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Research Article

Effects of Preoperative Preparation on the Vaginal Mucosa in Women with Genital Prolapse Associated with Genitourinary Menopausal Syndrome

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Abstract

Objective: To analyze the effect of preoperative preparation on vaginal architectonics and vaginal microbiota composition in women with stage III, IV genital prolapse POP-Q in combination with genitourinary menopausal syndrome.

Methods: A prospective randomized trial was conducted on the basis of the gynecological department of the Federal State Budgetary Institution of Research Institute of OMM of the Ministry of Health of Russia in the period 2018-2020. The study included 100 women aged 52 to 79 years with prolapse of the anterior vaginal wall of stage III, IV according to POP - Q. The first group are women who were given local hormone therapy (estriol and progesterone) in combination with lactobacteria at the preoperative stage (n = 35); the second group - local monotherapy with estriol (n = 35), the third group (control) - without previous preparation for surgery (n = 30). Material for morphological and immunohistochemical examination - biopsy of anterior vaginal wall mucosa was obtained during surgery. To determine the state of the receptor apparatus of the vaginal mucosa (ERα, ERβ), an immunohistochemical study was carried out. Real-time diagnosis based on polymeresis chain reaction was used to determine vaginal microbiocenosis.

Results: It has been found that the use of vaginal forms of estrogen and progesterone in combination with lactobacteria reduces the intensity of the inflammatory process in the vaginal mucosa, reduces the severity of dystrophic changes, and also statistically significantly increases the level of ERα expression and ERβ in all layers of the vaginal epithelium. Comparative characterization of *Candida* spp. after treatment, the observation groups showed a significantly lower number of yeast-like fungi in the first group with a statistically significant increase in lactobacillary flora.

Conclusion: This study revealed a significant positive effect of preoperative vaginal preparation using complex therapy with estriol, progesterone in combination with Lactobacillus on architectonics, local vaginal immunity and vaginal microbiota in women with severe postmenopausal genital prolapse.

Recommendations: As a local preoperative preparation of the vaginal mucosa, it is recommended to give preference to a complex approach - the use of local forms of estriol and progesterone in combination with lactobacteria.

Keywords: Genital Prolapse; Genitourinary Menopausal Syndrome

Abbreviations

GP: Genital Prolapse; GMS: Genitourinary Menopausal Syndrome; POP-Q: Pelvic Organ Prolapse Quantification; Immunohistochemical Study; SSE: Stratified Squamous Epithelium

Introduction

Aging of the population is one of the most important problems, which is now becoming extremely important not only for developed, but also for developing countries. Due to the increase in life expectancy around the world, the number of elderly and senile people has increased sharply. According to United Nations projections, the number of people over 60 will exceed one billion by 2025, which will be 15% of the total population [1]. Genital prolapse (GP) is one of the most pressing problems of this category of patients, in whom its frequency is 50-60% and, as a rule, is combined with various urogenital disorders, increasing to 80% in postmenopause due to estrogen deficiency [2]. The presence of drooping and prolapse of the genitals itself affects the physical and psychoemotional state of the woman, and in combination with genitourinary menopausal syndrome leads to social maladaptation, as well as to a decrease in quality of life [3].

The problem of choosing therapeutic tactics for genital prolapse in postmenopausal women is associated not only with concomitant diseases, but also with altered tissues in the vagina, the healing of which plays a key role as a result of surgical treatment [4]. The most clinically difficult is a group of women with severe genital prolapse in the postmenopausal period, who, on the one hand, have a long duration of the disease, on the other hand, significant structural changes in the tissues of the urogenital tract against the background of a deficiency of sex hormones.

Surgical correction, including vaginal access, is an effective treatment for GP. The result of reconstructive plastic surgery in patients is largely determined by the state of the tissues of the vaginal mucosa in the immediate area of surgery [5]. The presence of genitourinary menopausal syndrome (GMS) in women with severe genital prolapse worsens the prognosis of the postoperative course for several reasons. First, the likelihood of infectious complications is high. Second, the symptoms of GMS suggest a possible reduced tissue regenerative capacity. Third, matching the initially thinned mucosa may lead to incomplete epithelialization in the suture area.

Many pelvic floor surgeons describe a different percentage of complications associated with performing operations from 5 to 20% of cases [6,7]. The main postoperative complications are vaginal mucosal erosion, infectious complications and thinning of the postoperative suture, which occur due to incomplete healing of the vaginal epithelium against the background of the inflammatory process. Consequently, postmenopausal patients with genital prolapse prior to routine operative treatment, preoperative preparation for surgical treatment of pelvic organ prolapse is justified, aimed at restoring the functional state of hormone-dependent tissues of the urogenital system and stimulating the mechanisms of natural biological protection of the mucous membranes.

Currently, there are various methods of preoperative preparation. The effect of certain methods on the vaginal mucosa before surgery is debatable.

The above circumstances dictate the need to study the effectiveness of current methods of preparing vaginal tissues for surgical treatment of genital prolapse by vaginal access. One method of preoperative preparation is the use of local forms of estriol and progesterone in combination with lactobacteria [8-10].

To date, there is insufficient data on the effect of steroid hormones on the condition of the vaginal mucosa. Also, the mechanisms of influence of lactobacteria on the vaginal epithelium are not fully understood and contradictory. In this regard, this study to study the effect of preoperative preparation on the state of vaginal epithelium is very important.

Materials and Methods

An open-label, prospective, randomized controlled trial was conducted in 100 postmenopausal women with stage III, IV GP according to the POP-Q classification. Patients were randomized by envelopes to 3 groups (n = 100). The first group is women who were given local hormone therapy (estriol and progesterone) in combination with lactobacteria at the preoperative stage (n = 35). The second group is women who used local monotherapy with estriol as preoperative training (n = 35). The third group (control) is women with no prior preparation for surgery (n = 30). In preparation for operative treatment, women of group 1 in the preoperative period received three-component therapy, including local forms of hormones in combination with lactobacteria.

Preoperative preparation consists of intravaginal administration of capsules containing 0.2 mg estriol, 2 mg progesterone, Lactobacillus casei rhamnosus 2 * 107 cfu. Before surgery for 1 month 2 capsules once a day for 20 days, then 1 capsule once a day for 10 days. After surgical treatment, the capsule 1 continue therapy according to the 1 regimen once a day for 2 months. Patients in Group 2 used topical forms of estriol (0.5 mg estriol) as preoperative preparation.

To assess vaginal microbiocenosis, biological material was taken from the side wall of the vagina by scraping with a sterile urogynecological probe. The collected samples were placed in a transport medium containing saline. Precipitation of cells and bacterial agents was carried out by centrifugation at 13000G for 10 minutes. A culture study of genital biocenosis was carried out using molecular genetic methods (FEMOFLOR technology). The material was examined by real-time polymerase chain reaction using a set of reagents "Sample NK" and "Femoflor 16" of the company "DNA-Technology" (Russia). Real-time amplification was performed on a BIO-RAD IQ5 Multicolor Real-Time PCR Detection System (USA). The amount of DNA of the desired material in the sample was determined by software and expressed in equivalent genome (GE), which is proportional to the number of microorganisms (Table 1).

