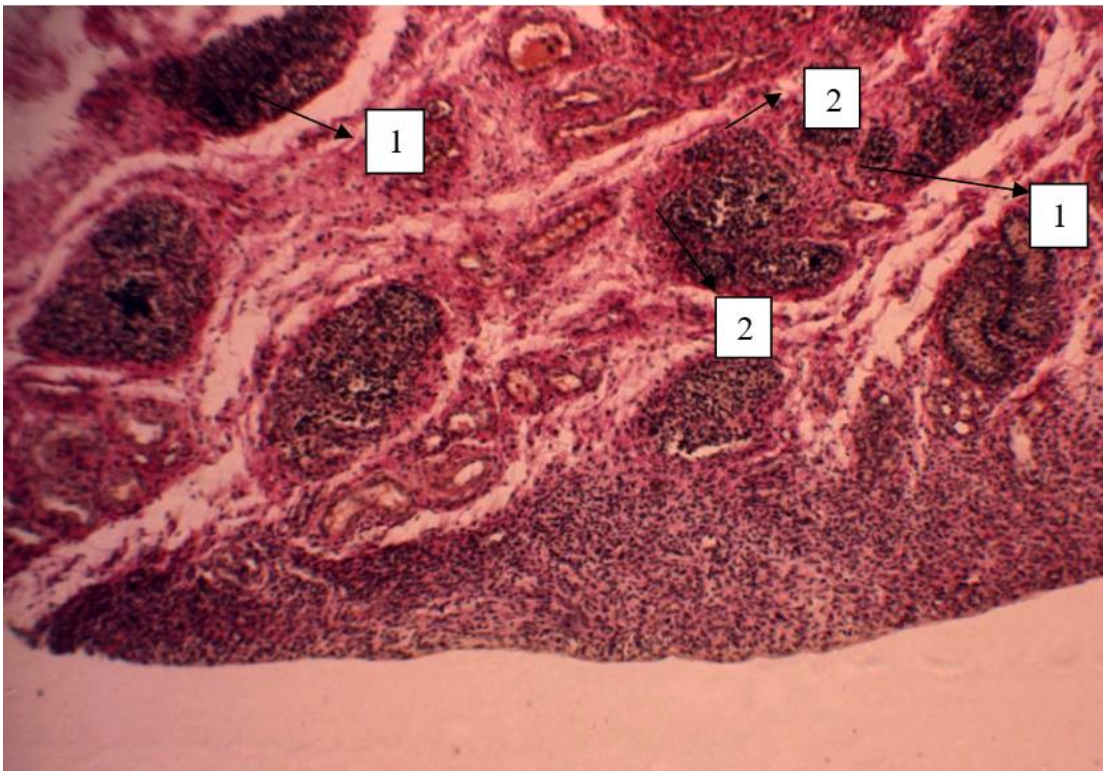
Pic. 7 – Endometrium surface epithelium and functional layer (future crypt),

proesterus (17-18 day of ovary cycle). (colored with picro-fuchsin, 10x10).

1.-High cylindrical epithelium is slightly wavy; 2.-Uterine glands with areas of stroma cells and gland epithelium division.



Pic. 8 – Endometrium surface epithelium and functional layer (future crypt), proesterus (17-18 day of ovary cycle). (colored with picro-fuchsin, 12x10)



Pic. 9 – Uterine glands of endometrium functional layer, proesterus (17-18 day of ovary cycle). (colored with picro-fuchsin, 28x10)

1. areas of stroma cells and gland epithelium division

2. strongly folded uterine glands

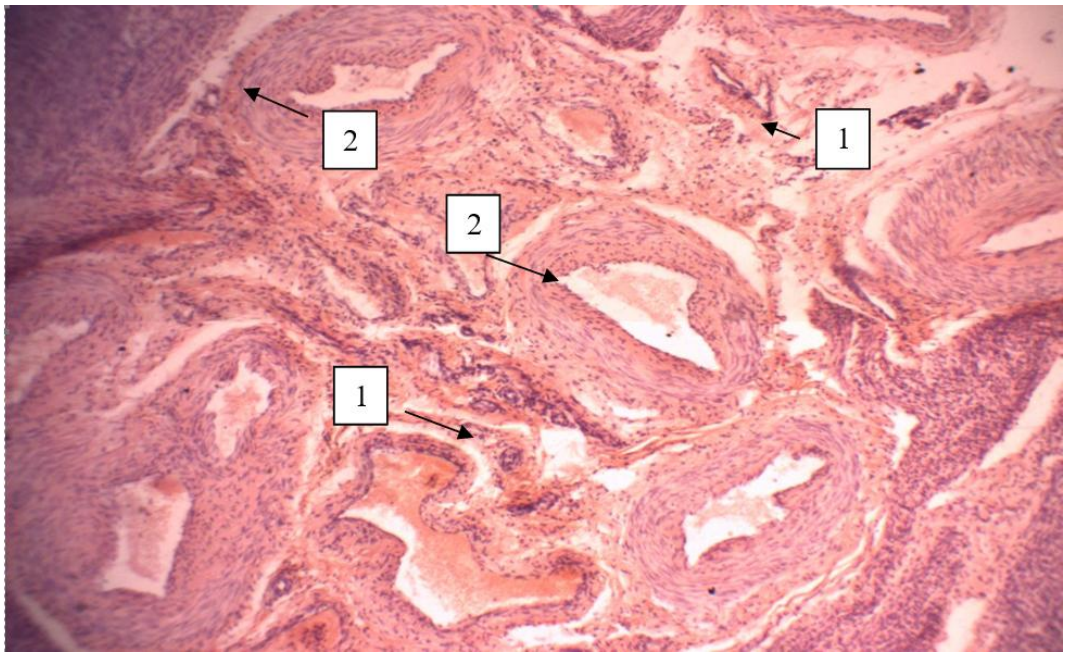
The histological examination of endometrium has shown | that in during metoestrus (7-8 day of ovary cycle) shows

