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Probiotics Application Prospects in the Treatment of Vaginosis: Long-Term Effects

Key words: *bacterial vaginosis, combination treatment, probiotics, Lactobacillus.*

Annotation: *to study the comparative efficacy of Lactoginal and Ecofemin used as part of combination therapy in patients with bacterial vaginosis and to evaluate the efficiency of Lactoginal monotherapy.*

Great importance is currently attached to vaginal diseases, especially caused by conditionally pathogenic flora, including bacterial vaginosis. The incidence of this pathology is 20 to 80% of all visits to the gynecology outpatient care. Numerous studies have established that vaginal dysbiosis and infections have a direct impact on the female reproductive function. There is an association between bacterial vaginosis and inflammatory diseases of the small pelvis, chorioamnionitis, spontaneous miscarriages (16,26), non-developing pregnancy, premature birth (4,7,16,19), chronic fetal hypoxia, weakness and disorganized labor activity, the development of metroendometritis, peritonitis, sepsis (2,3,7,12,14,16,23). Equally important is weight reduction and development of pneumonia in the newborn (3,4,7). In addition, the presence of bacterial vaginosis reduces the quality of life in women, creating intimate problems and discomfort in the sexual sphere. Normal vaginal microbiocenosis is represented mainly by lactobacilli, a small number of anaerobic and aerobic microorganisms that are in the state of dynamic equilibrium. Number of lactobacilli in a healthy woman is 10^7 — 10^8 CFU/ml. It is known that in the vaginal secretion there are several types of lactobacilli (*L. acidophilus*, *L. casei*, *L. crispatus*, *L. gasseri*, *L. jensenii*, *L. insers* and some others). In the process of life and disintegration of glycogen these bacteria produce lactic acid, synthesize peroxide, have the adhesive ability, but the intensity of these purposes is different in different strains. Maintenance of the colonization resistance is provided by acid-forming function of lactobacilli and ability to synthesize the hydrogen peroxide.

In the treatment of bacterial vaginosis antibiotics are widely used, however, their use does not guarantee long-term effect and is often accompanied by the disease recurrences, associated with the low number of lactobacteria and insufficient activity of normal microbiocenosis (1,5,6,8,9,11,13,15,17,18). The current course of bacterial vaginosis as a

recurrent disease creates certain difficulties for its treatment (6,16,17). There are often asymptomatic forms of bacterial vaginosis, which have as much influence on the reproductive system than as the forms having obvious clinical presentation (13,14,15,16,25).

Active and unsystematic use of antibiotics in modern medical practice leads to disturbance of vagina microbiocenosis. Currently, etiology of bacterial vaginosis is not fully established. However, it is known that the pathogenesis of the disease is associated with the presence of several microorganisms, with the content of lactic acid bacteria sharply reduced and the number of anaerobic, aerobic and microaerophilic microorganisms greatly increased. In bacterial vaginosis such microorganisms as *Gardnerella vaginalis*, *Atopobium vaginae*, *Mobiluncus spp.*, *Prevotella spp.*, *Peptostreptococcus spp.*, *Mycoplasma hominis*, *Ureaplasma urealiticum*, and others, are found. It is established that in bacterial vaginosis pathological mechanism is associated with the formation of bacterial biofilms, which are colonies of microorganisms attached to the vaginal epithelium and covering it completely or partially. These biofilms are often resistant to antibiotic therapy, contribute to the increase of pH and displace endogenous lactoflora. It is known that lactobacilli can also form useful biofilms, produce surfactant, synthesize hydroxyl radicals with antibacterial properties. In addition, lactobacilli are able to adhere to biofilms synthesized by pathogenic agents, while some types of lactobacilli contribute to the destruction of pathogenic biofilms (10,20,21,22).