Group	Indicators to be determined
Control	Material Take Control
	Positive control
	Internal Control Sample
Diagnosis of normocenosis	Total bacterial mass
	Lactobacillus spp. *
Aerobic microorganisms (optional anaerobes)	Family Enterobacteriaceae
	Streptococcus spp.
	Staphylococcus spp.
Anaerobic microorganisms (strict anaerobes)	*Gardnerella vaginalis*/*Peptostreptococcus* spp.
	Eubacterium spp.
	Sneathia spp./*Leptotrihia* spp./*Fusobacterium* spp.
	Megasphaera spp./*Veillonella* spp./*Dialister* spp.
	Prevotella bivia/*Porphyromonas* spp.
	Lachnobacterium spp./*Clostridium* spp.
	Mobiluncus spp./*Corynebacterium* spp.
	Atopobium vaginae
Mycoplasma group	*Mycoplasma* spp.
	Ureaplasma (*U. urealyticum* + *U. parvum*)
Mushrooms	*Candida* spp.

Table 1: Composition of Femoflor reagent kits.

Note: * - under spp. implies a wide group of microorganisms significant for the diagnosis of dysbiosis, which belongs to this genus, but may not fully correspond to the genus in its systematic understanding.

As part of this work, the proportion of lactoflora was evaluated to classify the type of microbiocenosis. With the proportion of lactobacilli in the total bacterial mass of 80% or higher, such microbiocenosis was conventionally called "normocenosis", and with the complete absence of lactoflora or a fraction of less than 80% - "dysbiosis".

Material for morphological and immunohistochemical examination was obtained with informed voluntary consent to participate in the study during vaginal access surgery for GP (taking a fragment of vaginal wall tissue). The material for the study was biopsies of the mucous membrane of the anterior vaginal wall. Biopsy of the anterior vaginal wall was performed during surgical treatment of prolapse, the size of the excised area is approximately 10×10 mm. After performing the vaginal wall biopsy, the resulting material was fixed in a 10% neutral buffered formalin solution for 24 hours. Subsequently, the material was dehydrated using the Leica TP1020 automatic station according to the standard scheme in alcohols of increasing concentration (70-95%), then in xylene and paraffin for the manufacture of histological and immunohistochemicals. The thickness of the serial sections was 3-7 μm. Histological examination of paraffin sections of the vaginal mucosa was performed in all women of the analyzed groups with hematoxylin Carazzi and eosin staining according to the standard method. Microdrugs were viewed and photographed at optimal magnification on an Axioplan 2 microscope ("CarlZeissJena," Germany) using a digital camera ("CarlZeissJena," Germany). In a morphological examination of the vaginal mucosa, a multilayer planar epithelium was evaluated. Particular attention was paid to such indicators as: areas of thinning of the epithelium, acanthotic strands (acanthosis), vacuole dystrophy of cells of the intermediate layer. Inflammatory changes in the vaginal mucosa were assessed by the presence of infiltration by plasmocytes and lymphocytes. The degree of mononuclear infiltration was defined as weak, moderate or severe. To determine the state of the receptor apparatus of the vaginal mucosa (ERα, ERβ), an immunohistochemical study was carried out. For the immunohistochemical study (IHC) study, a two-stage streptavidin-biotin-peroxidase method with antigen unmasking and the use of standard monoclonal and polyclonal antibody kits from Bond RTU Primary USA using a closed-type immunohistostainer Bond-maX (Leica, Germany) was used. In an IHC, antibodies to α and β estrogen receptors were used to evaluate estrogen receptor expression using primary monoclonal mouse anti-human antibodies manufactured by DakoCytomation in a standard 1:40 dilution for vaginal mucosal receptors. For immunohistochemical reactions, paraffin sections were treated according to a conventional method using mouse monoclonal antibodies to estrogen receptors - α (clone 6F-11), to estrogen receptors - β (clone 6F-12). The Super Sensitive Polymer-HPR Detection System (Biogenex) was used to visualize primary antibodies. The level of expression of ER α and ER β receptors (Bond RTU Primary) was determined in the samples. The results of the estrogen receptor response were identified by nuclear or membrane staining of the cells in brown for the corresponding markers with an estimate of the percentage of stained cells and the intensity of cell staining. Estrogen receptor expression was assessed on a 3-point scale (weak, moderate, and severe). To analyze the results of IHC reactions, the H-score histological counting method was used according to the formula: HS = 1a + 2b + 3c, where a -% of poorly stained cells, b -% of moderately stained cells, c -% of strongly stained cells, 1, 2, 3 - staining intensity, expressed in points. Statistical processing of the research results was carried out using the packages of application programs "MicrosoftExcel" (2013) and "StatisticaforWindows 6.0" (StatSoft, USA), as well as using the IBM SPSS Statistics 22 program. The work uses statistical methods: descriptive statistics, Student's test, methods for comparing shares, analysis of variance, the procedure for multiple comparisons of Scheffe (one of the options for accounting for the Bonferroni correction), Kruskal-Wallis analysis, discriminant analysis. Descriptive statistics show average values of indicators (M) and standard error of average (m). For measures whose distribution is not statistically significantly different from normal, the standard error was calculated using formulas for normal distribution. For dichotomous variables taking two values of 0 and 1, formulas based on binomial distribution were used to calculate the standard error. To check the normality of the distribution, the Kolmogorov-Smirnov criterion was used. The Student test for independent observations was used to compare the mean values in the two groups in the parameters with the normal distribution. Two levels of statistical significance were used: $\alpha = 0,05$ and $\alpha = 0,01$; differences are considered statistically significant if the p-value is less than 0.05 or the p-value is less than 0.01. Univariate analysis of variance (ANOVA) was used to compare mean values in more than two groups in measures with normal distribution. Statistical significance of the differences was assessed using the Scheffe test procedure for the two significance levels $\alpha = 0,05$ and $\alpha = 0,01$. For quantitative variables with a distribution other than normal, the Kruskal-Wallis procedure was used (a non-parametric method for comparing several groups). For indicators characterizing qualitative features, an absolute value and a relative value in percent were indicated, statistical hypotheses were tested using the chi-square test ($\chi 2$). The critical significance level of the differences (p), at which the null hypothesis of no difference was rejected and the alternative was accepted, was set to 0.025 (Kraskel-Wallis test) and $p < 0.05$ (Wilcoxon test).