absence of strict differentiation of functional layer on compact and spongy. Surface epithelium is flat or wavy, in areas of caruncle partially desquamated (pic 10.). Endometrium deep layer had a small number of unformed uterine glands, individually present in stroma cells; whereas the surface layer was close to be without glands (pic.10,11,12).

Dystrophic degradation of endometrium functional layer has been registered, they were shown by destruction of compact and spongy layers with strong thinning and dissolution of strict zoning. It, on our opinion, caused by hormone dependant cell desquamation of endometrium compact and spongy layers.

During meteoestrus, we have found, massive apoptic death, accompanied with karyopyknosis, karyorhexis and hyperchromatosis of cytoplasm. It is probably caused by development of hypoxic surface areas of functional layer, caused by vessel territories distress. Together with stroma cells apoptosis, we observed spindle cells with mitotic division, it proved that together with apoptosis were present the processes of proliferation, (pic.13).

Histochemichal analysis of endometrium during metoestrus (7-8 day of ovary cycle), shows tht deep functional layer during the period of living body development, there is no red-pink coloring specific for estrus (pink and yellow colors are dominant), proving the intensive loss of surface glicosaminglycans and high reactivity of acid proteoglicans in deep phase of collagen, whereas accumulation of protein-carbohydrate complexes in spongy layer is still present. Changes in proteoglycan matrix of connective tissue are found in surface areas of functional layer, they have a tendency to increase the number of cells with rich red coloring of comparatively deep layer (pic.14), it is probably connected with simultaneous dystrophic degenerative processes and regenerative processes into the stage of yellow body.



Pic.10 – Endometrium compact and spongy layers, metroestrus (7-8 day of ovary cycle) (colored by hematoxylin, 10x10).

