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# Changes in the microbiota of the genital tract and intestines in patients with adenomyosis and infertility

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## ABSTRACT

**Introduction:** Inflammation of the genital tract caused by a bacterial infection can affect the frequency of occurrence of hyperproliferative formations of the uterus, which in turn can lead to infertility. Changes in the microbiota of the vagina and intestines contribute to the formation of critical problems for women's reproductive health.

**Material and methods:** Microbiota composition was studied using microscopic and cultural bacteriological methods in genitalia and intestines in 64 women with adenomyosis and infertility and 30 healthy women.

**Results:** The obtained results indicate that the microbiota of the genital tract in patients with adenomyosis and infertility is characterized by a different frequency of pathological manifestations (bacterial vaginosis, vaginitis, intermediate type of microbiocenosis) and excessive proliferation of vaginosis-associated microorganisms. Associative forms of bacterial contamination of the genital tract are registered in sick women.

The formation of pathological intestinal microbiota in patients is largely due to a violation of the ratio between potentially pathogenic and protective species of bacteria.

**Conclusions:** In women with adenomyosis and infertility, a pathological microbiota of the genital tract is formed, which is characterized by an increase in the structure of vaginal bacteria of representatives of pathogenic aerobic and vaginosis-associated microorganisms, a deficiency of lactobacilli. Intestinal microbiota in patients is characterized by the formation of multicomponent associations, which with a significant frequency contain conditionally pathogenic microorganisms.

**Key words:** vaginal microbiota, intestinal microbiota, adenomyosis, infertility

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## Introduction

Among the diseases of the female reproductive system, adenomyosis of the uterus remains an urgent medical and social problem [1, 2]. Adenomyosis is often accompanied by infertility and habitual miscarriage [3–5].

Despite the existence of various theories of uterine adenomyosis, the pathogenesis of the disease and the mechanisms of its influence on reproductive function have not been fully explored [6, 7]. Pathological changes in the uterus can be accompanied by an imbalance of complex neuroendocrine relationships

in the hypothalamus-pituitary-ovary system, adrenal cortex, and thyroid gland, and dysfunction of the pelvic organs [8, 9]. There are publications about the potentially significant role of an infectious-inflammatory factor in the development of hyperproliferative syndrome and infertility [10, 11].

Thanks to the study of the microbiota of the vagina and uterine cavity, it was established that the composition of the microorganisms of the genital tract was disturbed in women with benign neoplasms [12–15]. It is known that patients with bacterial vaginosis have an increased risk of developing multiple uterine

fibroids [16]. This condition is characterized by sharp changes in the composition of vaginal microorganisms and is manifested in reduced numbers of lactobacilli against the background of active proliferation of anaerobic microorganisms. *Gardnerella vaginalis* and *Atopobium vaginae* are considered the most frequent causative agents of bacterial vaginosis [17, 18].

The composition of intestinal bacteria is considered the main determinant of human health and pathology [19]. Disruption of intestinal microbiota is accompanied by the development of allergic and immunopathological conditions [20–22]. Obligate intestinal microorganisms directly participate in many vital processes of the macroorganism and also have various systems of regulatory function [23–25].

Changes in the microbiota of the vagina and intestines, and inflammation associated with bacterial infection can affect the balance of cytokines, worsen reproductive outcomes, which can lead to infertility [26, 27]. Pathological processes in the uterus can occur with a high concentration of microorganisms and the presence of pathogenic strains [28–30].

The task of the work included the study of changes in the microbiota of the vagina and intestines in women with adenomyosis and infertility with the aim of developing complex therapy aimed at correcting the pathological microbial state.

## Material and methods

64 patients with adenomyosis and infertility and 30 healthy women were examined.

The condition of the microbiome of the vagina and cervical canal was assessed using microscopic and bacteriological research methods.

The use of the microscopic research method allows to determine the types of microbiocenosis: intermediate type, vaginosis, vaginitis [23]. The research material was stained with Gram and methylene blue.

Gardnerellosis was diagnosed by microscopy by staining smears according to Romanovsky, followed by counting „key” cells, performing an amine test, and determining pH.

Conducting microbiological analyzes by the cultural method and recording the results was carried out in accordance with order No. 234 of the Ministry of Health of Ukraine dated May 10, 2007.

