

## Role of microbiota correction in complex treatment of pregnant women with herpesvirus infection

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**Relevance.** Dysbiotic disorders and genital herpes are common among women of childbearing age, determines the urgency of enhancing the effectiveness of the treatment of these conditions.

**Purpose** – evaluation of probiotics efficacy in complex therapeutic and preventive measures for pregnant women with herpesvirus infection.

**Patients and methods.** The main group – 30 pregnant women with herpes infection to whom the complex of preventive measures with Florium probiotic containing 4 strains of lactobacilli was used; the comparison group – 30 pregnant women with prenatal care according to the Ministry of Health reports. The microscopic and cultural methods of examination were used in the studies of vaginal microecology. The intestine biocenosis disturbance level was evaluated by using cultural method and standard techniques.

**Results.** There was observed the reduction of opportunistic pathogens in the intestine (hemolytic *Escherichia coli*, *Proteus*, *Staphylococcus aureus*, *Klebsiella* and *Candida*) against the background of the increase of dominant flora (increased concentration of *Bifido*- and *Lactobacillus*). The incidence of complaints was decreased by 3 times (bloating, discomfort, constipation, mucus in the stool). The microbial landscape of the vagina has been improved: the incidence of *Lactobacillus* seeding was increased by 3 times; seeding rate pathogenic and opportunistic pathogens was reduced (*Staphylococcus aureus* and hemolytic, *Gardnerellas*, *Chlamydia*, *Ureaplazma*, *Mycoplasma* and *Candida*). The incidence of complaints was reduced by 2–3 times (excessive vaginal discharge, itching, swelling and redness of the mucosa). The 2-fold reduced incidence of placental insufficiency and pre-eclampsia as well as fetal distress testifies to the improvement of conditions for development of gestational process.

**Conclusions.** The results of efficacy and safety of probiotics in the complex treatment of pregnant women with herpes infection, allow us to recommend them for use in antenatal clinics and maternity homes.

**Key words:** pregnancy, dysbiosis, herpes, probiotics, treatment.

### Introduction

It is known that the mother is the main source of colonization of both normal and pathogenic microorganisms for a newborn. Recently, there are many pregnant women with bacterial vaginosis (20-25%). Bacterial vaginosis is one of the forms of vaginal microecology dysbiotic disorders caused under the influence of exogenous and endogenous factors. It is especially relevant for women with immunosuppression and herpesvirus infection particularly. From a position of microbiology, bacterial vaginosis should be treated as infectious inflammatory syndrome characterized by high levels of obligate anaerobes and sharp reduction of lactobacilli or their absence in the vaginal environment. It was proven that such imbalance of vaginal microflora in obstetric practice causes placental insufficiency, premature birth, growth retardation, postpartum endometritis and other obstetric complications [3].

Vaginal microbiota should not be considered as an isolated biotope, but in conjunction with a microbiome evaluation in women. Physiological immunosuppression during pregnancy and herpesvirus infection and alteration of acidity under the influence of gestation hormones disturb the microbial balance of biocenosis in all mucous membranes of the pregnant woman. Intestinal dysbacteriosis is revealed in 50-55% of women suffering from bacterial vaginosis. This dysbacteriosis testifies to the common dysbiotic process in the body with a dominant manifestation either in the genital or in the digestive system. The intestine is a reservoir for microorganisms associated with bacterial vaginosis. Severe intestinal dysbiosis may cause not only abnormal pregnancy and childbirth, but may also determine the formation of intestinal biocenosis in newborns. The intestine therefore is considered as a "target" for pathogenetic therapy [6].

For today the questions about the effect of combined disorders of vaginal and intestinal microbiocenosis on the development of obstetric and perinatal complications has not been sufficiently studied. Due to the lack of efficacy of therapeutic and preventive measures, it is necessary to look for preventive measures aimed at increasing resistance to infection of pregnant women.

The necessity to study and to treat mixed bacterial and viral infections of the lower genital tract (vulvovaginitis, cervicitis and bacterial vaginosis) and genital herpes infection is caused by their significant prevalence, insufficient efficacy of existing treatments associated with not quite studied processes of development of disease, drugs resistance, prolong course with frequent relapses and chronic process. A particular epidemiological threat is women asymptomatic herpes that causes the infection of the fetus, an increased number of spontaneous abortions, premature births and newborns with central nervous system disorders. Prolong herpesvirus persistence in humans and specific immunodeficiency generated in patients with recurrent genital herpes cause the addition of other urogenital tract infections [5].

