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**Clinical and morphological aspects of female reproductive system
with uterine leiomyoma after ulipristal acetate intake as
preoperative preparation**

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Introduction

Uterine leiomyoma (UL) is hormone-dependent tumor of myometrium. Topicality of reproductive health of women after surgery is determined by the tendency to the increase number of patients who have uterine leiomyoma, whom 50-55 % of clinical cases surgical treatment is indicated to renew fertility [3].

Nowadays in myoma (muscular tumor) pathogenesis complementary interrelation between estrogen and progesterone is necessary and important, which realize its influence on target tissues due to the mechanism of lipophilic hormones through specific cytosol receptors that initiate the linking of these steroids with receptor. At first there is an activation of hormone-receptor complex, which replaces into nucleus, links with acceptor areas of chromosome and provides gene's transcription. Messenger RNA that forms is designed for synthesis of specific proteins that define biological effect of steroid. One of these specific proteins is progesterone receptor. Estrogens are necessary for detection biological effect [16, 19].

In modern literature there are no practical recommendations for preoperative patients' management who are planned for conservative myomectomy, however there are medications which block progesterone receptors. One of these medications is ulipristal acetate (UA), which acts directly on progesterone receptors in leiomyoma, endometrium, hypophysis, inhibits ovulation without influence on the level of estrogen production and glucocorticoids [17]. Suppressing proliferation processes and strengthening apoptosis of leiomyoma cells, it does not act on outer layer of myometrium. Interaction of ulipristal acetate (UA) with progesterone receptors in the adenohypophysis does not affect prolactin and adrenocorticoid hormone production but suppresses follicle stimulated hormones production and luteinized hormones production. [7].

UA («Esmiia») refers to selective modulators of progesterone receptors of the second generation. Clinical examination which was done in 18 research centers in 4 countries of Europe, contained women of reproductive age who had at least one uterine leiomyoma from 3 to 10 s-m in diameter, uterus size was to 16 weeks of pregnancy, it was accomplished by menstrual bleeding, which indicated for uterine leiomyoma

surgery. Patients got four courses of three month treatment of UA in 10 mg /per day. After four courses of treatment the frequency of amenorrhea was 90%, the size of uterine leiomyoma decreased on 72%. Considerable and stable recession of myomatous nodules allowed refusing surgery in some part of patients [8].

Molecular and biological mechanisms of selective modulators of progesterone receptors, to which UA also refers, were investigated considerably in vitro [7]. UA inhibition of proliferative activity and apoptosis of leiomyocytus induction was proved, it was decreased the production of growth factor and collagen combined with synthesis of proteinase. Recently there are some papers where morphological changes in uterine leiomyoma (UL) after ulipristal acetate influence were described on clinical material [1,4,5].

In endometrium in most part of patients under UA influence there are reversible changes (during several weeks even after four courses of treatment) benign tumors, that received name ((progesterone receptor modulators) PRM-associated endometrial changes – endometrium changes which associated with progesterone receptors), that leads to amenorrhea. Menstrual period restores in 4-5 weeks after treatment. UL size can be to 6 months. Besides, UA treatment (therapy) decreases pain, which is associated with UL, improves woman's life [18].

It is known progesterone has protective action on estrogen-induced proliferation of endometrium, and endometrium is one of the main tissue targets for modulators of progesterone receptors. So, long lasting therapy by medications can be a cause of endometrium hyperplasia [11]. Unlike classic modulators of progesterone receptors, UA modulates the activity of progesterone receptors, epithelium of glands and cells of endometrium stroma, but doesn't block their activity as for example antigestagens [10]. As a result estrogen's influence on endometrium cells hyperplasia doesn't develop and form specific and reversible morphological changes of endometrium with signs of combined estrogen (minimally expressed proliferation) and progesterone (secretory changes) influence [18].

Performed investigations indicated, that in future this medication can realize individual therapeutic action and be a part of patient-specific (personified) treatment of uterine leiomyoma, and also other hormone-dependent diseases of female reproductive system.

The **aim** of our investigation was to decrease disorders of female reproductive function and save fertility in women after conservative myomectomy by establishing in clinical practice preoperative preparation of patients by UA medication and perform morphological investigation of myomatous tissue and endometrium in patients with UL after UA intake.

