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The effect of vaginal probiotic therapy on the outcome of papillomavirus infection

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ABSTRACT

Aim. To study the effect of vaginal probiotic therapy on the outcome of human papillomavirus (HPV) infection.

Materials and methods. The study included HPV-infected patients: 29 patients with normal vaginal flora and 146 patients with a deficiency of vaginal lactobacilli, of which 117 patients received vaginal probiotic therapy. In samples obtained before and after the therapy, the effect of the probiotic on the change in the ratio of living, apoptotic, and necrotic vaginal epithelial cells after preliminary exposure to oxidative stress was studied.

Results. It was found that probiotics reduce the number of infected epithelial cells that survived the oxidative damage and shift the balance of cell death forms towards apoptosis. Vaginal probiotic therapy in patients with a deficiency of lactobacilli increased the frequency of HPV elimination by 2.5 times and reduced the likelihood of treatment failure from 1.5 to 4 times, depending on the viral load. The probiotic therapy made the structure of HPV outcomes in Lactobacillus-deficient patients similar to that in patients with normal vaginal flora.

Conclusion. Vaginal probiotic therapy improves outcomes of HPV infection in patients with a deficiency of lactobacilli by reducing the number of survived infected cells and shifting the cell death pattern towards apoptosis.

Key words: apoptosis, epithelial cells, human papillomavirus, lactobacilli, Lactobacillus casei subsp. rhamnosus LCR35, probiotics, vagina.

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Conformity with the principles of ethics. All patients included in the research signed an informed consent to participate in the study. The study was approved by the Human Research Ethics Committee at Orenburg State Medical University (Protocol No. 149 of 05.10.2016).

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Влияние интравагинальной пробиотической терапии на течение папилломавирусной инфекции

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РЕЗЮМЕ

Цель – оценить влияние интравагинальной пробиотической терапии на течение папилломавирусной инфекции.

Материалы и методы. Исходы папилломавирусной инфекции оценивали у 29 пациенток с нормоценозом и 146 – с дефицитом нормофлоры, из них 117 женщин дополнительно интравагинально получали пробиотик. В пробах, полученных до и после терапии, изучали соотношение живых, апоптотических и некротических вагинальных эпителиоцитов после предварительной стимуляции апоптоза пероксидом водорода.

Результаты. Пробиотики снижают количество выживших инфицированных клеток и смещают баланс форм клеточной гибели в сторону апоптоза. Применение пробиотика у пациенток с дефицитом лактофлоры увеличило частоту элиминации вируса папилломы в 2,5 раза и повысило эффективность терапии в 1,5–4 раза в зависимости от вирусной нагрузки. Пробиотическая терапия у пациенток с дефицитом лактобацилл приблизила структуру исходов папилломавирусной инфекции к таковой группы пациенток с нормоценозом.

Заключение. Интравагинальная пробиотическая терапия улучшает исходы папилломавирусной инфекции у пациенток с дефицитом лактофлоры за счет снижения количества выживших инфицированных клеток и смещения структуры клеточной смерти в сторону апоптоза.

Ключевые слова: *Lactobacillus casei subsp. rhamnosus LCR35*, апоптоз, вирус папилломы человека, влагалище, лактобациллы, пробиотик, эпителиальные клетки.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Авторы заявляют об отсутствии финансирования при проведении исследования.

Соответствие принципам этики. Все пациенты подписали информированное согласие на участие в исследовании. Исследование одобрено локальным этическим комитетом ОрГМУ (протокол № 149 от 05.10.2016).

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INTRODUCTION

Human papillomavirus (HPV) is the most common sexually transmitted infection which contributes to the development of female genital tract cancer [1]. In most cases, papillomavirus is successfully eliminated, but in about 10% of infected women, HPV persists and can cause HPV-associated diseases of the genital tract, including cervical cancer [2]. It is not completely clear why HPV is eliminated in some cases but persists in other. Some of the likely reasons for this include individual differences in the state of the mucosal immune system [2, 3] and features of the microbial flora in the genital organs.