Considering the fact that dysbiotic disorders and genital herpes are common in women of childbearing age, it is clear that the improvement of the efficiency of the treatment of these conditions through improved pathogenetically reasonable methods of therapy is very important. Bacterial and viral pathogens have different effects on the state of immune resistance of the body. So if most opportunistic pathogens increase the tension of anti-infectious general and local immunity, then the viral disease have mosaic pattern of immunogenesis disorders due to pronounced ability of viruses to block the synthesis of protective proteins and functional activity of certain types of immune cells, which in turn reduces the effectiveness of the treatment and makes it incomplete [ 2].

Probiotics – the drugs containing live strains of normal intestinal microflora, plays a leading role in the normalization of intestinal microflora. Probiotics have an antagonistic effect against abnormal strains of intestinal microflora. It is caused by the action of metabolites of the normal bacteria, the most important of which are short chain fatty acids and lactic acid. Probiotics not only prevent colonization of pathogens, but also contribute to the stabilization of the epithelial barrier as well as prevent translocation of bacteria into the internal environment. The stimulation of the immune response of mucosa of gastrointestinal tract and other organs plays an important role in the mechanism of action of probiotics [1, 4].

**Purpose** – the evaluation of probiotics efficacy in complex treatment and preventive measures for pregnant women with herpesvirus infection.

### **Materials and methods**

To test the efficacy of probiotics in complex treatment and preventive measures we have selected 60 pregnant women with herpesvirus infection (HVI) on the 14-16<sup>th</sup> week of pregnancy. These 60 women were divided into two groups randomly: the main group - 30 pregnant women to whom the recommended complex of preventive measures with probiotics was used; the comparison group – 30 pregnant women with prenatal care according to the Ministry of Health reports. The control group involved 50 healthy pregnant women with the appropriate period of pregnancy but without HVI.

The microscopic and cultural methods of examination were used in the studies of vaginal microecology. When comparing the results obtained with the use of Kira's classification to prove the cultural studies and clinical parameters we recorded four types of condition of vaginal biocenosis: normocenosis, moderate normocenosis, moderate dysbiosis and expressed dysbiosis.

The intestine biocenosis disorder level was evaluated in accordance with the guidelines "Diagnosis and Treatment of intestinal dysbacteriosis" (Moscow, 1991).

Quantitative analysis of microflora was studied by seeding of serial cultivations of 1 cm<sup>3</sup> of material on the surface of solid differential-diagnostic mediums: egg-yolk salt agar, blood agar, Saburo medium for fungi, thioglycolic medium, Endo's and Ploskiryev's medium, Medium Blaurock (bifidobacteria) and MRS medium (lactobacilli) were used to study the normal microflora. Quantitative parameters of bacterial growth were converted to decimal logarithms.

Oral symbiotic Florium was included in the complex of treatment to restore the microflora of the vagina and intestine. The preparation is easy to use; it does not require any special instructions for use; the course of treatment is 1 week. Florium is a patented food supplement (European Patent PCT / EP2011 / 065877) in the form of capsules containing four strains of lactic acid bacteria that restore and maintain the balance of vaginal flora:

- Lactobacillus crispatus LBV88 no less than 100h109 CFU/g - 40 mg;
- Lactobacillus rhamnosus LBV96 no less than 100h109 CFU/g - 40 mg;
- Lactobacillus gasseri LBV150 no less than 100h109 CFU/g - 12 mg;
- Lactobacillus jensenii LBV116 no less than 100h109 CFU/g - 8 mg.

In addition to Lactobacillus the composition of Florium contains fructooligosaccharides which is a breeding ground for the growth and reproduction of its own microflora.

*Method of administration and dosage:* 1 capsule twice a day (in the morning and in the evening) 30 minutes before meals, drink water or other beverage. Duration of use is 1 week.