Materials and methods

The investigation was done during three months (January-March 2016) in the department of minimally invasive surgery of DNU" of Scientific Practical Centre of Preventive and Clinical medicine" of Government Control. General clinical, lab and special screenings were used: instrumental (ultra sound), immunological, biochemical, literature search, parametric and non-parametric methods of statistic analysis. Histological and immunohistochemical investigation was done, myomectomy was used, 9 patients after three month treatment by UA 5 mg per day (main group) were involved. Age of women was from 26 to 40 (average age was $35,8 \pm 4,2$). Experimental group contained 15 uterine leiomyoma in women without preoperative hormonal therapy or contraception. Patient's age was from 29 to 42 (average age was $38,6 \pm 3,4$). Patients of main group had sizes of dominant nodules before treatment contained $8,6 \pm 1,4$, after therapy was - $4,8 \pm 1,2$; experimental group had sizes of dominant node contained $5,2 \pm 1,54$. Nodules localized instrumentally and submucously in both groups. According to histological structure all nodules were simple uterine leiomyoma.

Parts of leiomyoma tissues and endometrium were fixed in 10% solution of neutral buffered formalin. Tissue processor STP-120 (histocentre) was used, EC-350 was used for perfusion of paraffin blockings, HM - 340E (Microm, Hamburg, Germany) was utilized for cutting paraffin blockings. Histological preparations were coloured by hematoxylin and eosin. Microscope Axioskop 40 with camera AxioCam MRc5 (CarlZeiss) was used.

Immunohistochemical investigation of estrogen receptors (DAKO, EP1), progesterone (DAKO, PgR636), marker of proliferative activity Ki-67 (DAKO, SP6), apoptosis inhibitor Bcl-2 (BCL-2 alpha Ab - 1 (100 / D5) was done in the tissue of leiomyoma in serial paraffin sections of 4-5mcm, and also visualization systems EnVision FLEX (DAKO) with diaminobenzidine was used. Coloring process by serial cycles of incubation of reagents and cleaning on beads was done in autostainer of Thermo Scientific. Preparations were colored by hematoxylin (hemalum). Tiny brown granules in the areas of antigen are the product of immunohistochemical reactions.

For estrogen and progesterone receptors, Ki-67 are nucleus of cells, for Bcl-2 – is cytoplasm and nucleus of cells.

Results of immunohistochemical reactions were estimated due to semiquantative morphometric method [15]. Intensity of coloring cells was visually estimated in grades from 0 to 3 (negative, poor, moderate, and expressed reaction) and the percent of positive coloring cells was calculated at each intensity point of coloring (1000 cells in 10 point of vision with the most expressed immunohistochemical reaction at extending microscope 400).

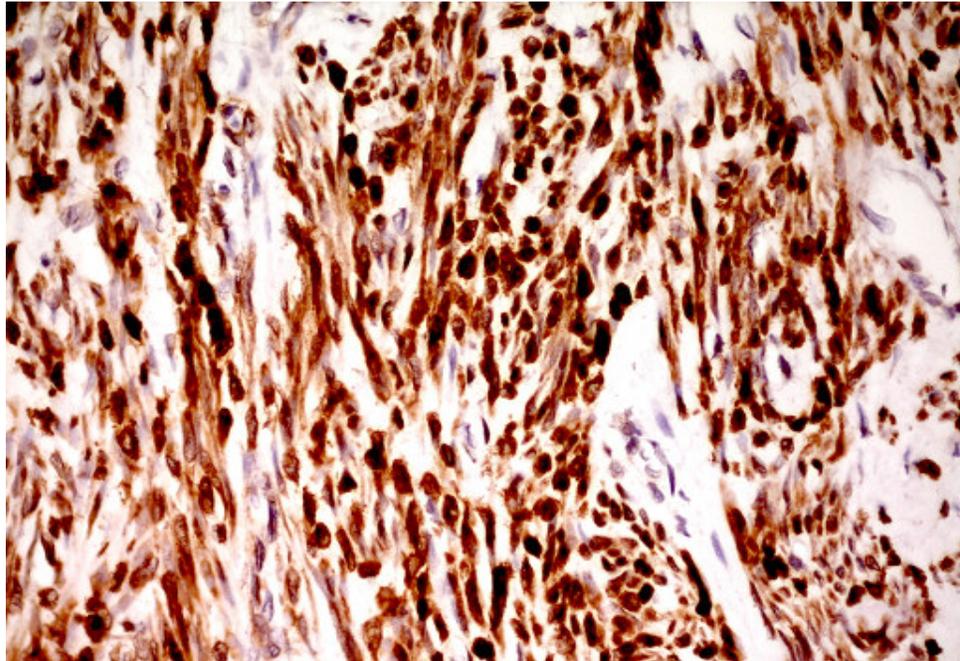
Medial area of expression in percents was also determined such as area ratio with immune positive cells/nucleus to general area cells/nucleus in the point of vision. Received parameters identify synthesis intensity and accumulation of investigated hormones and signal molecules in cells and tissues.