Sexually transmitted infections and bacterial vaginosis, in which the species composition of microorganisms in the vagina changes significantly, create a suitable environment for expansion of the virus [4–6]

and development of HPV-associated precancerous conditions and cancer [7]. The main manifestation of the imbalance in the vaginal microbial ecosystem is a pronounced deficiency of lactobacilli which protect the female reproductive tract from many pathogens, including viruses due to production of antimicrobial compounds and modulation of the mucosal immune system [2, 5].

Therefore, the concept of modifying vaginal bacterial communities using pre- and probiotics as a potential prospect for HPV infection management is widely discussed [8]. There are already successful examples of using probiotics for elimination of pathogens. In particular, the use of probiotics can prevent recurrence of vaginal yeast infection or bacterial vaginosis and increase the efficiency of metronidazole therapy for *Trichomonas vaginalis*; however, data on the possibility of using probiotics to control HPV infection are controversial.

The aim of the study was to investigate the effect of vaginal probiotic therapy on the progression of HPV infection. To achieve this aim, we studied how vaginal probiotic therapy affects the survival rate and the cell death pattern in primary cultures of vaginal epithelial cells, as well as the outcome of HPV infection.

MATERIALS AND METHODS

The study was conducted in accordance with the standards of good clinical practice and the principles of the Declaration of Helsinki, with the approval of the Human Research Ethics Committee at Orenburg State Medical University of the Ministry of Health of Russia (Protocol No. 149 of 05.10.2016). The study included 175 female patients with HPV infection who met the inclusion and exclusion criteria. The inclusion criteria were the following: high-oncogenic-risk HPV infection; age 18–40 years; a written informed consent to participate in the research and take pharmaceuticals. The exclusion criteria: smoking; immunodeficiency disorders; somatic symptom disorders at sub- and decompensated stages; menstrual dysfunction; pregnancy and lactation; taking hormonal contraceptives; individual allergic reaction to the drugs used in the study; therapy with antimicrobials or immunomodulators during the month preceding the study; a need for destructive methods of treating cervical pathology.

All the patients were treated with Allokin-Alpha® (State Research Institute of Highly Pure Biopreparations of the Federal Medical and Biological Agency) according to the following scheme: 1 mg subcutaneously, every other day, 6 injections per course, according to the manufacturer's instructions. In addition, patients with a deficiency of vaginal lactobacilli received a vaginal probiotic Lactoginal® (Laboratoires LYOCENTRE, SAS, France) containing *Lactobacillus casei subsp. rhamnosus LCR35* for 21 days according to the following scheme: 1 capsule 2 times a day for 7 days, then 1 capsule once a day for 14 days. The control group included 29 patients with a deficiency of vaginal lactobacilli who refused from vaginal probiotic therapy and received only immunomodulatory therapy. In accordance with the initial viral load, the patients were divided into three groups: 1) HPV $\leq 10^3$ cells, 2) HPV $10^{4–5}$ cells, 3) HPV $> 10^5$ cells, which were additionally divided into subgroups in accordance with the state of the vaginal flora: 1) normal flora; 2) flora deficient in lactobacilli.

Before treatment, all patients underwent a gynecological examination, cytology, and colposcopy, as well as an examination of the vaginal flora; the HPV copy number was measured, and the ratio of living, apoptotic, and necrotic vaginal epithelial cells was assessed. One month after the start of the treatment, the state of normal flora and the ratio of living, apoptotic, and necrotic vaginal epithelial cells were studied. Six months after the treatment, a gynecological examination, cytology, and colposcopy were carried out, and the HPV viral load was measured. All studies were performed on day 7–8 of the menstrual cycle. The progression of HPV infection was assessed by changes in the viral load and the cytology and colposcopy findings.

The presence and level of HPV were determined using a reagent kit for real-time polymerase chain reaction (PCR) manufactured by Lytech LLC (Russian Federation) on the MiniOpticon detection system (Bio-Rad, USA). The state of normal flora was assessed using the Nugent and Hay / Ison criteria and the number of bacteria of the genus Lactobacillus, determined by plating bacteria on MRS agar and then culturing in an atmosphere of 5% CO_2 for 48 hours. The belonging of microorganisms to the genus Lactobacillus was confirmed by real-time PCR with genus-specific primers [9].