1. areas of stroma cells and gland epithelium division

2. Endometrium arteriolas are spread, excessively wound

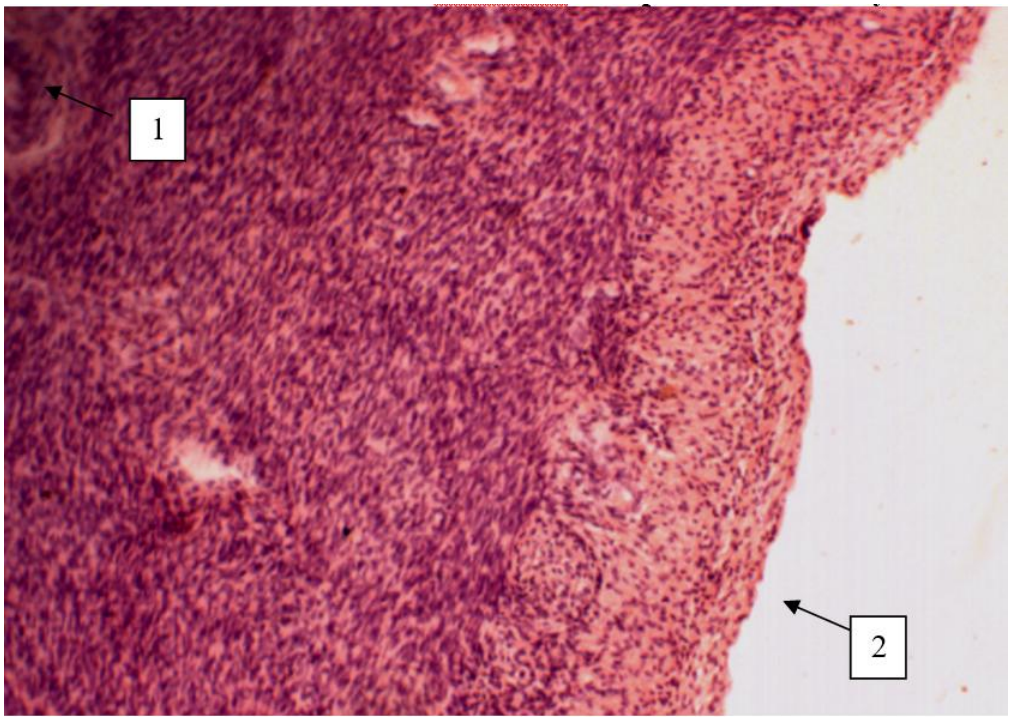
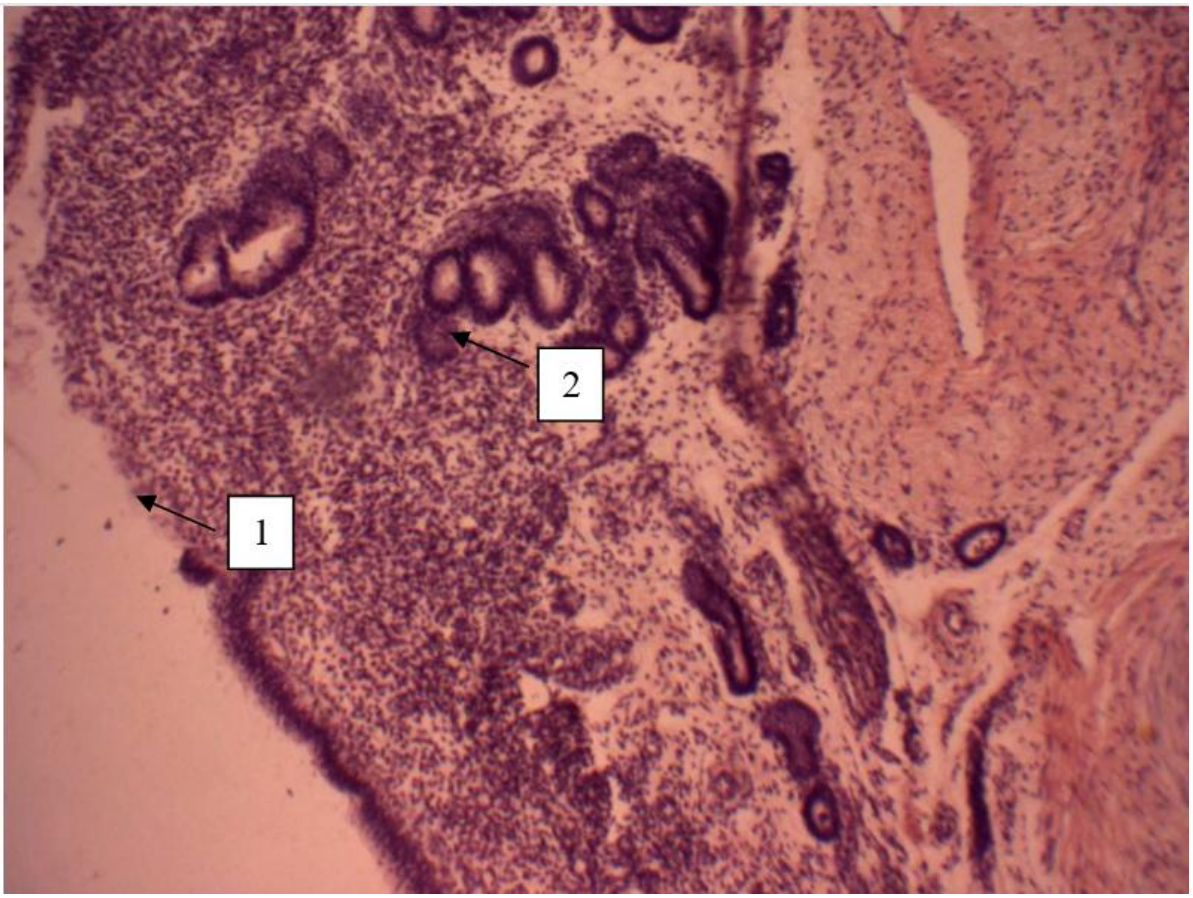