Results

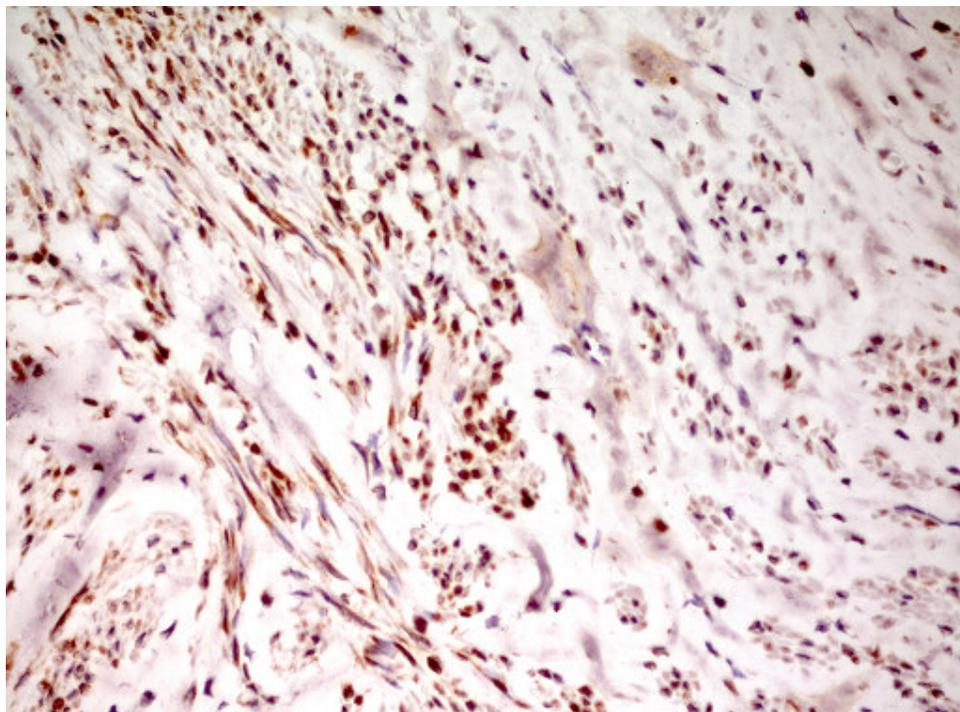
In group of patients with uterine leiomyoma without preoperative hormonal treatment progesterone receptors expressed in $76,4 \pm 6,8\%$ nuclei (**Picture 1**). Estrogen receptors were determined in $32,8 \pm 2,6\%$ nuclei. Our information coincides with V. O. Potapova information (2014), who define that myoma samples were characterized by considerably increased expression of progesterone receptors in comparison with estrogen ones [2].