Treatment for bacterial vaginosis during pregnancy is complicated due to its recurrent nature. Recent observations have made physicians to turn to probiotics as a second-line drug therapy. As a part of complex therapy probiotics may represent one of the alternatives for bacterial vaginosis treatment. This may be possible due to their ability to destroy biofilms created by pathogens, maintain the vaginal pH within normal limits (not more than 4.5) and enable formation of colonization resistance due to lactobacilli within them. The goal of treatment is not only to eradicate pathogenic microorganisms, but first and foremost, to provide and maintain long-term normal biocenosis with the help of lactobacilli. LACTOGINAL® is a tribiotic consisting of three components: prebiotic, *Lactobacillus casei rhamnosus* spp. 35 in the form of live culture, and eubiotics (active metabolites) generated in the process of vital activity of lactobacilli. *Lactobacillus casei rhamnosus* spp. 35 (24,25) break down glycogen and produce lactic acid, which lowers the pH of the vaginal discharge and makes it possible to resist the reproduction of conditionally pathogenic microorganisms, as well as contributes to the maintenance and recovery of normobiocenosis. Use of LACTOGINAL® promotes formation of protective biofilms by lactobacilli, which creates an obstacle for the adhesion of pathogenic microorganisms. Thus, “bacterial competition” leads to the restoration of normal microflora of the vagina. In addition, lactobacilli are able to synthesize hydrogen peroxide and other compounds with bactericidal and bacteriostatic action against pathogenic microbes. It is established that *Lactobacillus casei rhamnosus* spp. 35 shows bactericidal activity against *Gardnerella vaginalis*, *Prevotella bivia* and certain strains of *Candida albicans*.

The purpose of our research is to compare the effectiveness of LACTOGINAL® and ECOFEMIN® medications in the combined therapy of bacterial vaginosis, as well as to assess the effectiveness of LACTOGINAL® monotherapy. Unlike LACTOGINAL® tribiotic, ECOFEMIN® is represented in the form of soluble vaginal capsules containing *Lactobacillus*

acidophilus 10^8 — 10^9 CFU/ml and able to produce hydrogen peroxide. This strain is isolated from the vaginal microflora of healthy women, lactose is used as a medium.

One of the purposes of the study was to compare the efficiency of preparations containing various strains of lactobacilli in the therapy of bacterial vaginosis in women of reproductive age. Special attention is paid to studying long-term results of treatment and preventing recurrences.

MATERIAL AND METHODS

A total of 92 women aged from 18 to 35 years with confirmed diagnosis of bacterial vaginosis were involved in the study. Patients included in the study in a random order, were divided into three groups. The presence of bacterial vaginosis at the time of inclusion in the study was confirmed by the data of objective inspection and laboratory studies using Amsel criteria (21), pH-metry (pH > 4.5), revealing "key cells" in smears, and the data of FEMOFLOR® Real-time PCR test.

Pregnant patients as well as patients with STIs and vulvovaginal candidiasis were excluded from the study.

The first group consisted of 32 women who used Dalacin 2% vaginal cream followed by ECOFEMIN therapy, the second group included 30 patients using Dalacin® cream at the first stage and LACTOGINAL® at the second phase of therapy, the third control group was composed by the patients receiving LACTOGINAL monotherapy without antibiotics.

In Group 1, Dalacin® 2% cream was used intravaginally in the dose of 100 mg for 7 days, then one capsule of vaginal ECOFEMIN® twice a day for 6 days.

In Group 2, the treatment was carried out with Dalacin® cream intravaginally in the dose of 100 mg for 7 days, then vaginal LACTOGINAL® with a dose of one capsule in the morning and one capsule in the evening for 7 days.

In Group 3, the patients received the following treatment: for the first 7 days vaginal shower with saline was conducted in the morning and in the evening, for the following 7 days the patients were prescribed one LACTOGINAL® vaginal capsule twice a day.

To assess the effectiveness of the preparations used, the dynamics of complaints, the data of objective inspection, pH-metry of vaginal discharge, smear microscopy, FEMOFLOR® Real-time PCR test immediately after the treatment, in 1 month, 3 months, 6 months after the start of treatment were analyzed. Basic initial values in all three groups were comparable.