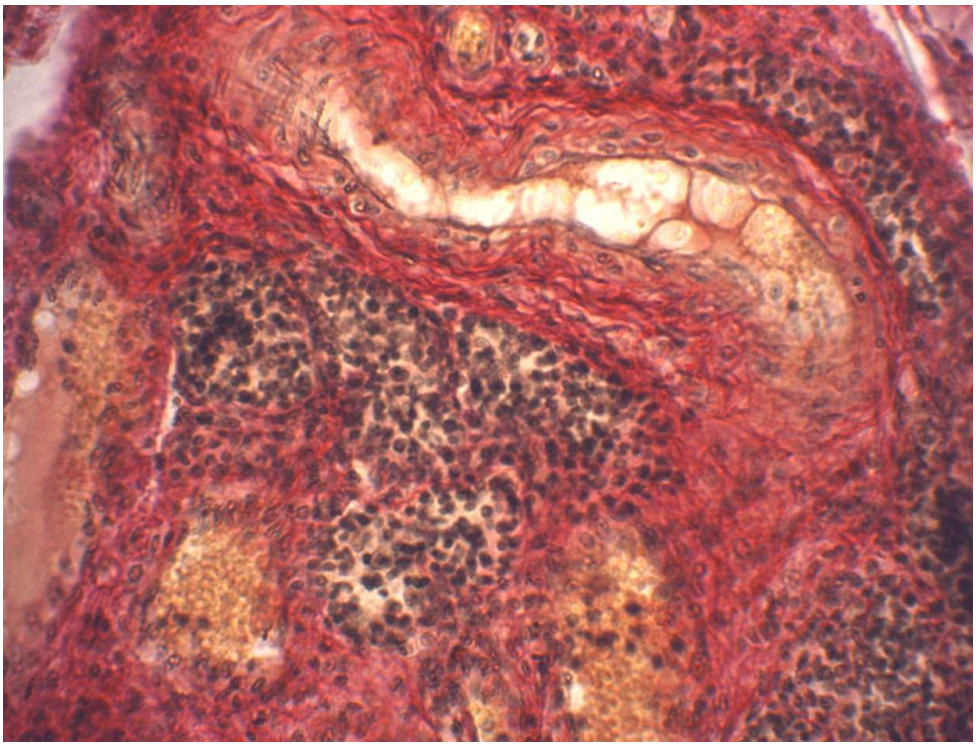
Рис. 11 – Endometrium compact and spongy layers in border caruncula area, metroestrus (7-8 day of ovary cycle) (colored by hematoxylin, 2x10).

1. Fragment of unformed uterine gland of deep layer; 2. Endometrium surface epithelium is slight wavy.

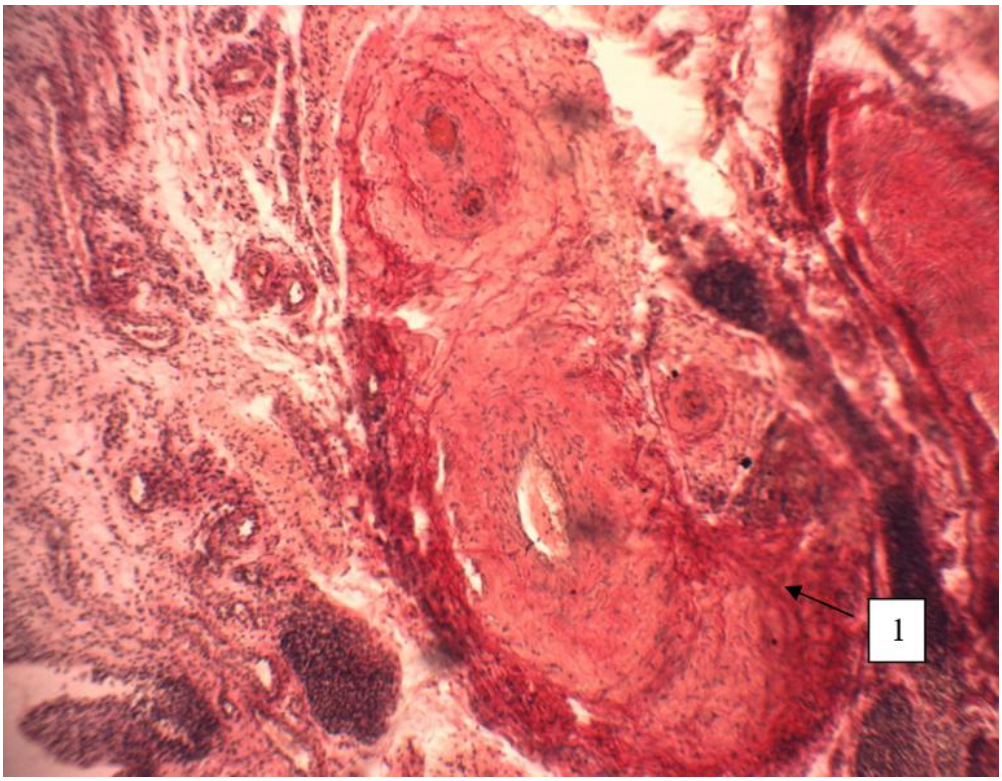


Pic. 12 – Endometrium compact and spongy layers of caruncula, metroestrus (7-8 day of ovary cycle) (colored by hematoxylin, 1x10).

1. areas of desquamated surface epithelium;2. unformed uterine gland of deep layer;



Pic. 13 – Blood vessels of endometriym spongy layer (sept,) metroestrus (7-8 day of ovary cycle) (colored by hematoxylin,10x10).



Pic. 14 Endometrium compact layer, metroestrus (7-8 day of ovary cycle) (colored with picro-fuchsin, 31x10).
1-Accumulation of protein-carbohydrate complexes is still present in spongy area of functional layer.