In experimental group of patients after UA therapy it was determined smaller sizes of smooth muscle cells and nuclei in UL; it was also detected focal sclerosis and hyalinosis of stroma of myomatous nodules. Reliable decrease of expression of progesterone receptors: $36,8 \pm 1,28\%$ ($p < 0,05$) (**Picture 2**) and unreliable decrease of expression level of estrogens was determined: $30,7 \pm 3,4\%$ ($p > 0,05$). Such tendency was described earlier when preparation intake with antiprogesterone effect – mifepristone; in immunohistochemical investigations considerable decrease number of progesterone receptors was determined, while level of estrogen receptors didn't change that allowed regressing of myomatous nodule in the result of direct antiprogesterone influence [9]. A. L. Tykhomyrov and co-authors (2014) note the increase of expression of progesterone receptors by nuclei of smooth cells at maintenance of level of estrogen receptors in patients who received UA [5]. According to authors' point of view there is a tendency to increase expression of progesterone receptors by cells of leiomyoma after UA therapy. It can be compensatory process. At this moment it is difficult to explain the mechanism of UA as selective modulator of progesterone receptors. Progesterone stimulates myoma growth, through the set of key genes that regulate apoptosis and proliferation. Progesterone stimulates growth factor (EGF) in cells of leiomyoma and inhibitor of apoptosis protooncogene bcl-2, as a result expression of proliferation markers in leiomyoma cells can increase, and apoptosis activity decreases. So, progesterone can affect myoma growth due to apoptosis

inhibition that causes increase of life cycle of tumoral cells, and also increases their proliferative activity [13].



Picture 1. Presents expression by nuclei of smooth muscle cells of leiomyoma of progesterone receptors. Patient is without hormonal treatment. Immunohistochemical investigation. X200.

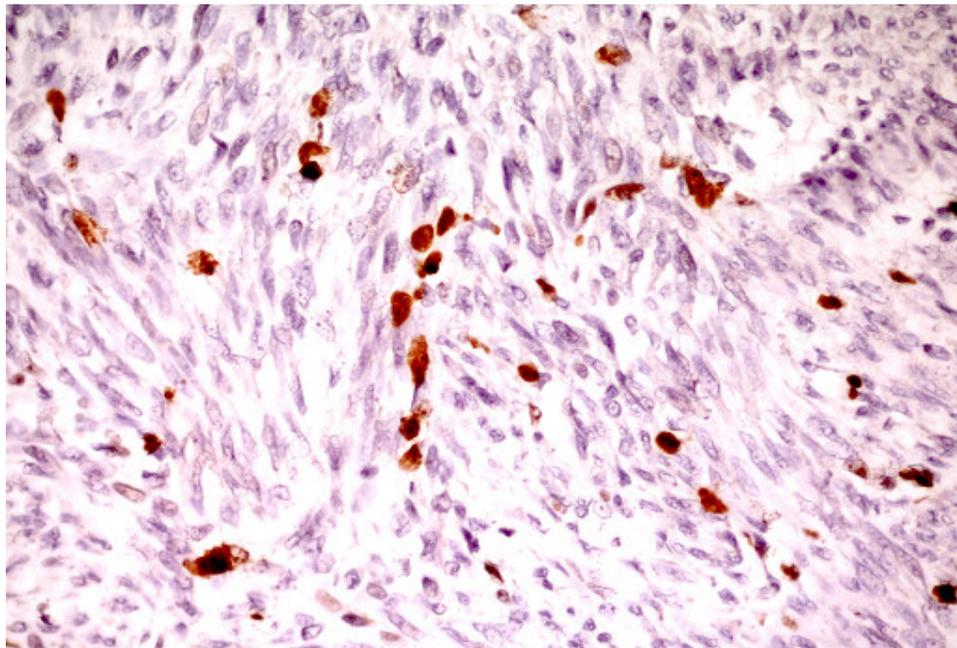


Picture 2. Presents expression by nuclei of smooth muscle cells of leiomyoma of progesterone receptors. Patient is after UA intake. Immunohistochemical investigation. X200.

Apoptosis inhibitor marker bcl-2 in control group was detected in $65,4 \pm 7,2\%$ cells. Considerable decrease apoptosis inhibitor bcl-2 - $42,6 \pm 3,2\%$ ($p < 0,05$) was detected in patients after UA intake, that do not coincide with A. L. Tykhomyrov data who note only low-level and statistically decrease of apoptosis inhibitor expression bcl-in the second group of patients who received UA [5].

Marker of proliferation Ki-67 in patients without hormonal treatment was determined in 11,8% of nuclei of smooth cells (**Picture 3**), and in women who got UA in 7,2% of leiomyoma cells (**Picture 4**), that coincides with A. L. Tykhomyrov data [5], who detected statistically decrease of proliferative activity of smooth muscle cells in patients after UA intake.

Endometrium morphology after UA intake is similar to estrogen-induced changes of endometrium or its hyperplasia, although structure's changes of glands, their epithelium, stroma and vessels of endometrium form specific morphological picture.