Results and Discussion

Clinical characteristics of the groups

The study involved 100 women aged 51 to 79 years (average age - 62.23 ± 5.79 years). The mean age of patients in Group 1 was 62,37 ± 1.02 years, in Group 2 61,60 ± 0,90 years, and in the control group 62,80 ± 1,11 let ($p > 0.05$). Analysis of anthropometric indicators: in group 1, the weight of women 70,5 ± 1,42 kg, in the second 70,91 ± 1.39 kg and in the third 72,15 ± 1.57 kg ($p > 0.05$). When studying somatic pathology in patients with GP, diseases of the respiratory system, gastrointestinal tract, as well as varicose veins of the lower extremities were most common. Respiratory diseases were statistically significantly more common in group 1 than in group 2 74.3% versus 48.6% ($p = 0.028$). Endocrine diseases were statistically significantly more common in group 1 than in group 3 42.9% versus 16.7% ($p = 0.023$). Eye diseases were statistically significantly less common in group 2 than in group 3 42.9% versus 70.0% ($p = 0.029$), with gastrointestinal diseases in group 2 significantly more common than group 3 97.1% versus 80% ($p = 0.027$). There were no statistically significant differences in the frequency of diseases of the cardiovascular system, urinary system, breast diseases in patients in the observation groups. When comparing the mean menopausal age and postmenopausal duration, it was found that there were no significant differences in these parameters between the groups of patients ($p > 0.05$). The median age at menopause in the follow-up groups was 53.8 years (Table 2). The duration of postmenopause was slightly longer in group 3 than in groups 1 and 2, but statistically insignificant 8,4 ± 0,82 years versus 8,34 ± 0.88 years and 8.20 ± 0,77 years ($p > 0.05$).

Indicators	Group 1 (n = 35)	Group 2 (n = 35)	Group 3 (n = 30)	p- level
Age of menopause, years	54,0 ± 0,26	53,0 ± 0,39	54,37 ± 1,8	$P_{1-2} = 0,771$ $P_{1-3} = 0,819$ $P_{2-3} = 0,473$
Duration of postmenopause, years	8,34 ± 0,88	8,20 ± 0,77	8,4 ± 0,82	$P_{1-2} = 0,617$ $P_{1-3} = 0,819$ $P_{2-3} = 0,886$

Table 2: Age of menopause and duration of postmenopause.

Morphological features of the vaginal mucosa depending on the type of local preoperative preparation

According to the design of the study, to study the morphological structure of the vaginal mucosa, a biopsy of the anterior vaginal wall was performed during surgery in women with GP. Further, the results of the study of biopsies of the vaginal mucosa in patients who underwent local preoperative preparation and in women without special training were compared.

In all patients with PG, the vaginal wall had a typical structure and was represented by mucous, muscular and adventitial membranes (Figure 1).

The mucosa was lined with a multi-layered flat epithelium (SSE) consisting of surface (collapsible), intermediate (glycogen-containing) and basal (mitotically active) layers.

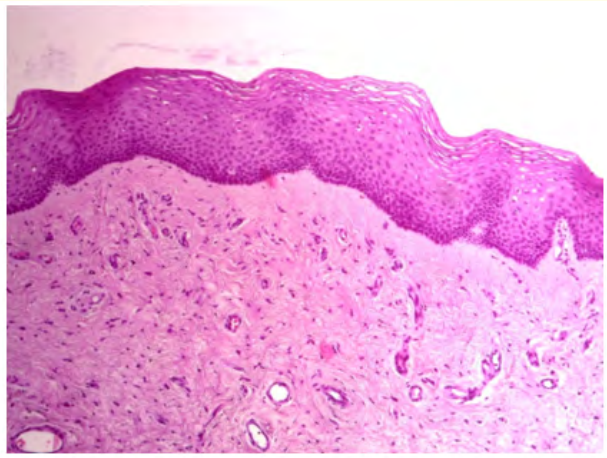

Figure 1: Vaginal wall. Hematoxylin and eosin coloration, light microscopy ×100.

A study of vaginal mucosal biopsies found obvious differences in the group with and without local mucosal preparation prior to surgery. In group 3, absolutely all vaginal wall biopsies (n = 30) showed morphological signs of inflammation in the form of plasmacytic-lymphocytic infiltration. While mononuclear infiltration occurred less frequently in groups 1 and 2 in 51.4% (n = 18) and 82.5% (n = 29) of cases, respectively. At the same time, in the group with local estriol monotherapy, lifocytic infiltrates were statistically significantly more common than in the group with estriol, progesterone and lactobacteria ($p < 0.001$). Infiltrate localization was most commonly reported in the perivascular zone and subepithelial zones. The degree of mononuclear infiltration was defined as weak, moderate, and pronounced. Weak diffuse mononuclear infiltration was observed in half of the cases in women who underwent local treatment with sex hormones in combination with lactobacteria (n = 9), while in the group with monotherapy with estriol in 31% of cases (n = 9), without preoperative preparation in 16.6% of cases (n = 5). Moderate lymphocytic infiltration occurred in more than 50% of cases in 2 (n = 16) and 3 (n = 17) groups, while 38.8% (n = 7) of moderate infiltration occurred in women in group 1 ($p < 0.001$). Severe lymphoid cell infiltration was statistically significantly more common in patients who did not undergo local preoperative preparation ($p < 0.001$). In samples taken after local preparation of the vaginal wall, a pronounced morphological reaction of inflammation is rarely established.

Non-uniform thickness of thinning-predominant MEPs was recorded at an equally high frequency in the first 85.7% (n = 12) and the second 78.9% (n = 15) groups, while in the third group, a decrease in the thickness of the cover epithelium was detected in 60.9% (n = 14), (Figure 2).

Figure 2: Morphological picture of the vaginal wall with areas of thinning of SSE. Hematoxylin and eosin coloration, light microscopy x 100.

Intracellular edema of epithelial cells with the appearance of vacuoles in the cytoplasm was observed more often in the group of women who did not receive local preoperative training and was detected in 39.1% of cases (n = 9), (Figure 3). At the same time, in 22.2% of cases (n = 2) in patients in the 3rd observation group, SSE with individual epithelial cells in the state of balloon dystrophy was determined, when the epithelial cell was maximally increased due to pronounced intracellular cytoplasmic edema with the preservation of only the cell envelope (Figure 4). Focal vacuole dystrophy of epithelial cells, in which the morphological pattern is visualized - the vacuole occupies almost the entire cell, pushing the nucleus to the periphery, was smaller in observation group 1 and 2 14.3% (n = 2) and 21.10% (n = 4), respectively. It is during dystrophic processes in the cells of the intermediate layer of SSE that the amount of glycogen, which is a substrate for lactobacteria, is reduced.