RESULTS AND DISCUSSION

Before the treatment the majority of patients in all the group complained of excessive foul-smelling discharge (30 (93.75%) in Group 1, 29 (96.66%) in Group 2, 29 (96.66%) in Group 3), itching and burning sensation in the genitals area were noted in 5 (15.62%), 4 (13.33%), 5 (16.66%) patients in Groups 1, 2, and 3, respectively. Discomfort during sexual intercourse was observed in 16 cases (50%) in Group 1, in 14 cases (46.66%) in Group 2, and in 15 cases (50%) in Group 3, all had positive Amsel criteria. Examination revealed foul-smelling grey homogeneous vaginal discharge 30 (93.75%), 29 people

(96.66%), 28 (93.33%) patients in Groups 1, 2, and 3, respectively. Vaginal pH was greater than 4.5 in all patients. (Table 1).

Table 1. Result of PH-metry in Groups 1,2,3 before treatment, in 1 month, 3 month and 6 months after the start of treatment.

Group 1

pH	Before treatment	After treatment	In 1 month	In 3 month	In 6 month
N 3,8-4,5	0	32	29	19	8
Abs.	0	100	91	59	25
%					

Group 2

pH	Before treatment	After treatment	In 1 month	In 3 month	In 6 month
N 3,8-4,5	0	30	30	29	28
Abs.	0	100	100	97	93,3
%					

Group 3

pH	Before treatment	After treatment	In 1 month	In 3 month	In 6 month
N 3,8-4,5	0	30	28	24	19
Abs.	0	100	93	80	63,3
%					

FEMOFLOOR® Real-time PCR test found a variety of gram-positive and gram-negative floras, but no signs of inflammation in patients of all groups were revealed. (Table 2). Before the treatment, all groups showed a reduction in the number of lactobacilli to 10^2 — 10^3 CFU/ml, an increase in the number of *Gardnerella vaginalis*, *Mobiluncus spp.*, *Atopobium vag.*, *Candida alb.* By the second day, all patients showed the improvement of their health. By the end of the course of therapy their complaints disappeared, pH-metric indices came to normal in 100% of patients in all three groups. Immediately after the course of treatment, positive results were received as expected, but it seemed particularly important to measure the duration of therapeutic effect over a period of 6 months and to assess the treatment methods used, to determine the risk of recurrence in each group.

Table 2. The results of FEMOFLOOR® Real-time PCR test in Groups 1,2,3 before treatment, after 1 month and 6 months after the start of treatment.

Group 1

Index	N bacterium	Before treatment	In 1 month	In 6 month
Lactobacillus	10^7 - 10^9 abs. %	18 56,2	29 90,6	21 65,6
Gardnerella vaginalis	0 - 10^2 abs. %	9 28,1	15 46,9	22 68,8
Atopobium vaginae	0 - 10^2 abs. %	17 53,1	26 81,2	28 87,5
Mobiluncus + Corynebacterium	0 - 10^2 abs. %	12 37,5	16 50	23 71,9

Group 2

Index	N bacterium	Before treatment	In 1 month	In 6 month
Lactobacillus	10 ⁷ -10 ⁹ abs. %	7 23,3	28 93,3	20 66,6
Gardnerella vaginalis	0-10 ² abs. %	9 30	18 60	24 80
Atopobium vaginae	0-10 ² abs. %	7 23,3	10 33,3	23 76,6
Mobiluncus + Corynebacterium	0-10 ² abs. %	8 26,7	19 63,3	27 90

Group 3

Index	N bacterium	Before treatment	In 1 month	In 6 month
Lactobacillus	10 ⁷ -10 ⁹ abs. %	8 26,6	28 93,3	27 90
Gardnerella vaginalis	0-10 ² abs. %	9 30	24 80	25 83,3
Atopobium vaginae	0-10 ² abs. %	4 13,3	8 26,6	28 93,3
Mobiluncus + Corynebacterium	0-10 ² abs. %	14 46,7	27 90	27 90

As seen from Table 2, despite the fact that the primary analysis of lactobacilli with respect to the norm in Group 1 was almost twice the values of Groups 2 and 3 (Group 1 – 56.2%, Group 2 – 23.3%, Group 3 – 26.6%), the results of treatment in Groups 2 and 3 were higher in relation to maintaining normoflora, and, which is very important, remained high over the long-term 6-month follows-up. Group 3 showed the most rapid and steady growth of lactobacilli (after 1 month - 93%, after 6 months - 90% of patients). The data are shown as a bar graph in Fig. 1.