Picture 3 presents expression by nuclei of smooth muscle cells of leiomyoma of Ki-67. Patient is without hormonal treatment. Immunohistochemical investigation. X400.

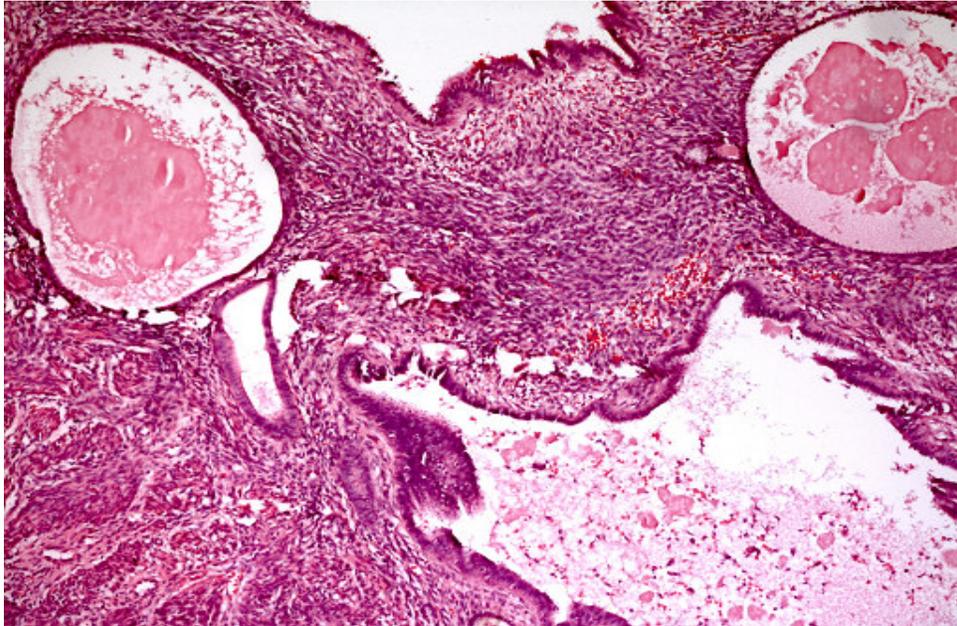
Picture 4. presents expression by nuclei of smooth muscle cells of leiomyoma of Ki-67. Patient is after UA intake. Immunohistochemical investigation x400.

In majority cases structure and location of glands varies in one biopsy material, major part of glands are considerably enlarged. Sulcated glands can be present sometimes festoon, star-shaped, which are responsible for secretory phase, and direct glands with narrow lumen. Cystous glands can be surrounded «collar» with dense localized stromal cells of augment shape. Hydrous secret is accumulated often in cystous glands (**Picture 5**). At progesterone receptor modulators (PRM), epithelium is inactive, or poor proliferative, with single mitosis (indirect nuclear division) (**Picture 6**). While investigating marker of proliferative activity Ki-67 in patients, who took UA, poor expression was detected in comparison with patients without hormonal treatment of uterine leiomyoma (**Picture 7, 8**).