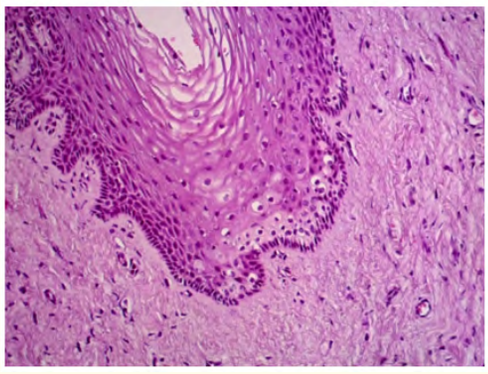

Figure 3: Morphological picture of the vaginal wall in stage III-IV PG according to post-menopausal POP-Q. PME cells with partial cytolysis are visible with the formation of liquid-filled vacuoles (vacuum dystrophy) in the cytoplasm. Hematoxylin and eosin coloration, light microscopy x 200.

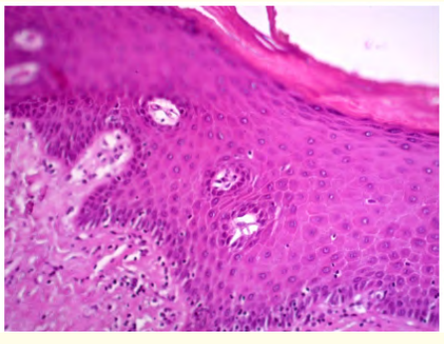

Figure 4: Morphological picture of the vaginal wall in stage III-IV PG according to post-menopausal POP-Q. SSE cells with complete cytolysis are visible with the formation of liquid-filled vacuoles (balloon dystrophy) in the cytoplasm. Hematoxylin and eosin coloration, light microscopy x 200.

The morphological peculiarity of the structure of the planar epithelium of the vaginal wall in the form of acanthotic strands was observed in all three groups. Acanthosis manifested itself morphologically as thickening and increasing the number of rows of the intermediate layer of SSE with elongation of epidermal outgrowths, in some cases penetrating deep into the underlying stroma (Figure 5). The basis of acanthosis in postmenopausal women against the background of deficiency of both estrogen and progesterone is a slowdown in the processes of differentiation of epithelial cells when their life expectancy increases.

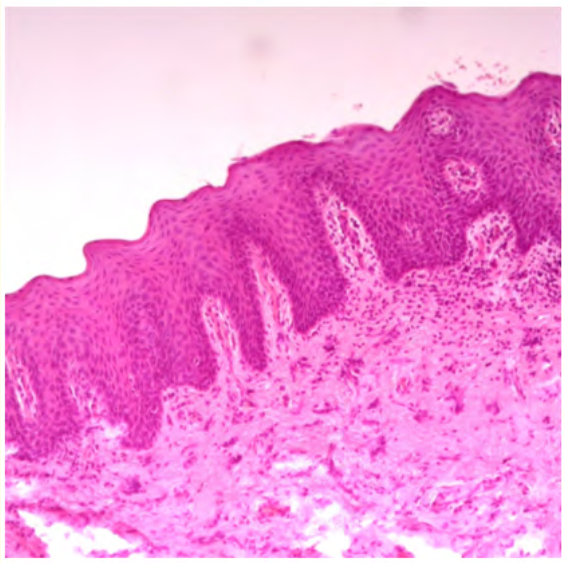

Figure 5: Morphological picture of the vaginal wall in stage III-IV GP according to post-menopausal POP-Q. High acanthotic strands are visible. Hematoxylin and eosin coloration, light microscopy x 200.

Histological examination of vaginal tissue biopsies in several cases visualized such a pathological morphological feature as sites of SSE keratosis (Figure 6).

The stratum corneum occurred in all follow-up groups with approximately the same frequency. In group 1, 20% (n = 7), in group 2, 22.8% (n = 8), in group 3, 26.7% (n = 8). Corneal sites consist of horn scales and are built of keratin fibrils and amorphous electropotential material. Keratinization of the multilayer flat vaginal epithelium in women with postmenopausal PG can be explained by the formation of horn scales against the background

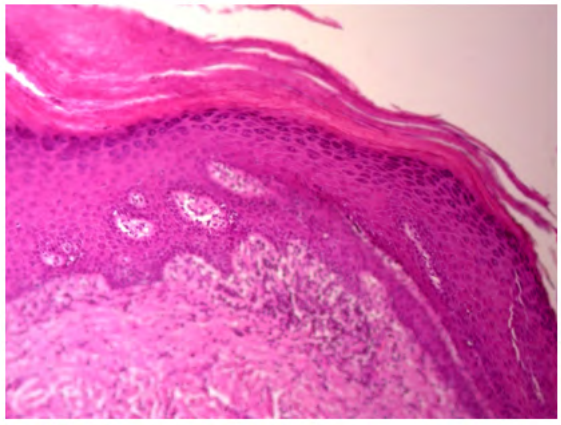

Figure 6: Morphological picture of the vaginal wall in stage III-IV PG according to post-menopausal POP-Q. Areas of keratinization are visible. Hematoxylin and eosin coloration, light microscopy x 200.

of a sluggish chronic inflammatory process.

Thus, physiological estrogen and progesterone deficiency in postmenopausal women causes impaired processes of differentiation as well as vaginal epithelial proliferation. Morphological signs of dystrophy, mild to moderate chronic inflammation outside of activity, explain the manifestation of symptoms of vaginal atrophy, and may also be the cause of unsatisfactory results of surgical treatment of GP in postmenopausal women. When performing surgical correction of genital prolapse by vaginal access, a linear incision of the vaginal mucosa is performed with subsequent wound suturing. Thus, a comparison of tissues with disturbed architectonics, pathological proliferation and differentiation of cells is obtained. Therefore, in such a vaginal epithelium, the processes of restoration and epithelization are damaged. In dystrophic processes, when alteration of cell ultrastructures occurs, regeneration at the molecular and ultrastructural levels is disrupted. Direct damage to the processes of molecular morphogenesis in women with postmenopausal GP may lie in the pathogenesis of complications of surgical treatment.

Immunohistochemical picture of the vaginal mucosa depending on the type of local preoperative preparation

According to the IHC results of the study of vaginal tissue biopsies taken during operative treatment, we identified an

immunopositive reaction to antibodies to estrogen receptors α and β, but there was a significant difference in the degree of their immunoreactivity and distribution in the vaginal mucosa (Table 3).

