

Correction of vaginal microflora in women with hyperandrogenia and reproductive losses at pregravid stage

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Summary. In modern conditions the role of endocrine pathology, including hyperandrogenia (HA) of various origin, was increased among the risk factors responsible for the high rate of perinatal morbidity and mortality. Infectious factor should be accented among the principal etiologic factors of fetal loss syndrome. Studying the state of the cervical canal in women with hyperandrogenia, including the high frequency (up to 34%) of functional isthmic-cervical insufficiency in these contingent of patients, appears to be essential. The secretion of cervical mucus is a barrier to ascending infection. The protective effects of mucous coating, and especially the local complex of antimicrobial protection that makes to investigate the status of vaginal microbiocenosis as a potential reservoir of pathogens that can cause a pathological process and promptly correct it at pregravid stage, are the main factors involved in the phenomenon of colonization resistance of the mucous membranes by the body.

Keywords: hyperandrogenia, miscarriage, microbiocenosis, probiotics, biotope.

The role of endocrine pathology, including hyperandrogenia (HA) of various genesis, was increased among risk factors responsible for the high rate of perinatal morbidity and mortality. Some studies results showed that HA is a risk factor for obstetric pathology, including placental insufficiency [1]. Pregnancy ensues mostly in obliterated «non-classical», «late onset» forms of hyperandrogenia as well as in its mixed forms.

One of the most common reasons of fetal loss syndrome are infectious diseases. The cervical mucus secretion is a barrier to ascending infection. The protective mucous coating effects and especially those of the local complex of antimicrobial protection are the main factors involving by the body to the phenomenon of colonization resistance of the mucous membranes. The study of the cervical canal state in women with hyperandrogenia, including the high frequency (34%) of functional isthmic-cervical insufficiency in these patient contingents, is particularly important. Inflammatory diseases of the lower internal genital organs (vaginitis, cervicitis) are characterized by obliterated latent course [1, 2].

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A peculiarity of urogenital infections is the increasing frequency of diseases occurring with the involvement of microorganisms that enter into the content of normal vaginal flora [3]. Under certain conditions, the normal microflora acquires pathogenic properties and its representatives turn into causative agents of a whole number of diseases that constrains to investigate the status of vaginal microbiocenosis as a potential reservoir of pathogens that can cause the pathological process [4, 5]. Studies conducted by V.M. Sidelnikova [6] showed that dysbiotic processes in the lower genital tract is the leading pathogenetic link in the mechanism of ascending endometrium infection, especially in women with isthmic-cervical insufficiency. Bacteriological examination of the cervical canal revealed a frequent association of several bacterial species, sterile inoculations are found only in 8% of women. Quantitative and qualitative content of the vaginal microflora depends on the age, endocrine and immune status of women.

Gram-positive and gram-negative aerobic, facultative aerobic and obligate-anaerobic microorganisms are involved in vaginal flora. Peroxide-producing lactobacilli dominate in the vaginal microbiocenosis content of healthy women in norm [3]. 7 species of lactobacilli are known, which are capable of preventing the development of infectious processes of the genitals. Some species of lactobacilli produce a biosurfactant blocking the pathogens adhesion to the vaginal mucous epithelium, as well as acids and bacteriocins, which inhibit the growth of various bacteria. A normal vaginal microflora is provided by colonization resistance (CR) [7, 8]. The main mechanisms of the CR vaginal biotope are acid formation and hydrogen peroxide production. An association between vaginal dysbiosis and unfavorable pregnancy outcome is demonstrated by some investigators. Clinical presentations of infection of the lower genital tract occur only in 25-30% of women by apparent imbalance in the vaginal microbiocenosis. About 50% of the vaginal microbiocenosis composition abnormalities occur without clinical presentations, remain undiagnosed and therefore untreated. Asymptomatic disease forms sometimes influence on the reproductive health more than symptomatic ones. Miscarriage is characterized not by monoinfection but by a combined urogenital infection that often occurs in subclinical form, making it difficult to identify [4].

Pregavid preparation has an utmost significance for the course and outcome of the next pregnancy for women with a history of miscarriage and detected urogenital infection. Detection of clini-

cal and microbiological signs of vaginal dysbiosis requires etiotropic treatment, the ultimate goal of which is to restore the vaginal biotope [9]. Drugs of oral and vaginal mode of application are used for vaginal dysbiosis treatment. For this purpose, probiotics are often used, some of them are oriented on the vaginal mode of application (Acilact, Lactobacterin and others), the use of which does not always lead to a favorable clinical and therapeutic effect as lactobacilli and bifidus bacteria, isolated from the gastrointestinal tract of humans, while getting into an unusual habitat, have low adhesive properties towards vaginal epithelial cells [10, 11]. A probiotic medicine for peroral administration, that normalizes the vaginal microflora, Vagisan («Jadran» Drug Company, Croatia), appeared in the arsenal of doctors. It consists of unique lactobacilli strains (*Lactobacillus Rhamnosus* GR-1 and *Lactobacillus Reuteri* RC-14), which were isolated from the distal urethra and vagina of healthy women by Canadian scientists A.W. Bruce and G. Reid (1980). A peculiarity of these lactobacilli strains use is their high adhesiveness towards vaginal epithelial cells. These lactobacillus strains preserve their vital capacity after passage through the gastrointestinal tract and can successfully colonize the vagina and displace pathogenic flora, at a necessary dose of probiotic 10^9 CFU/ml [5, 6].