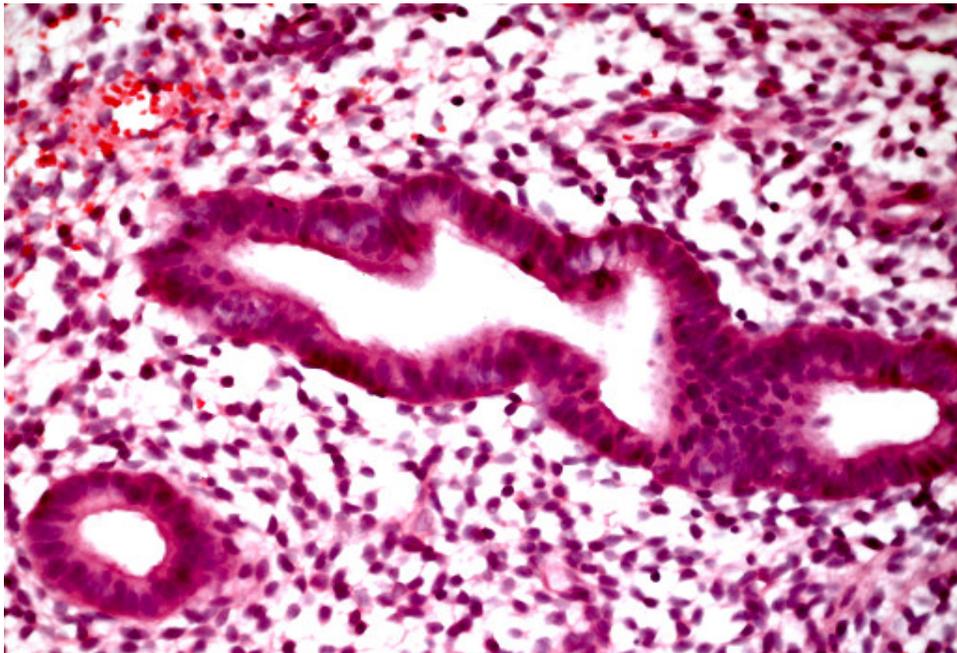
However the structure of glands is responsible for secretory phase, glandular epithelium is usually flattened, cuboidal, or prismatic without signs or with poor expressed nuclei stratification with single mitosis

(indirect nuclear division) (**Picture 7**). Part of epithelial cells is located in cytoplasm of basal vacuole; secretory epithelium is present with signs of apocrine secretion, especially in cystic glands.

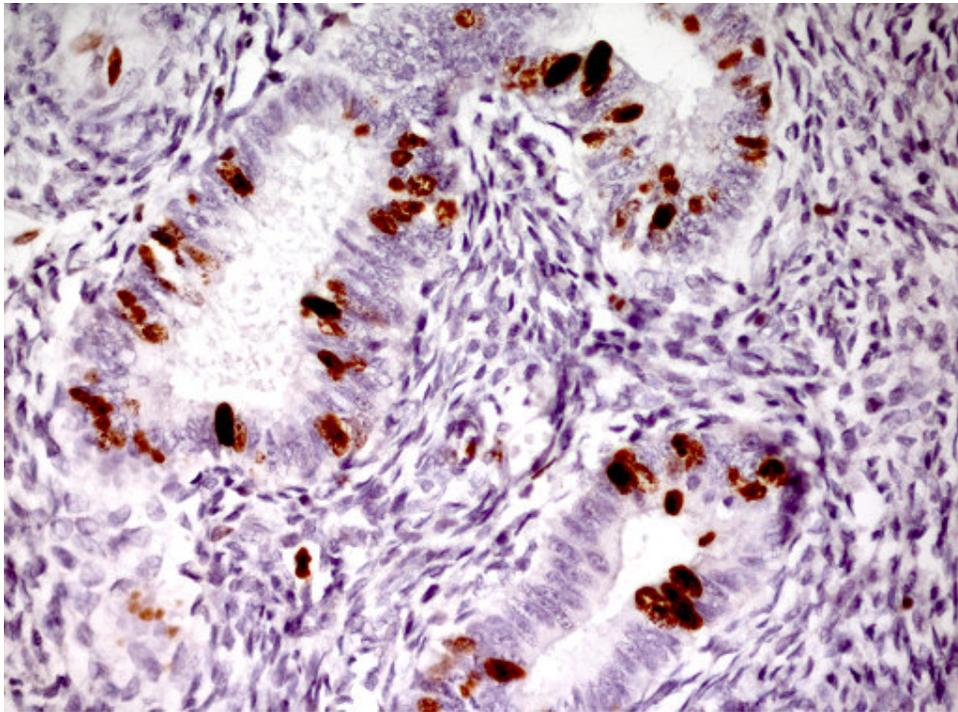
There is stroma with dense cell location, low proliferative activity, without signs of perivascular reaction. Glandular correlation is not disrupted but glands are distributed unevenly. Arteries accumulation is with thickened walls.