Indicators	Group 1 (n = 35)	Group 2 (n = 35)	Group 3 (n = 30)	p-level
ERα in the surface layer	62,17 ± 4,53	43,11 ± 4,88	13,23 ± 2,81	P_{1-2} = 0,006 P_{2-3} = 0,001 P_{1-3} = 0,001
ERα in the intermediate layer	50,78 ± 4,58	43,86 ± 5,09	23,63 ± 3,99	P_{1-2} = 0,316 P_{2-3} = 0,001 P_{1-3} = 0,003
ERα in the basal layer	48,17 ± 5,28	42,49 ± 5,53	22,07 ± 4,61	P_{1-2} = 0,459 P_{2-3} = 0,001 P_{1-3} = 0,007
ERβ in the surface layer	39,00 ± 4,33	31,57 ± 4,61	15,70 ± 3,72	P_{1-2} = 0,244 P_{2-3} = 0,001 P_{1-3} = 0,011
ERβ in the intermediate layer	41,60 ± 4,64	40,20 ± 5,16	22,17 ± 4,47	P_{1-2} = 0,841 P_{2-3} = 0,004 P_{1-3} = 0,012
ERβ in the basal layer	39,14 ± 4,71	41,34 ± 5,84	23,93 ± 5,24	P_{1-2} = 0,770 P_{2-3} = 0,034 P_{1-3} = 0,032

Table 3: Immunohistochemical measures of estrogen receptor expression of α and β in different layers of SSE in women of the examined groups (H-score, M ± m, p).

When examining estrogen α receptor expression in all layers of the vaginal epithelium, there was a tendency for a greater degree of expression in women in the group who underwent preoperative preparation using sex hormones and lactobacteria. A statistically significant increase in the level of ERα expression in the surface layer of the vaginal epithelium was demonstrated in women with three-component preoperative preparation compared with the group of women receiving estriol monotherapy (62,17 ± 4,53% versus 43,11 ± 4,88%; p = 0.006) and in comparison, with the control (62,17 ± 4,53% versus 13,23 ± 2,80% in the control; p = 0.000004) (Figure 7).

Also noteworthy is the statistically significant large expression of ERα in the surface layer of SSE in women who used estriol only before surgery compared to a group of women without preoperative training 43,11 ± 4,88% versus 13,23 ± 2,80% (p = 0.001).

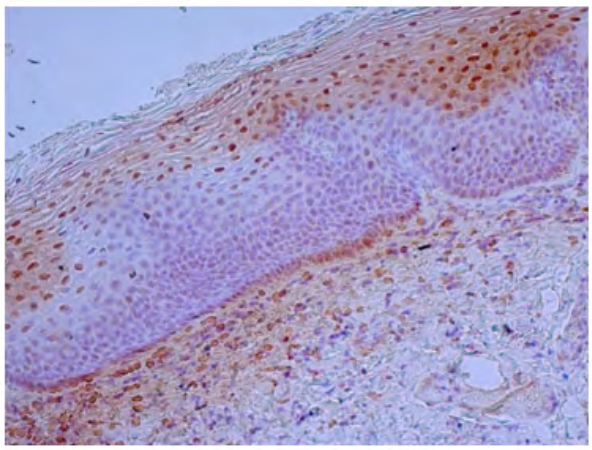

Figure 7: Vaginal wall. Pronounced ERα expression in the surface layer. IHC method with antibodies to estrogen α, uvelich. ×200.

When examining samples of vaginal mucosa in women with stage III-IV GP according to post-menopausal POP-Q, the high expression of ERα in the intermediate layer of the epithelium in groups of women who underwent local preoperative preparation (50,77 ± 4,58% versus 23,63 ± 3,99% in the control; p = 0.003326 in group 1 and 43,86 ± 5,09% versus 23,63 ± 3,99%), statistically significantly exceeding the control values (Table). There were no statistically significant differences in the comparison of patients in Group 1 and 2, however, the level of ERα expression in the intermediate layer was higher in patients in Group 1. Thus, the visual immunohistochemical picture of the vaginal mucosa is determined: in women who did not undergo local operational preparation, there was a tendency to reduce ERα expression in all layers of the epithelium (Figure 8), while high expression of ERα in the basal, intermediate and surface layers is determined after complex preoperative preparation (Figure 9).

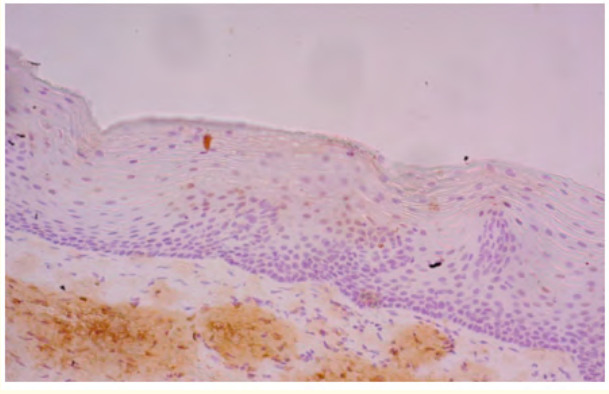

Figure 8: Vaginal wall. Very weak expression of ERα in the vaginal epithelium of control patients. IHC method with antibodies to estrogen α, increase in ×200.

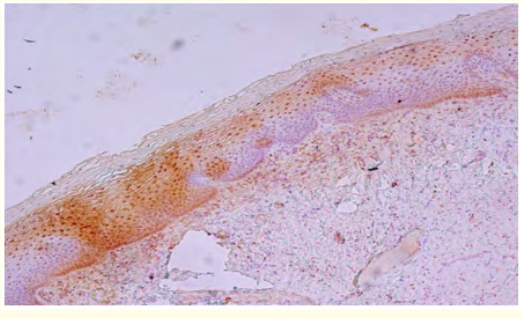

Figure 9: Vaginal wall. Pronounced expression of ERα in the basal, intermediate and surface layer in patients of group 1. IHC method with antibodies to estrogen α, increase in ×100.

After treatment, group 1 women showed a statistically significant increase in ERβ expression in all layers of the vaginal epithelium: in the surface layer 39,00 ± 4,33% versus 15.70 ± 3,718% (p = 0.0109), in the intermediate layer 41,60 ± 4,64% versus 22,17 ± 4,47% (p = 0.0117), in the basal layer 39,14 ± 4,70% versus 23,93 ± 5,24% (p = 0.0323).

According to the obtained results, the expression of ERβ in the vaginal epithelium of patients of groups 1 and 2 did not differ significantly among themselves. Despite this, the expression of β estrogen receptors in the surface and intermediate layers was greater in the group of women with therapy with local forms of sex hormones and lactobacteria (Figure 10).

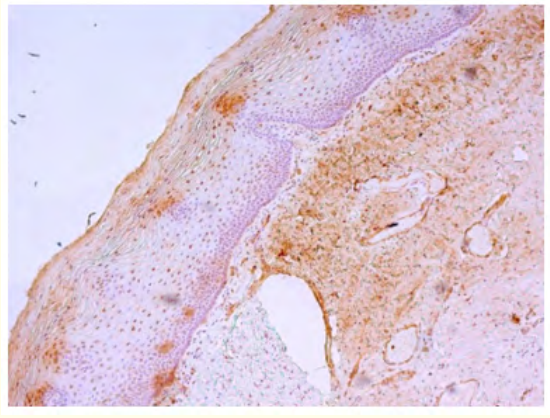

Figure 10: Vaginal wall. Expression of ERβ in predominantly superficial layers of vaginal SSE. IHC method with antibodies to estrogen β, increase in ×100.