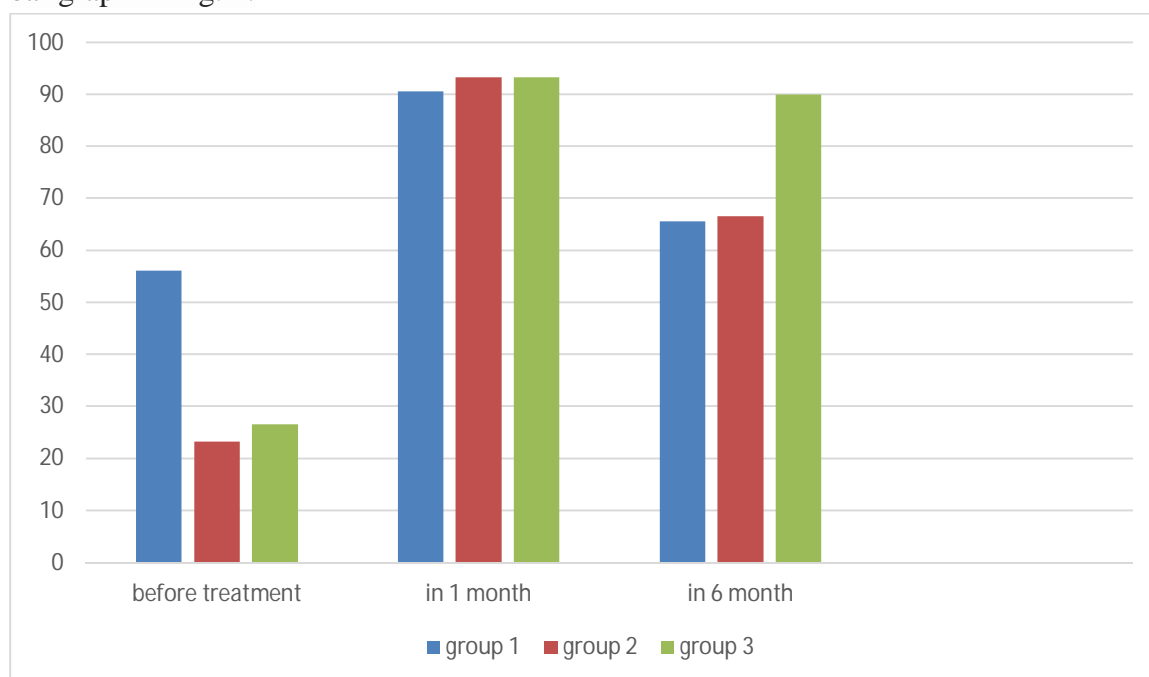


Fig.1. Growth of lactobacilli in Groups 1,2,3 before and after the treatment

Despite the equal initial number of patients with an increased rate of *Gardnerella vag.* in all groups, in a month after the start of therapy the result in Groups 2 and 3 was significantly higher (Group 1 – 46.9%, Group 2 - 60%, Group 3 - 80%), and this positive result was maintained over the period of 6 months (in Groups 1 and 2 indices have improved up to 69% and 80% respectively, and in Group 3 this value was 83%), (Fig. 2), that probably has a positive effect on the prevention of recurrences. Despite the fact that the standard therapy includes antibacterial therapy or metronidazol, in some cases (intolerance, allergic reaction) antibiotics have to be discontinued. The research results show that in most cases the LACTOGINAL® therapy can be effective enough, especially in the prevention of recurrence, provided that the complete course of therapy (Fig.2) is followed. It is possibly connected with the confirmed *in vitro* fact of generation of *Lactobacillus casei rhamnosus* spp. 35 of Lactocin-160 bacteriocin, which is active against a number of pathogens in vaginal microflora. According to some researches, the bactericidal activity of Lactocin is conditioned by Lactocin-induced disturbances in cell membranes of pathogenic microorganisms, probably due to the formation of pores leading to ATF leakage (26).