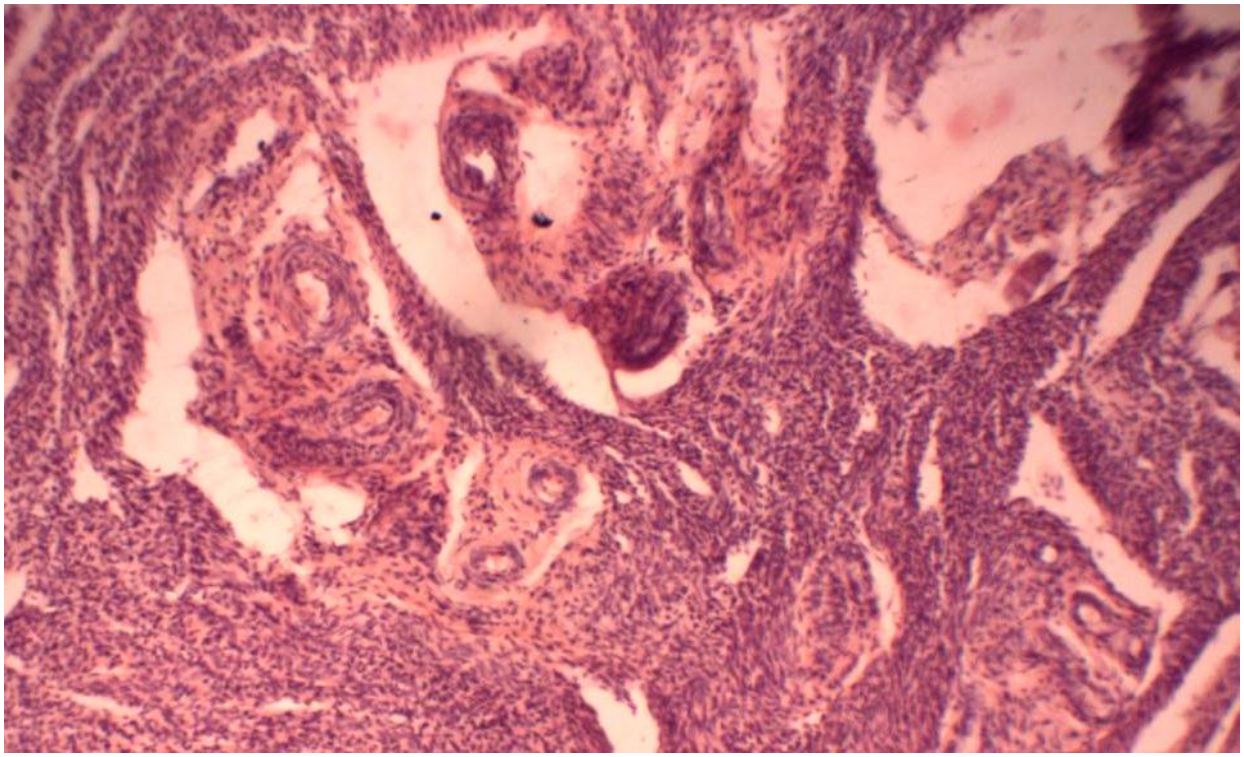
The histological examination of postmorbid endometrium shows differentiation of stroms cells, vessels and

uterine glands. There is no visible differentiation of functional layer on compact and spongy, allocation of uterine glands in stroma is chaotic and irregular. Focality and multi colors are present in morphological picture of functional layer, it has glands of different development stage and areas of stroma inappropriate for any stage of ovary cycle (pic.15-19). There are individual, having signs of dystrophy and pseudostratification cells of surface epithelium, desquamated, their nucleus are partly destroyed (pic. 16).