The primary culture of stratified squamous epithelial cells was obtained by scraping the vaginal portion of the cervix after removing exfoliated cells with sterile normal saline. The cultures were transported in thermal containers in 1 ml of Hanks' Balanced Salt Solution (HBSS). To eliminate the accompanying microbial flora, the epithelial cells were washed once with a tenfold volume of phosphate-buffered saline.

The state of the epithelial cells was assessed after preliminary stimulation of apoptosis by triple exposure to H_2O_2 (50 μM) with an interval of 1 hour [10]. The ratio of apoptotic, living, and necrotic cells was determined by fluorescence microscopy using a kit containing Annexin V–Fluorescein with Propidium Iodide (BioVision, USA) according to the manufacturer's instructions.

Data are presented as absolute and relative (abs. (%)) incidence (outcomes of infection) and mean and standard deviation (state of epithelial cells). GraphPad Prism 6.0 software was used for statistical analysis and normal distribution testing. Normality of data distribution was tested using the Kolmogorov – Smirnov test. To assess the significance of differences between the incidence of different outcomes, the Fisher's exact test was used. The Mann – Whitney test was used for the ratio of apoptotic, living, and necrotic cells. The threshold for the statistical significance was set as a two-tailed *p*-value of 0.05.

RESULTS

Depending on the results of determining the viral load and the state of the vaginal flora, the patients were divided into the following groups: 29 patients with a normal number of lactobacilli and 29 patients with a deficiency of lactobacilli, who refused from probiotic therapy and were treated with immunomodulators, as well as 117 patients with a deficiency of vaginal lactobacilli receiving both immunomodulatory and probiotic therapy.

Table

Characteristics of patients according to the state of normal flora, HPV viral load, and the type of therapy

Viral load, DNA copies / 10^5 cells	Number of patients			Total
	with a deficiency of lactobacilli		with a normal number of lactobacilli	
	treated without probiotics	treated with probiotics		
$\leq 10^3$	47		13 (21.67%)	60
	9 (15.00%)	38 (63.33%)		
10^{4-5}	52		9 (14.75%)	61
	11 (18.03%)	41 (67.21%)		
$>10^5$	47		38 (70.37%)	54
	9 (16.67%)			

No significant differences in the basic demographic and anthropometric parameters between the groups were identified. By the end of the study, 6 women had dropped out: 4 due to failure to appear for the final follow-up examination and 2 (HPV> 10^5) due to indications for destructive therapy. Therefore, data on the structure of HPV outcomes will be presented for 141 patients in the treatment group and 28 patients in the control group.

Effect of probiotic therapy on the ratio of living, apoptotic, and necrotic vaginal epithelial cells

In samples from the patients with lactobacilli deficiency receiving probiotic therapy, the proportion of living infected cells remained practically unchanged, while the proportion of apoptotic cells increased, and the proportion of necrotic cells decreased (Fig.1). These processes were more pronounced in patients with high viral load.

In samples obtained from the patients with lactobacilli deficiency who refused from probiotic therapy (except for those who had high viral load), an increase in the survival rate of infected cells was observed (Fig.1). Reduction of the number of dead cells took place due to a decrease in necrosis, while the relative proportion of apoptotic cell death increased (Fig. 1). In the group of patients with high viral load, the changes were minimal and did not have statistical significance.

Therefore, inclusion of probiotics in the combination therapy for HPV infection prevented an increase in the proportion of survived infected cells and shifted the balance of cell death forms towards apoptosis.

Effect of vaginal probiotic therapy on outcomes of HPV infection

The results of a final follow-up examination (6 months after the beginning of the observation) showed that the state of the normal flora in the vagina affects the outcomes of HPV infection. Therefore, the frequency of complete HPV elimination in patients with normal flora was 2–2.5 times higher than in patients with deficiency of lactobacilli who did not receive probiotic therapy (Fig. 2). Vaginal probiotic therapy in patients with deficiency of lactobacilli caused an increase in the frequency of HPV elimination by 2.5 times and reduced the likelihood of therapy failure from 1.5 to 4 times, depending on the viral load (Fig. 2).