The aim of our study was to evaluate the effectiveness of Vagisan in treating vaginal dysbiosis in women with HA and habitual pregnancy loss by pregravid preparation.

Material and Methods

65 women with HA and history of reproductive losses, vaginal dysbiosis diagnosis, participated in this study. Vaginal microflora correction was performed on the final stage of pregravid preparation after bowel bacteria overgrowth elimination, extragenital nidi of infection sanation, medical hormonal homeostasis correction according to the form of androgens domination. The 1-st group included women (n=31) who were treated with Vagisan for a vaginal dysbiosis at a dose of by one capsule twice a day *per os* for a period of two weeks. The 2-nd group (control group) comprised patients (n=34) who received treatment for a vaginal dysbiosis with antibacterial medicines for topical administration (Betadine, Hexicon, Tergynan, Polygynax, Klion, Macmiror).

All women had the following tests: bacteriology (inoculation from cervical canal and vagina to the flora, ureaplasma and mycoplasma; polymerase

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chain reaction for the detection of chlamydial antigen, inoculation of the vagina consist) and virology (cytomegalovirus and herpes antibodies), determination of E2 and P levels in blood on the 19th-21st day of menstrual cycle, and also interferon status study, homocystein, hemostasis (coagulogram), ultrasound. The age of women in both groups averaged 30.4 years. In the 1-st group habitual miscarriage in the past history was observed in 75.0% of women, in the control group in 59,1%. Cervical lesions dominated among gynecological disorders in both groups. 42.2% of the women in the 1-st group had ectopia and cervicitis, in the control group in 41.2%. Bacteriological examination detected a high specific weight of microbial associations in both groups: in the 1-st group in 70.9% of women, in the 2-nd group in 64.7%. To evaluate the effectiveness of treatment of vaginal dysbiosis a repeated bacteriological examination (inoculation from cervical canal and the vagina consist) was carried out two weeks after completion of Vagisan treatment. A bacteriological study was carried out in the control group one week after cancelling suppositories.

Results of the study

The results of the study are presented in **Table 1** and **2**. As shown by Table 1, in the group (after vaginal dysbiosis treatment with Vagisan) the number of women has increased in which colonization of lactobacilli in the cervical canal increased from 13.3 to 46.2% ($p \leq 0.05$) at bacteriological test; while lactobacilli colonization in the cervical canal was not significantly increased (after topical an-

Table 1. Results of bacteriological examination of the cervical canal before and after treatment with Vagisan (%)

Pathogen	1-st group before treatment	1-st group after treatment	2-nd group before treatment	2-nd group after treatment
<i>Gardnerella vag.</i>	12.9	0	11.8	0
<i>Lactobacilla sp.</i>	13.3±6.2	46.2±9.8	20.6±6.9	41.2±8.4
<i>E. coli</i>	35.5±8.6	18.5±7.4	32.4±8.0	20.6±6.9
<i>Streptococcus sp.</i>	16.7±6.7	3.8±3.7	35.3±8.2	11.8±5.5*
<i>Staphylococcus aur.</i>	13.3	0	5.9	0
<i>Corynebacterium sp.</i>	16.7±7.8	3.8±3.7	23.5±5.8	14.7±6.0
<i>Enterococcus sp.</i>	40.0±8.9	11.5±6.3*	35.3±8.2	11.8±5.5*
<i>Streptococcus B</i>	23.3±7.7	15.4±7.1	11.8±5.5	14.7±6.0
<i>Candida</i>	13.3±6.2	7.4±5.1	8.8±4.8	17.6±6.5
<i>Klebsiella sp.</i>	6.4±4.5	11.5±6.1	2.9	0
<i>Staphylococcus epid.</i>	16.7±6.7	3.8±3.7	29.4±7.8	23.5±7.3

Note. Here and in Table. 2: * — the difference in indices before and after treatment is meaningful ($p < 0.05$).