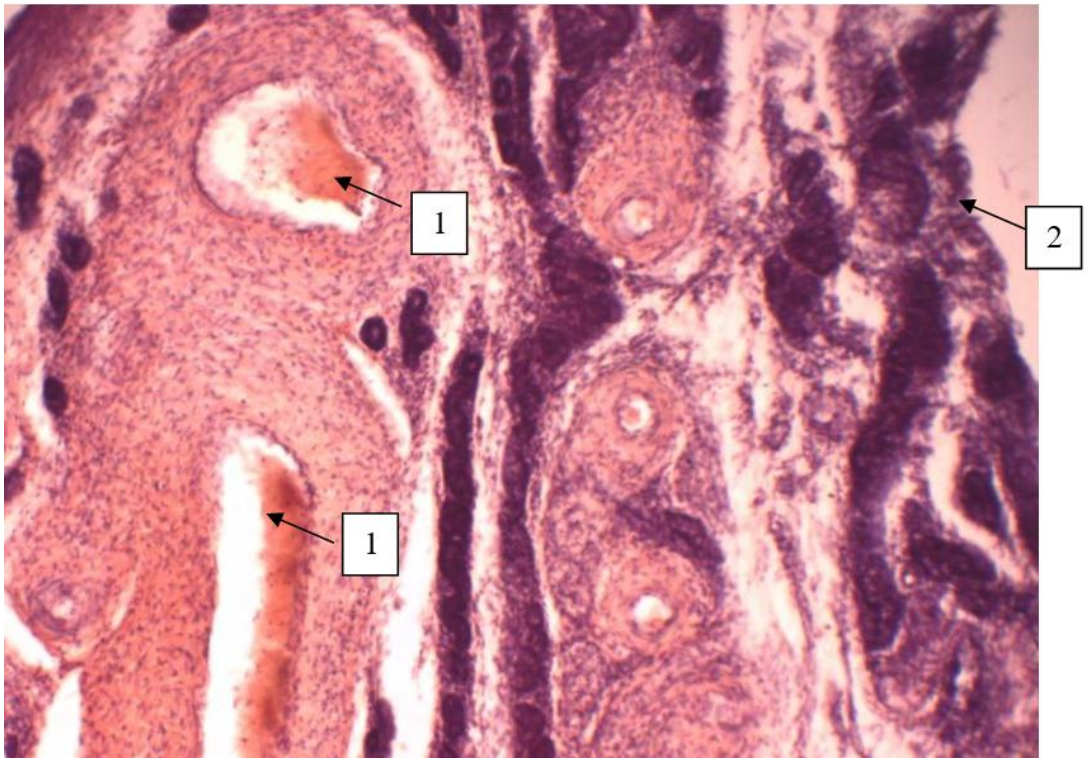
In most casese uterine glands had different shapes, with atrophic epithelium covering, strongly twisted with numerous brunches, being so close to each other, sometimes pushing out the stroma (pic.16,18). Gland epithelium is explanate, low, prismatic. Cell nucleus are prolate and e[tanded with signs of polymorphism. Scientists consider that anaplastic cells of gland epithelium lose the ability for adequate answer on hormonal stimulation (Tomitova, 2011).

As it is known, condition of spiral arteries is the most important factor for significant characteristics determining adequacy of gestagenic effect. Studing the spiral arteries of postmorbid epithelium was found a distopography and disorganization of territory vessels, thickening of walls caused by intimal hyperplasia, prevascular fibrosis. The stasis and obliteration is present, in vessels old thrombotic masses are present (рис.16, 17). On our opinion, postmorbid endometrium is formed because of the discord in hierarchy central mechanisms of feedback, causing slowdown and dissonance in circulating sex hormones influence. Histochemichal analysis of postmorbid endometrium showed prevalence of yellow colors in functional layer. It proves the intensive loss of surface glicosaminglicans and high reactive activity of acid proteoglicans of deep stage fibrous proteins matrix (pic.16-18). According to scientific data, endometrium cells in physiological condition characterized by fuchsinophily being colored with picro-fuchsin, because of reactive activity of basic proteins, absorbing picric acid from picro-fuchsin mixture coloring them in yellow. Surface micro phase of postmorbid endometrium cells, loses acid proteins which bind basic fuchsin, that is why they are colored in yellow (Selivanov, 2003).

On our opinion condition of posmorbid endometrium is a result of compensatory adaptive reaction of tissue reaction, as an answer to apoptosis disorders and changes in irritability of receptors to sex hormones.

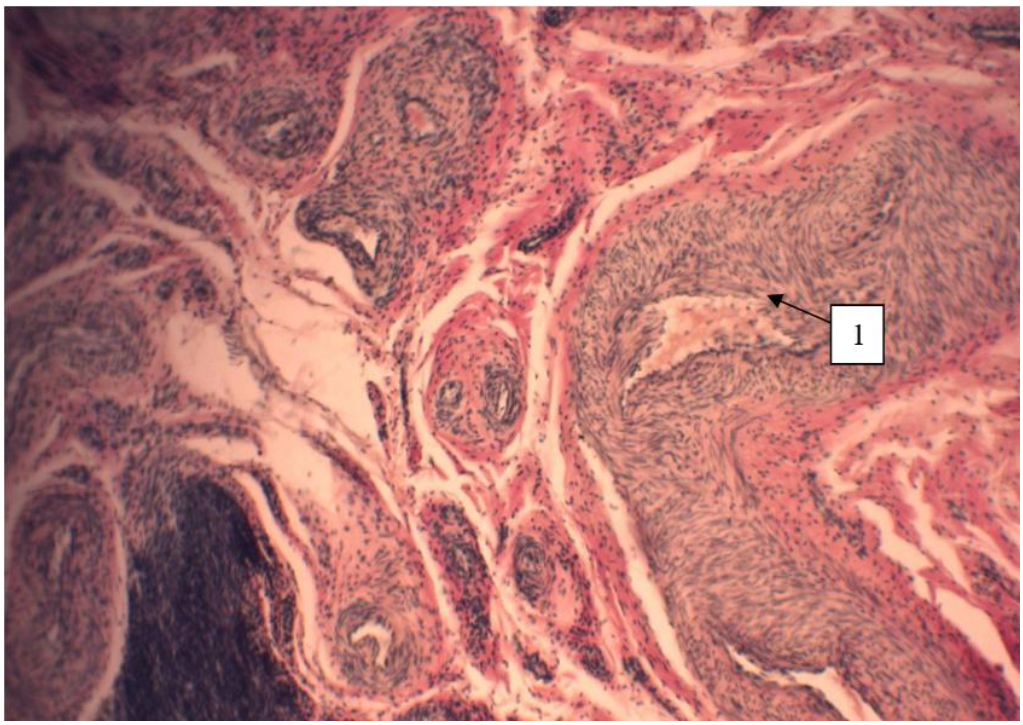


Pic. 15 – Endometrium compact layer and spongy layers, postmorbid condition colored by hematoxylin, 2x10).