### **Results and discussions**

After the treatment with probiotics the intestinal microecology of the women from the main group has been improved as evidenced by the reduced incidence of pathogens sowing. The incidence of sowing of Proteus (20.0% to 10.0%), Staphylococcus aureus (16.7% to 6.7%) fungus Candida spp. (36.7% to 16.7 %, p <0,05) was decreased by 2 times.

According to the Table 1, bifidobacteria and lactobacilli concentrations in the women of the main group have been increased. Thus, the number of lactobacilli in women after treatment was  $6,8 \pm 0,4$  Lg CFU/ml versus  $4,3 \pm 0,5$  Lg CFU/mL before treatment ( $p < 0,05$ ), bifidobacteria -  $6,6 \pm 0,6$  Lg CFU/ml versus  $4,3 \pm 0,4$  Lg CFU/ml, respectively ( $p < 0,05$ ). Against the background of dominant flora growth the reduction of opportunistic pathogens was recorded: in the women of the main group the significantly low concentrations of hemolytic *Escherichia coli*, however, *Staphylococcus aureus*, *Klebsiella* and fungi of *Candida spp.* were recorded. According to the analysis of laboratory and clinical parameters, the proportion of women of the main group with intestinal dysbiosis of various severity was decreased from 66.7% to 46.7%. Before treatment 40.0% of women with HVI complained about intestinal dysbiosis clinical manifestations, the most common of which were abdominal discomfort, constipation and mucus in feces. After the recommended treatment these complaints were observed only in 12.0% of pregnant women. As a result of complex treatment of women of the main group the microbial landscape of the vagina was improved. When a significant inhibition of normal microflora was recorded in the treatment of women of both the main group and the comparison group, then after the course the situation has improved. Thus, before the treatment bifidobacteria (CFU at 10<sup>4</sup> and above) were detected only in 10.0% and 13.3% of women of the main group and the comparison group, but after the recommended treatment the rate was increased up to 40,0% ( $p < 0,05$ ), and in the women of the comparison group, it remained at the same level (16.7%). Before the treatment Lactobacilli, which presence is essential for diagnosis of vaginal biocenosis, were detected in 13.3% and 16.7% of women, respectively, but after treatment they were detected in 46,7% ( $p < 0,05$ ) and 20,0%. As a result the incidence of sowing of significant levels of pathogenic and opportunistic pathogens was decreased (*Staphylococcus aureus* and hemolytic, *Gardnerellas*, *Chlamydia*, *Ureoplasma*, *Mycoplasma* and fungi of *Candida spp.*).

Table 1

**Quantitative parameters of intestinal microecology in the dynamics of treatment, Lg cfu/ml**

Microorganisms	Group of women studied				
	Main group		Comparison group		Control
	Before treatment	After treatment	Before treatment	After treatment	
<i>E. coli</i>	$6,2 \pm 0,4$ *	$6,8 \pm 0,3$	$6,0 \pm 0,4$ *	$6,1 \pm 0,5$ *	$7,6 \pm 0,5$
<i>E. coli haemol.</i>	$5,6 \pm 0,5$ *	$3,2 \pm 0,4$ n#	$5,5 \pm 0,4$ *	$5,1 \pm 0,5$ *	$2,6 \pm 0,3$
<i>Enterobacter cloacal</i>	$6,9 \pm 0,6$	$6,6 \pm 0,6$	$7,0 \pm 0,4$	$6,7 \pm 0,5$	$6,4 \pm 0,4$
<i>Klebsiella pneumoniae</i>	$7,0 \pm 0,5$ *	$5,4 \pm 0,4$ n#	$7,3 \pm 0,4$ *	$6,7 \pm 0,5$ *	$4,9 \pm 0,3$
<i>Proteus vulgaris</i>	$3,9 \pm 0,4$ *	$2,8 \pm 0,4$	$4,1 \pm 0,3$ *	$3,8 \pm 0,4$ *	$2,4 \pm 0,2$
<i>Staphylococcus epidermidis</i>	$5,8 \pm 0,4$	$6,0 \pm 0,5$	$5,6 \pm 0,6$	$5,9 \pm 0,4$	$6,2 \pm 0,5$
<i>Staphylococcus aureus</i>	$5,2 \pm 0,5$ *	$3,0 \pm 0,5$ n#	$5,4 \pm 0,4$ *	$5,2 \pm 0,6$ *	$2,1 \pm 0,5$
Fungi of <i>Candida spp.</i>	$6,4 \pm 0,4$ *	$4,8 \pm 0,6$ n#	$6,7 \pm 0,5$ *	$6,3 \pm 0,7$ *	$3,8 \pm 0,4$
<i>Bifidobacterium</i>	$4,3 \pm 0,4$ *	$6,6 \pm 0,6$ n#	$4,5 \pm 0,5$ *	$4,9 \pm 0,6$ *	$8,4 \pm 0,3$
<i>Lactobacterium</i>	$4,3 \pm 0,5$ *	$6,8 \pm 0,4$ n#	$4,0 \pm 0,5$ *	$4,5 \pm 0,3$ *	$7,6 \pm 0,2$