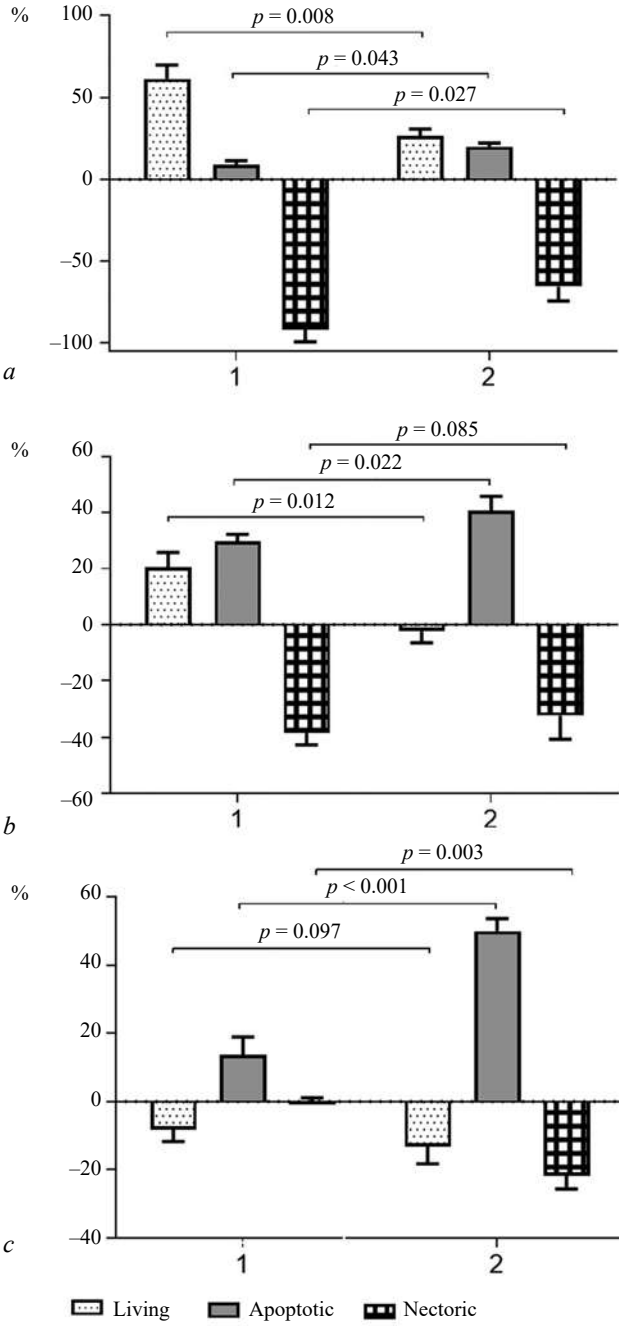

Fig. 1. Change in the ratio of living, apoptotic, and necrotic vaginal epithelial cells under the effect of therapy: Y-axis – compared with the baseline level (before therapy), %; X-axis – type of therapy: without a probiotic (1), with a probiotic (2). Baseline viral load, DNA copies / 10^5 epithelial cells: $\leq 10^3$ (a*),* $10^{4–5}$ (*b),* $>10^5$ *(c)*