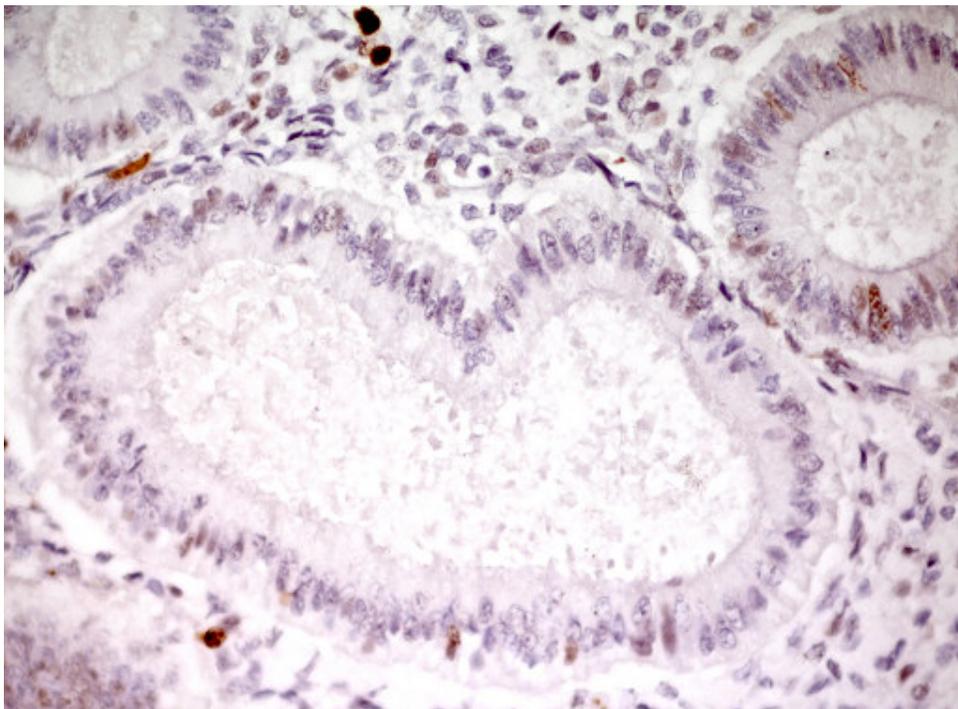
Picture 5 presents endometrium of patient after UA intake. Cystic glands are enlarged with poor proliferative flattened epithelium. Coloring with hematoxylin and eosin. x200.



Picture 6. Presents endometrium of patient after UA intake. Endometrium glands are with poor proliferative epithelium. Coloring with hematoxylin and eosin. x200.



Picture 7. presents expression by nuclei of epithelial cells of endometrium of Ki-67. Patient is without hormonal treatment. Immunohistochemical investigation x400.



Picture 8. Presents low expression by nuclei of epithelial cells of endometrium of Ki-67. Patient is after UA intake. Immunohistochemical investigation x400.

Progesterone receptor modulators (PRM-associated endometrial changes – endometrium changes) which associated with progesterone receptors are present in 60% of patients with symptomatic uterine leiomyoma, who were treated during three months by UA. Endometrium changes are reversible and regress independently during several weeks after therapy [18]. The main cause of progesterone receptor modulators (PRM) is damage of size and shape and also poor proliferative flattened and inactive secretory or neutral epithelium [14, 16,18]. At estrogen-induced changes of endometrium or its hyperplasia enlarged glands are lined considerably proliferative, not flattened epithelium with signs of pseudostatification and mitosis figures; epithelium of major part of glands is characterized for middle or late stages of proliferation phase.

Conclusions

Patients who took UA in smooth muscle cells of leiomyoma are accomplished by decrease of expression of progesterone receptors, markers of apoptosis inhibitor bcl-2 and proliferative activity of protein Ki-67. So, in smooth muscle cells of myoma under UA influence there is decrease of progesterone receptors number, so its action reduces as a result, apoptosis provocation and decrease of proliferation processes so there is involution of uterus.

Endometrium during UA therapy has characteristic changes, which should be differentiated with estrogen-induced changes or hyperplasia, so in the direction to pathohistological investigation it is necessary to indicate UA therapy. Pathologist who is incompetent with progesterone receptor modulators (PRM) morphology can diagnose improperly estrogen-induced changes of endometrium or its hyperplasia for which damages of structure and glands location with cystous enlargement of their lumen that can precipitate incorrect patient's management.

In **perspective** it is necessary to continue study of morphological changes, in particular, immunohistochemical peculiarities of myomatous tissue and endometrium in patients after UA (ulipristal acetate) therapy for full study of mechanism of its action.

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