Notes: \* - a reliable difference regarding the rate of healthy pregnant women ( $P < 0,05$ );  
 ^ - a reliable difference regarding the rate before treatment ( $P < 0,05$ );  
 # - a reliable difference regarding the rate of women of the comparison group ( $p < 0,05$ ).

Table 2

**The results of microscopic examination of the vagina in the dynamics of treatment, abs. (%)**

Parameter	Group of women studied				
	Main group		Comparison group		Control
	Before treatment	After treatment	Before treatment	After treatment	
Type 2 smear (inflammatory)	25 (83,3)*	18 (60,0)*	27 (90,0)*	24 (80,0)*	18 (36,0)
Leucocytes:					
<20	5 (16,7)*	8 (26,7)	6 (20,0)*	5 (16,7)*	20 (40,0)
20-50	12 (40,0)	15 (50,0)	12 (40,0)	12 (40,0)	25 (45,0)
>50	13 (43,3)*	7 (23,3)	12 (40,0)*	13 (43,3)*	5 (10,0)
Sticks of Doderlein: available	4 (13,3)*	7 (23,3)*	5 (16,7)*	6 (20,0)*	30 (60,0)
single	5 (16,7)	8 (26,7)	5 (16,7)	4 (13,3)	5 (10,0)

absent	21 (70,0)*	15 (50,0)	20 (66,7)*	20 (66,7)*	15 (30,0)
Candida	12 (40,0)*	6 (20,0)*	14 (46,4)*	12 (40,0)*	4 (8,0)
Gardnerella	7 (23,3)*	4 (13,3)*	8 (26,7)*	6 (20,0)*	1 (2,0)
Chlamydia	7 (23,3)*	5 (16,7)*	8 (26,7)*	7 (23,0)*	1 (2,0)

Note: \* - a reliable difference regarding the rate of healthy pregnant women ( $P < 0,05$ ).

Table 3

**Clinical manifestations of vaginal microbiota disorders and the results of additional tests, abs. (%)**

Parameter	Group of women studied				
	Main group		Comparison group		Control
	Before treatment	After treatment	Before treatment	After treatment	
Pruritus	9 (30,0)*	3 (10,0) ^	8 (26,7)*	7 (23,3)*	3 (6,0)
Swelling, hyperemia	5 (16,7)*	2 (6,7)	7 (14,0)*	7 (14,0)*	2 (4,0)
Profuse discharge	10 (33,3)*	4 (13,3)^	8 (26,7)*	9 (30,0)*	3 (6,0)
Vaginal pH is more than 4.5	12 (40,0)*	5 (16,7)^#	11 (36,7)*	10 (33,3)*	4 (8,0)
Positive amine test of vaginal discharge	8 (26,7)*	3 (10,0)n#	8 (26,7)*	8 (26,7)*	2 (4,0)

Notes: \* - a reliable difference regarding the rate of healthy pregnant women ( $P < 0,05$ );

^ - a reliable difference regarding the rate before treatment ( $P < 0,05$ );

# - a reliable difference regarding the rate of women of the comparison group ( $p < 0,05$ ).

Table 4

**Complications of pregnancy in women studied, depending on the treatment, abs. (%)**

Complications	Group of women studied		
	Main group	Comparison group	Control
Threatened miscarriage	9 (30,0)*	12 (40,0)*	4 (8,0)
Threat of premature birth	5 (16,7)*	8 (26,7)*	2 (4,0)
Placental insufficiency	7 (23,3)*n	12 (40,0)*	4 (8,0)
Anemia	6 (20,0)	10 (33,3)*	9 (18,0)
Fetal distress	5 (16,7)*n	11 (36,7)*	3 (6,0)
Pre-eclampsia	3 (10,0)	6 (20,0)*	2 (4,0)
Pathology of the amniotic fluid	5 (16,7)*	8 (26,7)*	2 (4,0)

Notes: \* - a reliable difference regarding the rate of healthy pregnant women ( $P < 0,05$ );

^ - a reliable difference regarding the rate of women of the comparison group ( $p < 0,05$ ).