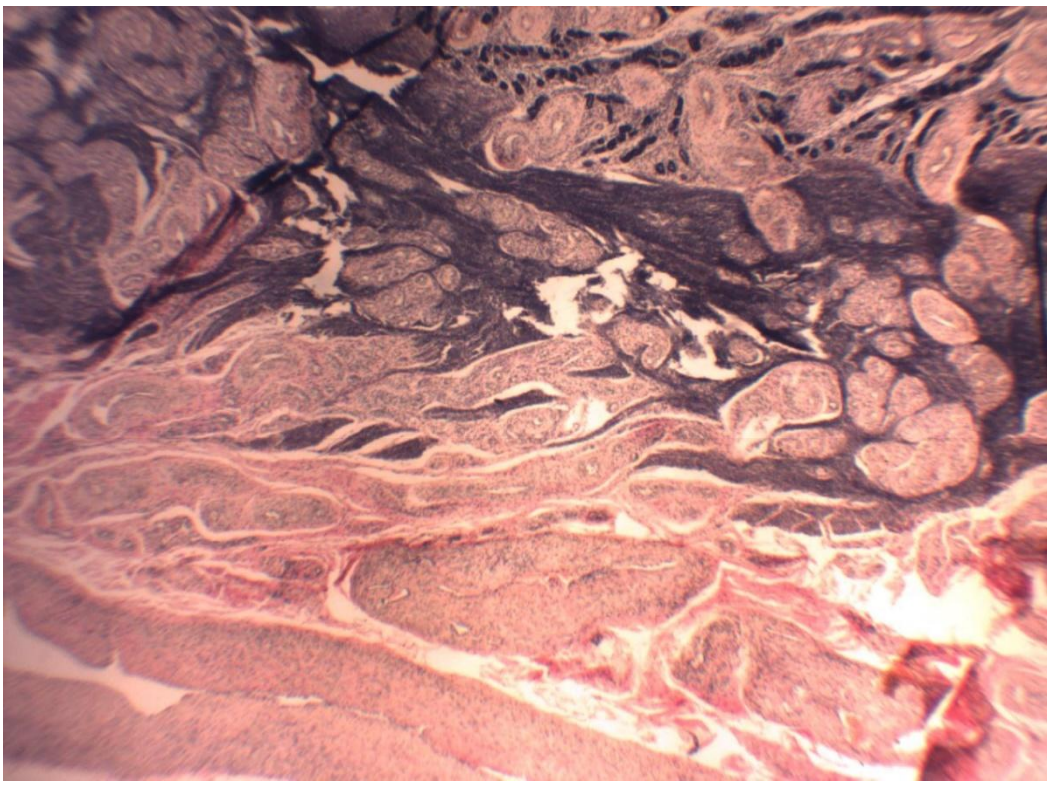


Pic. 16 – Blood vessels in endometrium spongy postmorbid condition (colored by hematoxylin, 1x10).

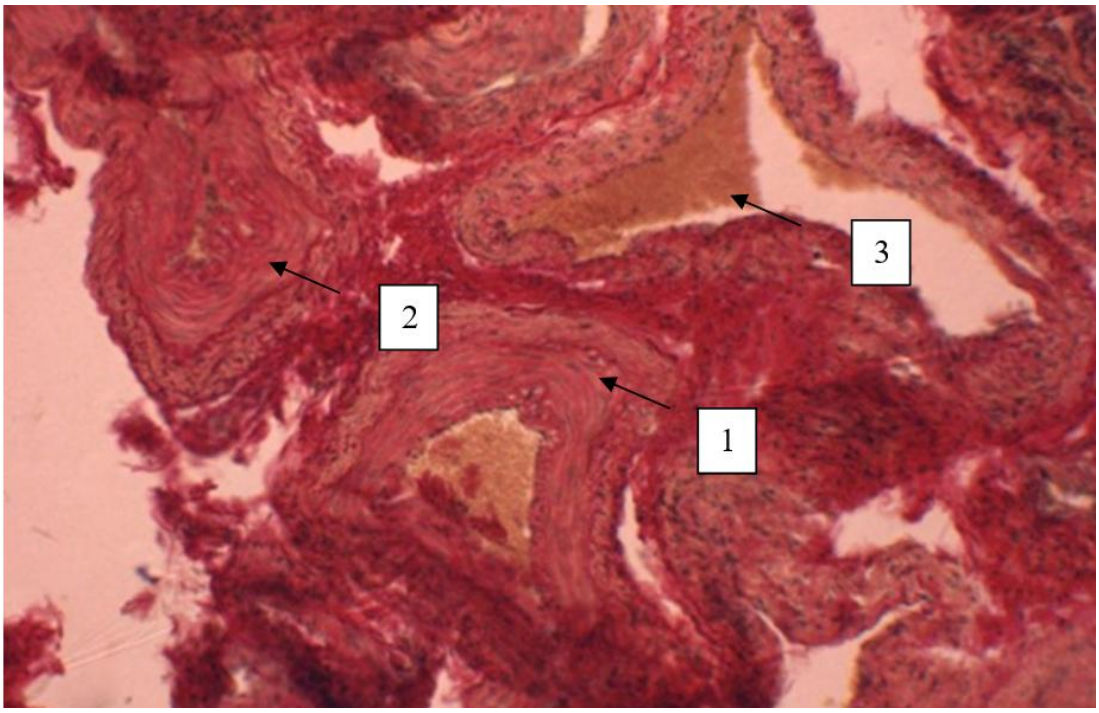
1. Stasis and post thrombotic masses in vessels; 2. Surface epithelium cells.