Against the background of motor therapy with estrogen-containing drugs, there was a statistically significant increase in ERβ expression in all layers of the vaginal wall epithelium: in the surface layer 31,57 ± 4,60% versus 15,7 ± 3,72% (p = 0.0001), in the intermediate layer 40.20 ± 5,16% versus 22,17 ± 4,47% (p = 0.0039), in the basal layer 41,343 ± 5,84% versus 23,93 ± 5,24% (p = 0.0342), (Figure 11, 12).

Vaginal microbiota status after local preoperative preparation in follow-up groups

Significant improvements in the structure of the vaginal microbiome according to the "FEMOFLOR-16" test was observed over time (Table 4).

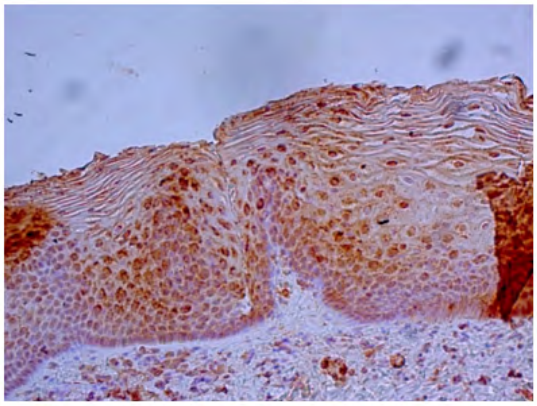

Figure 11: Vaginal wall. Pronounced expression of ERβ in all layers of the vaginal wall epithelium. IHC method with antibodies to estrogen β, increase in ×200.

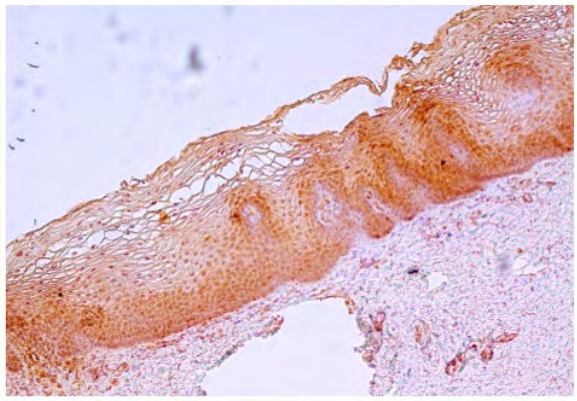

Figure 12: Vaginal wall. Expressed expression of ERβ in SSE in the background of acantotic strands. IHC method with antibodies to estrogen β, increase in ×100.

Indicators	Group 1		Group 2		Group 3		P-level	
	Before surgery (1)	After surgery (2)	Before surgery (3)	After surgery (4)	Before surgery (5)	After surgery (6)	Before surgery	After surgery
Total bacterial mass, GE/ml	7,07 ± 0,15	7,19 ± 0,16	6,25 ± 0,20	5,90 ± 0,16	5,48 ± 0,27	5,97 ± 0,25	p_{1-3} = 0,001 p_{1-5} = 0,001 p_{3-5} = 0,022	p_{2-4} = 0,001 p_{2-6} = 0,001
Normoflora, GE/ml								
Lactobacillus spp.	6,64 ± 0,18	6,50 ± 0,13	4,29 ± 0,36	3,19 ± 0,28	1,37 ± 0,30	3,30 ± 0,37	p^{1-3} = 0,001 p^{1-5} = 0,001 p^{3-5} = 0,022	p_{2-4} = 0,001 p_{2-6} = 0,001
Facultative aerobic microorganisms, GE/ml								
Enterobacterium spp.	3,23 ± 0,23	2,16 ± 0,25	2,98 ± 0,18	2,44 ± 0,17	3,08 ± 0,27	2,80 ± 0,20	p > 0,05	p > 0,05
Streptococcus spp.	2,70 ± 0,18	2,80 ± 0,23	3,19 ± 0,26	2,86 ± 0,27	3,30 ± 0,28	3,63 ± 0,18	p > 0,05	p_{2-6} = 0,008 p_{4-6} = 0,023
Staphylococcus spp.	2,87 ± 0,13	2,51 ± 0,31	3,13 ± 0,14	1,86 ± 0,19	2,36 ± 0,24	2,35 ± 0,17	p_{1-5} = 0,053 p_{3-5} = 0,005	p > 0,05
Obligate anaerobic microorganisms, GE/ml								
Gardnerella vaginalis/Prevotella bivia/ Porphyromonas spp.	4,36 ± 0,29	3,78 ± 0,30	3,88 ± 0,28	3,94 ± 0,33	3,99 ± 0,37	2,93 ± 0,24	p > 0,05	p_{2-6} = 0,036 p_{4-6} = 0,020
Eubacterium spp.	4,25 ± 0,23	3,30 ± 0,21	3,84 ± 0,26	2,16 ± 0,29	3,49 ± 0,33	1,12 ± 0,14	p > 0,05	p_{2-4} = 0,002 p_{2-6} = 0,001 p_{4-6} = 0,004

Sneathia spp./*Leptotrihia* spp./*Fusobacterium* spp.	2,44 ± 0,31	1,97 ± 0,26	2,14 ± 0,29	1,62 ± 0,26	1,96 ± 0,36	1,45 ± 0,24	p > 0,05	p > 0,05
Megasphera spp./*Veilonella* spp./*Dialister* spp.	3,72 ± 0,24	2,06 ± 0,24	2,92 ± 0,24	1,75 ± 0,24	2,39 ± 0,32	1,95 ± 0,26	p_{1-3} = 0,022 p_{1-5} = 0,001	p > 0,05
Lachnobacterium spp./*Clostridium* spp.	3,09 ± 0,26	2,55 ± 0,25	2,45 ± 0,20	2,58 ± 0,32	2,26 ± 0,24	2,64 ± 0,23	p_{1-3} = 0,058 p_{1-5} = 0,025	p > 0,05
Mobiluncus spp./*Corynebacterium* spp.	3,64 ± 0,22	2,20 ± 0,23	3,51 ± 0,27	2,38 ± 0,23	2,60 ± 0,24	2,78 ± 0,22	p_{1-5} = 0,002 p_{3-5} = 0,015	p > 0,05
Peptostreptococcus spp.	3,28 ± 0,24	2,84 ± 0,24	2,92 ± 0,27	2,49 ± 0,21	2,44 ± 0,35	2,50 ± 0,24	p_{1-5} = 0,046	p > 0,05
Atopobiumvaginae	1,05 ± 0,26	1,70 ± 0,28	1,17 ± 0,26	0,96 ± 0,25	0,51 ± 0,16	0,57 ± 0,20	p_{3-5} = 0,046	p_{2-4} = 0,052 p_{2-6} = 0,002
Mycoplasmas, GE/ml								
Mycoplasma (hominis+ genitalium)	0,36 ± 0,20	0,43 ± 0,17	0,53 ± 0,17	0,59 ± 0,17	0,27 ± 0,12	0,75 ± 0,23	p > 0,05	p > 0,05
Ureaplasma (urealyticum+parvu)	2,26 ± 0,43	0,48 ± 0,15	2,30 ± 0,40	0,61 ± 0,24	1,18 ± 0,30	0,83 ± 0,28	p_{1-5} = 0,054 p_{3-5} = 0,035	p > 0,05
Yeast-like mushrooms, GE/ml								
Candida spp.	1,17 ± 0,17	0,35 ± 0,09	1,24 ± 0,20	2,44 ± 0,25	2,14 ± 0,25	2,78 ± 0,26	p_{1-5} = 0,001 p_{3-5} = 0,005	p_{2-4} = 0,001 p_{2-6} = 0,001