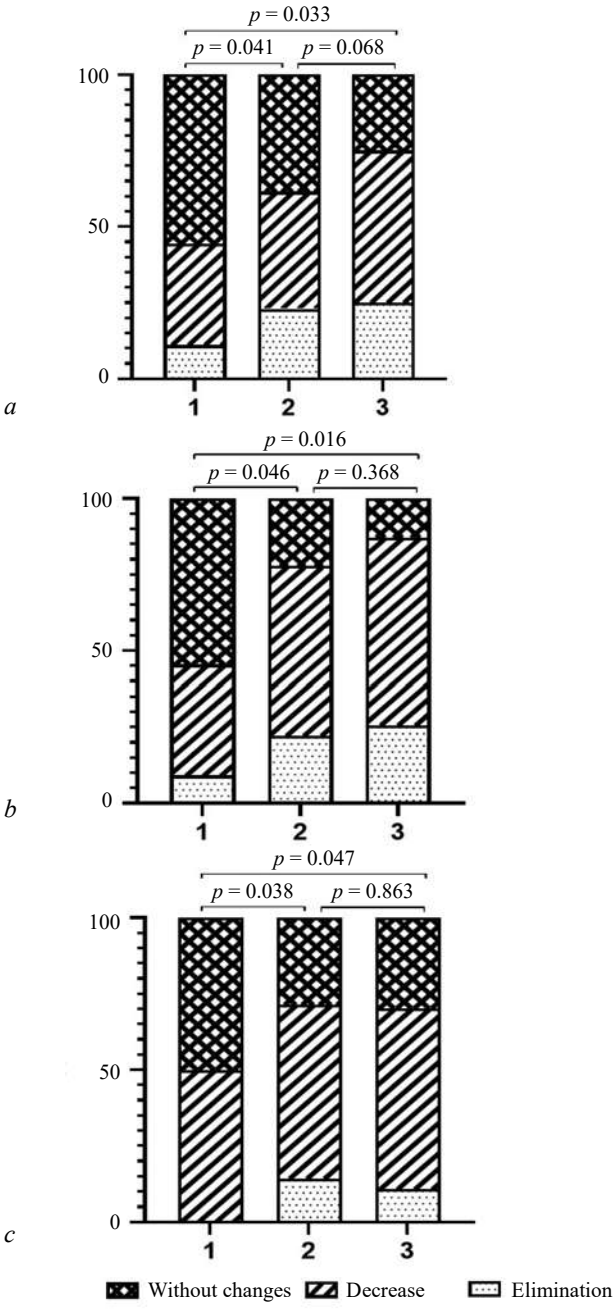

Fig. 2. Structure of HPV infection outcomes. Y-axis – proportion of outcomes, %; X-axis – the state of normal flora and the type of therapy: lactobacilli deficiency, without a probiotic (1), normal flora, without a probiotic (2), deficiency of lactobacilli, with a probiotic (3). Baseline viral load, DNA copies / 10^5 epithelial cells: $\leq 10^3$ (*a),* $10^{4–5}$ (*b),*; $>10^5$ (*c*)

The structure of HPV infection outcomes in Lactobacillus-deficient patients receiving probiotic therapy was similar to that in the group of patients with normal flora, and sometimes even exceeded it. Thus, inclusion of vaginal probiotic therapy in treatment of HPV-associated diseases improves their outcomes.

DISCUSSION

Therefore, the use of probiotics in Lactobacillus-deficient HPV-infected patients increases the likelihood of virus elimination. This effect can be determined by several mechanisms. Firstly, the stimulating effect of probiotics on synthesis of host defense fac-

tors [11] and an increase in their biological activity is known [12]. Secondly, metabolites of probiotic strains have a direct antiviral effect [13]. Thirdly, lactobacilli are able to selectively suppress cancer epithelial cells by increasing apoptosis without affecting healthy epithelial cells [14]. Our study showed that probiotic therapy also reduced the survival rate of infected cells by increasing apoptosis, while the proportion of other cell death forms decreased. At the same time, the shift in the balance of cell death forms towards apoptosis was most pronounced in samples obtained from patients with high viral load. Apparently, the shift in the cell death pattern from necrosis to apoptosis is also important for the elimination of the virus. It is known that necrosis, unlike apoptosis, is accompanied by the release of inflammatory mediators [15], and this adversely alters the environment of lactobacilli [16] and induces carcinogenesis [17].

We found that the effect of a probiotic containing the *LCR35* strain on HPV elimination is similar to the effect of normal flora and even slightly exceeds it. Perhaps this is determined by a higher concentration of lactobacilli during vaginal use of the probiotic or individual features of the probiotic strain *LCR35*. The first hypothesis is supported by the data that an oral probiotic does not affect HPV outcomes [18], since it cannot provide the required level of lactobacilli in the vagina. The second hypothesis is supported by data on the ability of the probiotic strain *LCR35* to potentiate the effect of antimicrobial drugs and significantly increase the likelihood of pathogen elimination [19]. In addition, a direct inhibitory effect of surfactants and lactate produced by the probiotic strain on reproduction of the papilloma virus is possible [11, 20].