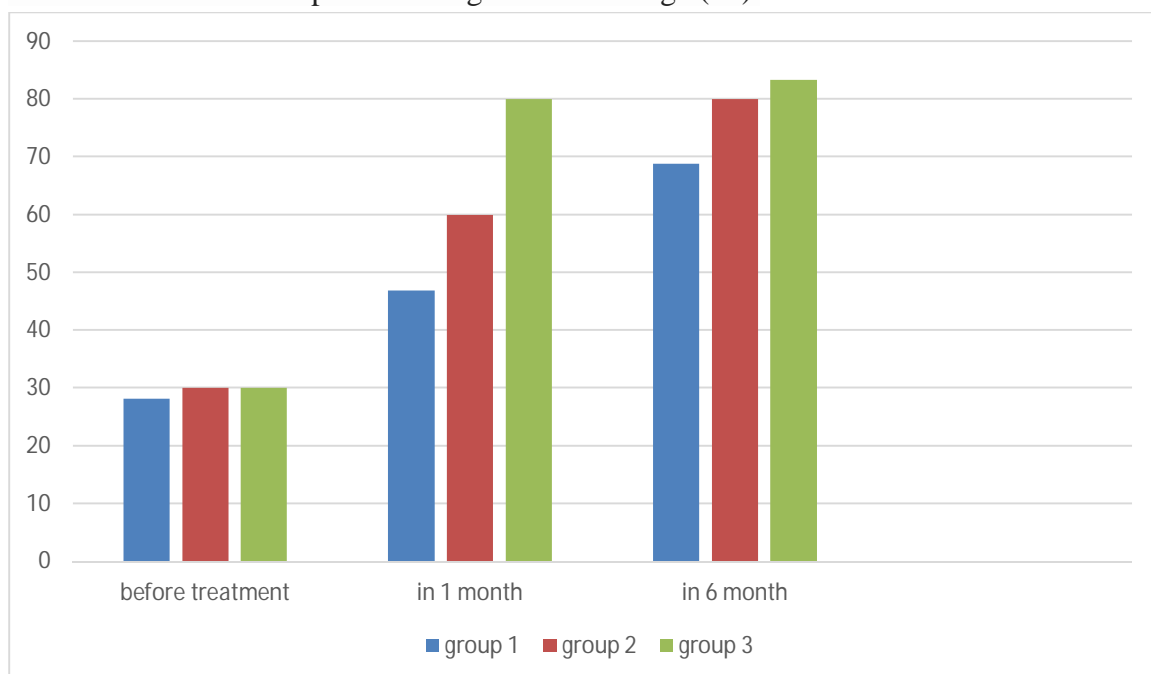


Fig.2. Growth of *Gardnerella vaginalis* in Groups 1,2,3 before and after the treatment

Although in the primary analysis of *Atopobium vag.* with respect to the norm the number of patients with permissible *Atopobium vag.* level in Group 3 were the smallest (13.3%), unlike Group 1- 53.1% and Group 2 – 23.3%, the highest result after 6 months was in Group 3 - 93%.

It should be noted that generally the influence of lactobacillus strains on *Atopobium vag.* requires a longer period of time compared to other pathogens. (Fig.3.)