Table 4: Dynamics of the vaginal microbiota before and after surgery with various types of preoperative preparation.

In the observation group where complex preoperative preparation of the vaginal mucosa with estriol, progesterone in combination with L. casei rhamnosus was performed, a statistically significant dominance of lactoflora in the structure of the vaginal microbiome was observed. Number of *Lactobacillus* spp. in 1 group statistically significantly exceeds this indicator in 2 and 3 groups at the stage, both before the operation on the background of the preparation of the vaginal mucosa, and 3 months after the operation. The number of lactobacillary flora in patients of Group 1 with three-component therapy significantly increased compared to estriol monotherapy and patients without local preparation for surgery 6,64 ± 0.18 GE/mL versus 4,29 ± 0,36 GE/mL (p = 0.01), 6,64 ± 0.18 GE/mL versus 1,37 ± 0.30 GE/mL (p = 0.01). After surgical treatment with *Lactobacillus* spp. in 1 group were within normal values compared to 2 and 3 groups, where this indicator was sharply reduced.

A statistically significant lower amount of *Streptococcus* spp. was detected in the postoperative follow-up groups of optional aerobic microorganisms. Group 1 vs Group 3 2,80 ± 0,23 GE/mL vs 3,63 ± 0,18 GE/mL (p = 0.008) and Group 2 vs Group 3 2,86 ± 0,27 GE/mL vs 3,63 ± 0,18 GE/mL (p = 0.023). It should be said that aerobic microbial parameters were within normal values in all observation groups.

During the comparative analysis of the number of obligate-anaerobic microorganisms before the operation, statistically significant differences of some microorganisms were revealed on the background of the preoperative preparation. *Megasphera* spp./*Veilonella* spp./*Dialister* spp. were found in a greater statistically significant amount in group 1 compared to groups 2 and 3 3,72 ± 0,24 GE/mL versus 2,92 ± 0,24 GE/mL (p = 0.02), and versus 2,39 ± 0,32 GE/mL (p = 0.01). Higher significant concentration of

Mobiluncus spp./*Corynebacterium* spp. and *Atopobium vaginae* in women after preoperative pretreatment was detected in group 2 compared to group 3 3,51 ± 0,27 GE/mL versus 2,60 ± 0,24 GE/mL (p = 0.015) and 1,17 ± 0,26 GE/mL versus 0,51 ± 0,16GE/ml (p = 0.046). A statistically significant high concentration of *Lachnobacterium* spp./*Clostridium* spp., *Mobiluncus* spp./*Corynebacterium* spp. и Peptostreptococcus spp. in patients in group 1 compared to the control group 3,09 ± 0,26 GE/mL versus 2,26 ± 0,24GE/ml (p = 0.025), 3,64 ± 0,22 GE/mL versus 2,60 ± 0,24 GE/mL (p = 0.002), and 3,28 ± 0,24 GE/mL versus 2,44 ± 0,35 GE/ml (p = 0.046). During the analysis of comparison of the number of obligate-anaerobic microorganisms after the operation, statistically significant differences of several microorganisms were detected in the study groups. Amount of *Eubacterium* spp. and *Atopobium vaginae* after surgery was statistically significantly greater in group 1 women compared to groups 2 and 3 3,30 ± 0,21 GE/mL versus 2,16 ± 0,29 GE/mL (p = 0.002) and versus 1,12 ± 0,14 GE/mL (p = 0.001); 1,70 ± 0,28 GE/mL vs 0,96 ± 0,25 GE/mL (p = 0.052) and vs 0,57 ± 0,20 GE/mL (p = 0.002). When comparing the amount of *Gardnerella vaginalis/Prevotella bivia/Porphyromonas* spp. in the study groups, the amount of this microorganism was statistically significantly less in women of group 3 than in group 1 and in the second group 2,93 ± 0,24 GE/mL versus 3,78 ± 0,30 GE/mL (p = 0.036) and versus 3,94 ± 0,33GE/ml (p = 0.020), respectively. It is important to emphasize that the number of obligate anaerobic microorganisms in the study groups did not exceed the normal limits.

When studying the Mycoplasma group, no statistically significant differences were found in the observation groups, both before and after surgery. A statistically significant lower level of Ureaplasma (urealyticum + parvu) was detected in the control group of patients compared with groups 1 and 2 1,18 ± 0,30GE/ml against 2,26 ± 0,43GE/ml (p = 0.054) and against 2,30 ± 0,40 GE/mL (p = 0.035), respectively. There were no significant differences in the follow-up groups after operative treatment when studying the dynamics of mycoplasmas. Comparative characterization of *Candida* spp. both before and after operative treatment in the follow-up groups revealed a statistically significant lower number of yeast-like fungi in group 1 compared to group 2 and 3 (Table).

It was found that after preoperative preparation, the number of *Candida* spp. Group 1 and 2 were statistically significantly lower compared to the control group 1,17 ± 0,17 GE/mL versus 2,14 ± 0,25 GE/mL and 1,24 ± 0,20 GE/mL versus 2,14 ± 0,25 GE/mL. Comparative characterization of *Candida* spp. prior to operative treatment, the follow-up groups showed statistically significant lower numbers of yeast-like fungi in Group 1 compared to Group 2 and 3 1,18 ± 0,30GE/ml versus 2,26 ± 0,43GE/ml (p = 0.001) and versus 2,30 ± 0,40 GE/mL (p = 0.005). Number of yeast-like fungi of the genus *Candida* spp. after operative treatment remained statistically significantly smaller in the groups using local forms of sex hormones - in group 1 0,35 ± 0,09 GE/mL (p = 0.001), in group 2 2,44 ± 0,25 GE/mL (p = 0.001), in group 3 2,78 ± 0,26 GE/mL.