Pic. 17 – Blood vessels in endometrium spongy postmorbid condition (colored with picro-fuchsin, 07 x40). 1- Perivascular fibrosis



Pic. 18 – Endometrium compact layer and spongy layers, postmorbid (colored with picro-fuchsin, 01 x04).



Pic. 19 – Endometrium compact layer and spongy layers, postmorbid (colored with picro-fuchsin, 01 x04).

1. Intimal hyperplasia; 2. Stasis and obliteration; 3. Old intravascular (organized) thrombotic masses with loss of glicosaminglycan phase and which reactive activity of acid glicosaminglycans.

Conclusions.

1. During estrus high functional activity, activation of physiological secretory processes and intensification of protein lipid metabolism, with accumulation of protein carbohydrate

mixtures in matrix, is present.

2. In endometrium during the predicted proestrus, we can find an activation of regeneration processes, together with the beginning of remodulation of connective tissue matrix endometrium. Such condition accompanied with accumulation of surface glicosaminglycans and increase of reactive activity of acid proteoglycans of deep phase of fibrous matrix proteins.

3. Endometrium of cows during metestrus is charachterized by simultaneous dystrophic degenerative changes caused by hormone dependant cell desquamation, and cell proliferation initiation.

4. In postmorbid condition endometrium shows differentiation of stroma cells, vessel territories and uterine glands, reduction of proliferation processes, depression of regenerative ability and neoangenogenesis.

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Структурно-морфологічні зміни ендометрію відносно стадії статевого циклу та стану статевої функції корів

У статті проаналізовано структурно-морфологічні зміни ендометрію відносно стадій статевого циклу та стану статевої функції корів.

Задачею наших досліджень було визначення та обгрунтування структурно-морфологічних змін ендометрію залежно від стадій статевого циклу та стану статевої функції маточного поголів'я корів дослідних господарств під час прояву ними статевої циклічності.

Матеріалом для досліджень були фрагменти ендометрію верхньої третини рогів матки корів. Зразки тканин ендометрію відбирали у вимушено забитих тварин віком 3-10 років, на 0 день статевого циклу (еструс), 7-8 день статевого циклу (розквіт жовтого тіла), 17-18 день статевого циклу (передбачувана тічка), а також, у клінічно здорових тварин, що перехворіли на ендометрит та затримку посліду. Для оглядової мікроскопії фарбування гістологічних препаратів проводили гематоксилін-еозином, а для вивчення структури сполучної тканини - пікрофуксиновою сумішшю за Ван-Гізоном.

Встановлено, що під час еструсу реєструється висока функціональна активність, активація фізіологічних секреторних процесів та інтенсифікація білково-ліпідного обміну клітин СОМК, що супроводжується депонуванням у матриксі білково-вуглеводних сполук. В СОМК під час передбачуваного проеструсу спостерігається активація регенеративних процесів, які супроводжуються початком розвитку ремодуляції сполучнотканинного матриксу ендометрію. Такий стан проявляється накопиченням поверхневих глікозаміногліканів та збільшенням реакційної здатності кислих протеогліканів глибокої фази фібрилярних білків матриксу.

Ендометрій корів під час метеструсу характеризується поєднанням одночасних дистрофіко-дегенеративних змін, пов'язаних з гормонозалежною клітинною десквамацією, та ініціацією клітинної проліферації. За постморбідного стану СОМК, спостерігається роздиференціювання клітин строми, судиннх територій та маткових залоз, зниження процесів проліферації, пригніченням регенеративної здатності та неоангіогенезу.

Перспективою подальших досліджень е необхідність з'ясування механізму розвитку морфофункціональних змін постморбідної СОМК за неплідності корів та опрацюванні на цій основі обґрунтованих методів корекції.

Ключові слова. *корови, структурно-морфологічні зміни ендометрію, постморбідна СОМК.*

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