CONCLUSION

Therefore, vaginal probiotic therapy improves the outcomes of HPV infection by reducing the number of survived infected cells and shifting the balance of cell death forms towards apoptosis.

REFERENCES

1. Drolet M., Bénard É., Boily M.C., Ali H., Baandrup L., Bauer H., Beddows S., Brisson J., Brotherton J.M.L., Cummings T., Donovan B., Fairley C.K., Flagg E.W., Johnson A.M., Kahn J.A., Kavanagh K., Kjaer S.K., Kliewer E.V., Lemieux-Mellouki P., Markowitz L., Mboup A., Mesher D., Niccolai L., Oliphant J., Pollock K.J., Soldan K., Sonnenberg P., Tanton C., Brisson M. Population-level impact and herd effects following human papillomavirus vaccination programmes: a systematic review and meta-analysis. *The Lancet Infectious Diseases*. 2015; 15 (5): 565–580. DOI: 10.1016/S1473-3099(14)71073-4.
2. Murall C.L., Rahmoun M., Selinger C., Baldellou M., Berna C., Bonneau M., Bernat C., Bonneau M., Boué V., Buisson M., Christophe G., D'Auria G., Taroni F.D., Foulongne V., Froissart R., Graf C., Grasset S., Groc S., Hirtz C., Jaussent A., Lajoie J., Lorcy F., Picot E., Picot M.-C., Ravel J., Reynes J., Rousset T., Seddiki A., Teirlinck M., Tribout V., Tuaillon E., Waterboer T., Jacobs N., Bravo I.G., Segondy M., Boulle N., Alizon S. Natural history, dynamics, and ecology of human papillomaviruses in genital infections of young women: protocol of the PAPCLEAR cohort study. *BMJ Open*. 2019; 9 (6): e025129. DOI: 10.1136/bmjopen-2018-025129.
3. Huh W.K. Human papillomavirus infection: a concise review of natural history. *Obstetrics & Gynecology*. 2009; 114 (1): 139–143. DOI: 10.1097/AOG.0b013e3181ab6878.
4. Da Silva C.S., Adad S.J., de Souza M.A.H., Barcelos A.C.M., Terra A.P.S., Murta E.F.C. Increased frequency of bacterial vaginosis and *Chlamydia trachomatis* in pregnant women with human papillomavirus infection. *Gynecologic and Obstetric Investigation*. 2004; 58 (4): 189–193. DOI: 10.1159/000079822.
5. Kero K., Rautava J., Syrjänen K., Grenman S., Syrjänen S. Association of asymptomatic bacterial vaginosis with persistence of female genital human papillomavirus infection. *European Journal of Clinical Microbiology & Infectious Diseases*. 2017; 36 (11): 2215–2219. DOI: 10.1007/s10096-017-3048-y.
6. Gillet E., Meys J.F., Verstraelen H., Bosire C., De Sutter P., Temmerman M., Broeck D.V. Bacterial vaginosis is associated with uterine cervical human papillomavirus infection: a meta-analysis. *BMC Infectious Diseases*. 2011; 11 (1): 10. DOI: 10.1186/1471-2334-11-10.
7. Norenhag J., Du J., Olovsson M., Verstraelen H., Engstrand L., Brusselaers N. The vaginal microbiota, human papillomavirus and cervical dysplasia: a systematic review and network meta-analysis. *BJOG: an International Journal of Obstetrics and Gynaecology*. 2019. DOI: 10.1111/1471-0528.
8. Mitra A., MacIntyre D.A., Marchesi J.R., Lee Y.S., Bennett P.R., Kyrgiou M. The vaginal microbiota, human papillomavirus infection and cervical intraepithelial neoplasia: what do we know and where are we going next? *Microbiome.* 2016; 4 (1): 58. DOI: 10.1186/s40168-016-0203-0.
9. Balashov S.V., Mordechai E., Adelson M.E., Sobel J.D., Gygax S.E. Multiplex quantitative polymerase chain reaction assay for the identification and quantitation of major vaginal lactobacilli. *Diagnostic Microbiology and Infectious Disease*. 2014; 78 (4): 321–327. DOI: 10.1016/j.diagmicrobio.2013.08.004.
10. Pletyushkina O.Yu., Fetisova E.K., Lyamzaev K.G., Ivanova O.Yu., Domnina L.V., Vysokikh M.Yu., Pustovidko A.V., Alekseevsky A.V., Alekseevsky D.A., Vasilyev Yu.M., Merphy M.P., Chernyak B.V., Skulachev V.P. Hydrogen peroxide produced inside mitochondria takes part in cell-to-cell transmission of the apoptotic signal. *Biochemistry.* 2006; 71 (1): 75– 84 (in Russ.).
11. Arena M.P., Capozzi V., Russo P., Drider D., Spano G., Fiocco, D. Immunobiosis and probiosis: antimicrobial activity of lactic acid bacteria with a focus on their antiviral and antifungal properties. *Applied Microbiology and Biotechnology*. 2018; 102 (23): 9949–9958. DOI: 10.1007/s00253-018-9403-9.