Discussion

Since the majority of patients suffering from genital prolapse are women of menopausal and postmenopausal age, the role of estriol and progesterone in ensuring a favorable postoperative prognosis cannot be overestimated. Urogenital disorders in peri- and postmenopausal women caused by atrophic and dystrophic processes in hormone-dependent tissues of the lower third of the genitourinary tract - the bladder, urethra, vagina, ligamentous apparatus of the small pelvis and pelvic floor muscles should be leveled before surgical treatment. In women, during the aging of the reproductive system, as estrogen saturation decreases, atrophy of the vaginal epithelium progressively worsens, resulting in a deficiency of lactobacilli, which leads to a change in the pH of the medium [11]. So in our study, a critical decrease in lactobacilli in the vagina in women with genital prolapse in postmenopausal women was demonstrated. Alkalinization of vaginal contents and insufficiency of the lactobacilli pool increase the likelihood of inflammatory diseases of the genital tract and urinary system, mainly with ascending infection.

It is known that in postmenopausal age, against the background of hypoestrogenism, the number of lacto- and bifidobacteria in the vagina decreases, while the composition of the microflora becomes scarce with the dominance of obligate anaerobic bacteria [12,13]. It is possible that just such features of the bacteriological status of the vaginal epithelium of the operating area can be the cause of infectious and inflammatory complications after surgical treatment of HP. In conditions of deficiency of sex hormones, there is a violation of the proliferation of the epithelium of the vagina and urethra, a decrease in the synthesis of glycogen, a change in the nature of the vaginal secretion, and the possible addition of a secondary infection.

Estrogen deficiency leads to inhibition of proliferative processes in the vagina, as a result of which the number of epithelial layers of its mucous membrane is significantly reduced. Our study showed that thinning of the vaginal mucosa is one of the main morphological characteristics of the vaginal epithelium in postmenopausal women with genital prolapse. At the same time, the number decreases and the rate of maturation of surface epithelial cells, the main suppliers of glycogen, slows down. A decrease in the concentration of glycogen, which is nutrition for lactobacilli, leads to their partial or complete disappearance from the vaginal biotope and a change in the normal acidic environment of the vagina to an alkaline one.

A number of authors have proved that in postmenopausal women with genital prolapse, the vaginal microflora is characterized by the absence of the main symbiont - lactobacilli, which are the dominant representative of the studied biotope in women of reproductive age [14]. Hummelen R. *et al*. found that in healthy postmenopausal women *L. iners* and *L. crispatus* predominate in the cluster of lactobacilli [15]. HP exacerbates the existing dysbiosis against the background of mucosal atrophy and estrogen and progesterone deficiency in postmenopausal women. In a bacteriological examination of the vagina, among facultative anaerobic bacteria, a fairly high proportion is occupied by corynebacteria and coagulase-negative staphylococci (about 70%) [16].

In the course of this study, when conducting a comparative analysis of the number of obligate anaerobic microorganisms after surgery against the background of ongoing preoperative preparation, statistically significant lower concentrations were revealed for such microorganisms as *Eubacterium* spp., *Gardnerella vaginalis* and *Atopobium vaginae*.

Non-clostridial anaerobic bacteria isolated from the vagina are normal symbionts of this biotope. However, if the evolutionary balance is disturbed, they can act as potential pathogens and, in particular, cause postoperative complications, since they have a full arsenal of various pathogenicity factors. In 2019, in their study, O. A. Borovleva., *et al.* determined the composition and characteristics of the vaginal microbiota in postmenopausal women with genital prolapse [17]. In postmenopausal patients with genital prolapse, in comparison with healthy women, dysbiotic changes in the vaginal microbiota are more pronounced, which is due to the gaping of the genital fissure, dystopia of the genital organs, and circulatory disorders. In 10% of postmenopausal women without HP, lactobacilli are recorded in the vagina. Among aerobic bacteria, coagulase-negative staphylococci dominate; among non-clostridial anaerobic bacteria, *Eubacterium* spp. and *Peptostreptococcus* spp. In the vaginal microbiota in postmenopausal women with genital prolapse, there are more pronounced dysbiotic changes, characterized by an increase in the frequency of detection of coagulase-negative staphylococci, *Corynebacterium* spp., *Eubacterium* spp., *Peptostreptococcus* spp. and a decrease in some taxa of anaerobic microorganisms (*Propionibacterium* spp. and *Bacteroides* spp.). Immunological aspects of vaginal ecology in women are of particular interest to both domestic and foreign researchers. The state of inflammation of the vaginal mucosa, concomitant atrophy of the vaginal epithelium are an unfavorable background for surgical treatment for two reasons: firstly, the likelihood of infectious complications is high, and secondly, the possible sluggish regenerative capacity of tissues can affect the outcomes of surgical treatment of women with genital prolapse.

The results of 19 randomized trials, which included more than 4 thousand patients, showed the effectiveness of local estrogen therapy in the treatment of vulvovaginal atrophy, both in monotherapy and in combination with systemic menopausal hormone therapy and pessaries [18]. Due to the short time of exposure to receptors, estriol does not affect the endometrium of the uterus and does not cause cell proliferation, in this regard, the administration of the drug is considered safe.

It has also been demonstrated in clinical studies that estrogen-containing drugs enhance the proliferative activity of the vaginal epithelium, which is necessary for the successful healing of the vaginal wall at the site of the mesh implant [19]. In 2015, German scientists performed a study to study changes in the expression of estrogen and progesterone receptors in the posterior wall of the vaginal mucosa after the use of local hormone therapy [20]. In addition, the expression of steroid hormones among women without the use of hormone therapy at various reproductive ages was studied. It was found that local use of estrogen leads to an increase in the expression of alpha-estrogen receptors and progesterone receptors in the vaginal mucosa in postmenopausal women, but the expression of beta-estrogen receptors does not change [21]. This explains the fact that the proliferation of vaginal tissue is mediated by estrogen receptor alpha and thus improves the status of surgical treatment for genital prolapse. That is why

preoperative preparation using hormonal therapy allows you to take a fresh look at the possibilities of restoring the vaginal mucosa in women who will undergo surgical correction of genital prolapse. Indeed, the saturation of the vaginal epithelium with estrogen and progesterone provides sufficient tissue regeneration, affecting the rate of collagen renewal.

Conclusion

The study revealed a significant positive effect of preoperative vaginal preparation using complex therapy with estriol, progesterone in combination with Lactobacillus on the architectonics of the vaginal mucosa and vaginal microbiota in postmenopausal women with severe genital prolapse.

Recommendations

When addressing postmenopausal patients suffering from stage III-IV genital prolapse according to POP-Q, planning to perform surgical correction of genital prolapse, it is necessary to give preference to an integrated approach as a local preoperative preparation of the vaginal mucosa - the use of local forms of estriol and progesterone in combination with lactobacilli.

Conflict of Interest

No conflict of interest.

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