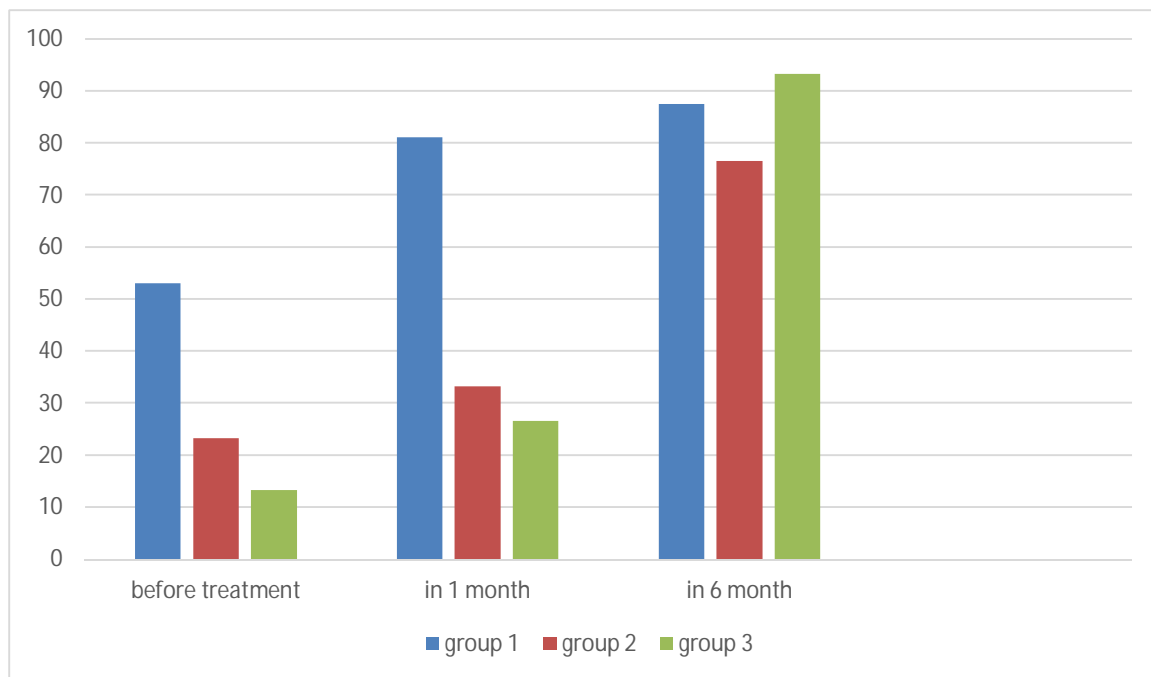


Fig.3. Growth of *Atopobium vaginae* in the patients of examined groups before and after the treatment

Analysis of treatment results concerning quantitative composition of *Mobiluncus* (Fig.4.) showed the situation similar to the volume of *Gardnerella vag.* in all groups: 1 month after the start of therapy result in *Mobiluncus* in Groups 2 and 3 was significantly higher (Group 1 - 50%, Group 2 – 63.3 %, Group 3 - 90%), and the result in varying degrees of effectiveness lasts for 6 months (Group 1 – 71.9% , Group 2 - 90%, Group 3 - 90%) (Table 2), which can be regarded as a higher result for the prevention of recurrent bacterial vaginosis. This may be explained by the idea that the treatment of bacterial vaginosis needs combination of the acidification of the vaginal environment and supplement of lactobacilli to restore normocenosis in vagina. Given that LACTOGINAL vaginal capsules consist of products of lactobacilli life, probably this explains higher results in Groups 2 and 3.

It should be noted that the results obtained can be interpreted only as a tendency to permanent cure, because there were no statistically significant differences confirmed ($P > 0,005$) in the groups. However, this study can be considered as a pilot project, aimed at attracting the specialists' attention to the development of new alternative treatments for bacterial vaginosis. In future the arrangement of a large-scale study is possible.

CONCLUSION

The results obtained can be interpreted as an evidence that the use of probiotics in the treatment for bacterial vaginosis significantly improves the results of treatment and promotes normalization of vaginal biocenosis. Current researches often assess treatment results obtained immediately after the therapy. This research set the task to monitor the long-term effect of the treatment and the opportunity to find ways to minimize the risk of recurrence in the future. Regarding the different activity of lacto-bacteria (*Lactobacillus acidofilus* and *Lactobacillus casei rhamnosus* spp.), the choice of a probiotic in the complex therapy of bacterial vaginosis should be determined on the basis of activity of lactobacilli composing the

drug. LACTOGINAL® contains Lactobacillus strains, which are able not only to adhere to vaginal epithelium, maintaining the pH of the vaginal discharge, but also, according to some authors, have direct bactericidal effect on pathogenic microorganisms (24,25).

Thus, administration of LACTOGINAL® as a monotherapy for bacterial vaginosis in women who cannot use antibiotics widely due to allergic reactions and other conditions seems very promising and requires further study.

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