12. Sgibnev A.V., Kremleva E.A. Modification of enzymatic and antimicrobial activity of lysosomes by metabolites of vaginal lactobacilli. *Journal of Microbiology, Epidemiology, and Immunobiology*. 2018; 4: 21–27 (in Russ.). DOI: 10.36233/0372-9311- 2018-4-21-27.
13. Cadieux P., Burton J., Gardiner G., Braunstein I., Bruce A.W., Kang C.Y., Reid G. Lactobacillus strains and vaginal ecology. *JAMA*. 2002; 287 (15): 1940–1941. DOI: 10.1001/jama.287.15.1940.
14. Motevaseli E., Shirzad M., Akrami S.M., Mousavi A.S., Mirsalehian A., Modarressi M.H. Normal and tumour cervical cells respond differently to vaginal lactobacilli, independent of pH and lactate. *J. Med. Microbiol*. 2013; 62 (7): 1065–1072. DOI: 10.1099/jmm.0.057521-0.
15. Bordignon V., Di Domenico E., Trento E., D'Agosto G., Cavallo I., Pontone M., Pimpinelli F., Mariani L., Ensoli F. How human papillomavirus replication and immune evasion strategies take advantage of the host DNA damage repair machinery. *Viruses*. 2017; 9 (12): 390. DOI: 10.3390/v9120390.
16. Kremleva E.A., Sgibnev A.V. Proinflammatory cytokines as regulators of vaginal microbiota. *Bulletin of Experimental Biology and Medicine*. 2016; 162 (1): 75–78. DOI: 10.1007/s10517-016-3549-1.
17. Mangino G., Chiantore M.V., Iuliano M., Fiorucci G., Romeo G. Inflammatory microenvironment and human papillomavirus-induced carcinogenesis. *Cytokine & Growth Factor Reviews.* 2016; 30: 103–111. DOI: 10.1016/j.cytogfr.2016.03.007.
18. Ou Y.C., Fu H.C., Tseng C.W., Wu C.H., Tsai C.C., Lin H. The influence of probiotics on genital high-risk human papilloma virus clearance and quality of cervical smear: a randomized placebo-controlled trial. *BMC Womens Health*. 2019; 19 (1): 103. DOI: 10.1186/s12905-019-0798-y.
19. Sgibnev A.V., Kremleva E.A., Shchetinina Yu.S., Cherkasova Yu. I. Combined sue of antimicrobials and probiotics as a way to enhance the efficacy of therapy for genital infections. *Obstetrics and Gynecology*. 2018; 4: 113–118 (in Russ.). DOI: 10.18565/aig.2018.4.113-118.
20. Gao Y., Liu W., Wang W., Zhang X., Zhao X. The inhibitory effects and mechanisms of 3, 6-O-sulfated chitosan against human papillomavirus infection. *Carbohydrate Polymers*. 2018; 198: 329–338. DOI: 10.1016/j.carbpol.2018.06.096.

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