Table 2. Vagina microbiocenosis before and after Vagisan treatment in women with miscarriage (%)

Microflora	1-st group		2-nd group	
	before treatment	after treatment	before treatment	after treatment
Only lactobacilli	6.7±4.5	18±7.4	8.8±4.8	11.8±5.5
Lactobacilli prevail	43.3±8.9	73.1±8.8*	35.3±8.2	50.0±8.6
Lactobacilli in smaller numbers	20.0±7.2	7.7±5.1	5.9±4.0	5.9±4.9
Other microflora predominates	33.3±8.3	11.5±6.1*	17.6±6.5	29.5±7.8
«Key» cells	10.0	0	20.6±6.9	20.6±6.9
Yeast-like cells	10.0±5.4	11.5±6.1	11.8±5.5	5±7.3

tibacterial therapy) in the control group of women. *Enterococcus sp.* colonization was reduced by 3.5 times: from 40.0 to 11.5% ($p \leq 0.05$). *Gardnerella vag.* and *Staphylococcus aur.* were eliminated after treatment.

No increase in fungi discharge of *Candida* genus was observed during vagina dysbiosis treatment with Vagisan. There is a tendency towards a decrease in colonization of such microorganisms like *Streptococcus B*, *E. coli*, *Corynebacterii sp.* and *Streptococcus sp.* According to Table 2 data, 2 weeks after vagina dysbiosis treatment with Vagisan, lactobacilli were prevailing in 73.1% of women. In the control group, a significant increase in lactobacilli was not observed. The number of women with predominance of other microflora (from 33.3 to 11.5%) was decreased by 3 times. Vagisan use led to total disappearance of «key» cells, whereas they remained in 5.9% of women in the control group. No increase in yeast-like cells discharge was observed after vagina dysbiosis treatment with Vagisan, whereas a tendency to their increase (from 11.8 to 23.5%) was demonstrated in the control group. The results of the study suggest that Vagisan can be successfully used for vagina dysbiosis treatment as monotherapy in patients with HA and miscarriage. Owing to its high efficiency and tolerance, absence of side effects, this drug can be recommended for use in general clinical practice.

Conclusions

1. Vagisan application leads to increasing by 3.5 times lactobacilli colonization in the cervical canal, reducing by 3.5 times *Enterococcus sp.* colonization, promotes disappearance of *Gardnerella vag.* and *Staphylococcus aur.* from the vagina microbiocenosis, does not increase the growth of *Candida* genus fungi.

2. According to the data of the examined vagina composition, after vagina dysbiosis treatment with Vagisan, lactobacilli prevail in 73.1% of women.
3. The data show a beneficial effect of oral probiotic Vagisan application in the treatment of vagina dysbiosis in women with reproductive losses and HA.

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Коррекция вагинальной микрофлоры женщины с гиперандрогенией и репродуктивными потерями на прегравидарном этапе

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Резюме. В современных условиях среди факторов риска, обуславливающих высокий уровень перинатальной заболе-

ваемости и смертности, возросла роль эндокринной патологии, в том числе гиперандрогении (ГА) различного генеза. Среди основных этиологических факторов синдрома потери плода следует отметить инфекционный. Особого внимания заслуживает изучение состояния цервикального канала у пациенток с гиперандрогенией, учитывая высокую частоту (до 34%) функциональной истмико-цервикальной недостаточности у этого контингента больных. Барьером на пути восходящей инфекции является слизистый секрет шейки матки. Основными факторами колонизационной резистентности слизистых оболочек являются протективные эффекты слизевого покрытия и, особенно, локального комплекса антимикробной защиты, что заставляет исследовать микробиоценоз влагалища как потенциальный резервуар возбудителей, которые могут вызывать патологический процесс, и своевременно его корректировать на прегравидарном этапе.

Ключевые слова: гиперандрогения, невынашивание беременности, микробиоценоз, пробиотики, биотоп.

Корекція вагінальної мікрофлори жінок з гіперандрогенією та репродуктивними втратами на прегравідарному етапі

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Резюме. У сучасних умовах серед чинників ризику, що обумовлюють високий рівень перинатальної захворюваності та смертності, зросла роль ендокринної патології, в тому числі гіперандрогенії (ГА) різного генезу. Серед провідних етіологічних чинників синдрому втрати плода слід зазначити інфекційний. На особливу увагу заслуговує вивчення стану цервікального каналу в пацієнток із гіперандрогенією з огляду на високу частоту (до 34%) функціональної істміко-цервікальної недостатності в цього контингенту хворих. Бар'єром на шляху висхідної інфекції є слизовий секрет шийки матки. Основними чинниками клонізаційної резистентності слизових оболонок є протективні ефекти слизового покриття та, надто, локального комплексу антимікробної захисту, що змушує досліджувати микробиоценоз піхви як потенційний резервуар збудників, які можуть викликати патологічний процес, і вчасно його коригувати на прегравідарному етапі.

Ключові слова: гіперандрогенія, невиношування вагітності, микробиоценоз, пробиотики, біотоп.