The effectiveness of treatment and the results confirmed by the microscopic examination of the vagina (tab. 2): the proportion of women with a leucocytes' number more than 50 was decreased by 2 times. After treatment the presence of sticks of Doderlein was observed in 23.3% of women versus 13.3% of women before treatment. It testified to the improvement of protective capabilities and created conditions for growth inhibition of pathogens (fungi of *Candida* spp., *gardnerella*, *chlamydia*).

The clinical picture of the dynamics of treatment also proved its effectiveness. The incidence of complaints of women in the main group was reduced, significantly (Table. 3). According to our data, before the treatment the pregnant women with HVI often complained about the profuse vaginal discharge. Such complaints have been recorded in a third of women. After the treatment this proportion was decreased by 2 times (14.0% versus 24.0% in the comparison group,  $p < 0,05$ ); the proportion of women who complained about pruritus was decreased by 3 times, and the proportion of women who complained about mucosal swelling and congestion was decreased by 2 times. Clinical manifestations have been proven by the results of additional tests. Before the treatment the content of vaginal pH was more than 4.5 in more than a third of women with HVI, and after the recommended treatment rate was decreased by more than 2 times (from 40,0% to 16,7%,  $p < 0,05$ ). Amine test of vaginal discharge has shown the positive results in 26.7% of women, and after the recommended treatment – in 10,0% ( $p < 0,05$ ) of women.

The improvement of the general condition and microbiota of the woman of the main group allowed to improve the conditions for the development of gestational process. The pregnancy was aggravated in 33.3% of pregnant

women of the main group versus 53.3% of women of the comparison group ( $p < 0,05$ ). The incidence of placental insufficiency was decreased by approximately 2 times (from 40.0% to 23.3%) and the incidence of pre-eclampsia (tab. 4) as well as fetal distress were also decreased by approximately 2 times from 36.7% to 16.7%. No side effects or complications associated with the intake of probiotics were recorded. According to the integrated analysis of laboratory and clinical parameters, the performed treatment with the use of probiotics in 93.3% of women can be considered effective. The results of efficacy and safety of the probiotics in the complex treatment of pregnant women with herpesvirus infection allow to recommend these probiotics to use in antenatal clinics and maternity homes.

## Conclusions

The performed treatment of pregnant women with herpesvirus infection with use of probiotic helped to improve the state of microbiota.

There was observed the reduction of opportunistic pathogens in the intestine (hemolytic *Escherichia coli*, *Proteus*, *Staphylococcus aureus*, *Klebsiella* and *Candida*) against the background of the increase of dominant flora (increased concentration of *Bifido*- and *Lactobacillus*). According to the analysis of laboratory and clinical parameters, the proportion of women with intestinal dysbiosis of various severity was decreased from 66.7% to 46.7%; the incidence of complaints was reduced by 3 times (bloating, discomfort, constipation, mucus in feces). The microbial landscape of the vagina has been improved: the incidence of *Lactobacillus* seeding was increased by 3 times; seeding rate pathogenic and opportunistic pathogens was reduced (*Staphylococcus aureus* and hemolytic, *Gardnerellas*, *Chlamydia*, *Ureaplasma*, *Mycoplasma* and *Candida*). The incidence of complaints was reduced by 2–3 times (excessive vaginal discharge, itching, swelling and redness of the mucosa). The improvement of the general condition and microbiota allowed to improve the conditions for the development of gestational process. The incidence of placental insufficiency, pre-eclampsia as well as fetal distress was decreased by approximately 2 times. The results of efficacy and safety of the probiotics in the complex treatment of pregnant women with herpesvirus infection allow to recommend these probiotics to use in antenatal clinics and maternity homes.

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