The following differential diagnostic media were used for the study: 5% blood agar, yolk-salt agar for staphylococci, Endo’s medium for enterobacteria, Sabouraud’s agar for *Candida* fungi, MRS medium for *Lactobacillus*, Blaurok’s medium for *Bifidobacterium*.

The content of conditionally pathogenic microflora in the studied material was expressed as the number

of colony-forming units in 1 mL (CFU/mL) of biological material.

The taxonomic position of microorganisms was determined in accordance with „Bergey’s Identifier of Bacteria” based on their cultural and morphological characteristics. Further identification of detected microorganisms was carried out on an automatic microbiological analyzer BD BBL Crystal (USA).

Determination of the number of lactic acid bacteria on the MRS medium was carried out after 24–48 hours of incubation at a temperature of  $37 \pm 1^\circ\text{C}$ . *Lactobacillus* in smears that were stained according to Gram *spp.* had the appearance of Gram-positive rods.

To identify staphylococci, yolk-salt agar was used, on which staphylococci grew in the form of yellow or white convex colonies. The study took into account the pigmentation of the colonies and the presence of lecithinase activity.

For the identification of yeast-like fungi, the cups with crops were incubated in a thermostat at a temperature of  $37 \pm 1^\circ\text{C}$  for three days, then the characteristic colonies — dense, opaque, milky in color — were microscopically examined in a 40% solution of caustic soda. For further identification of isolated yeast-like fungi, MIKROLA-TEST „Candidatetest 21” kits were used [Erba Lachema s.r.o. (Czech Republic)].

The state of the intestinal microbiota was assessed based on the results of a bacteriological examination of stools. The content of the main representatives of obligate microflora, as well as the content of anaerobes and *Candida spp.*

Determination of anaerobic microorganisms was carried out according to the methodological recommendations of „Laboratornaya diagnostics purulent — inflammatory diseases caused by asporogenic anaerobic by microorganisms”, Kharkiv, 1985.

The anaerobic microflora of the genital tract was studied with strict adherence to the technique of anaerobic cultivation. Thioglyc medium, blood agar with glucose, liver broth, Kitt-Tarozzi medium were used for research. Anaerocult system (Merck, Germany) and Anaerogaz gas packs were used to create anaerobic conditions. For further identification of isolated anaerobic microorganisms, MIKROLA-TEST „Anaerotest 23” kits [Erba Lachema s.r.o. (Czech Republic)].

The statistical processing of the obtained research results was carried out using the standard computer package „Data Analysis” Microsoft Excel for Windows 2007. The value of the arithmetic mean value (M), the mean error of the mean value (m), the level of probability disagreements (p). The reliability of the obtained data was assessed by the generally accepted method using the Student’s criterion. Reliability was considered established if its probability was equal to at least 95% (0.05).

## Results

Examination of material from the vagina and cervical canal of women with adenomyosis and infertility revealed various manifestations of microbiota disorders.

An intermediate type was found in 23.4% of women: a slight or moderate increase in the level of leukocytes (5–25 in the field of view), a slight increase in the content of Gram-positive and Gram-negative microflora, and a decrease in the number of lactobacilli.

Vaginosis was registered in 39% of examined patients. Vaginosis was manifested in a significant increase in the number of „key” cells and was accompanied by destructive changes in epithelial cells, a significant increase in the content of Gram-positive cocci, Gram-negative rods, a moderate increase in the number of leukocytes (15–25 in the field of view).

Vaginitis was found in 37.5% of patients. This condition of the vaginal microbiota was characterized by an increase in the number of leukocytes (50–100 in the field of view), neutrophils, and an increase in the number of Gram-positive coccal and Gram-negative

bacillus microflora, and an increase in the frequency of registration of fungi.

The application of the cultural bacteriological method of studying the state of the microbiota of the vagina in women made it possible to establish that the composition of the microbial spectrum with the highest frequency included microorganisms of the phyla *Firmicutes*, *Proteobacterium* and *Enterobacteriaceae* families.

Among representatives of the phylum *Firmicutes* had a significant specific gravity of staphylococci. Quantitative indicators of registration of these microorganisms exceeded the normal indicators (lg 4.0 — lg 5.4 CFU/mL) (Tab. 1).

A microbiological study of the contents of the vagina indicates an increase in the qualitative and quantitative level of seeding of certain types of enterobacteria (*Escherichia coli*, *E. coli heme + Kl. ebsiella spp.*). Quantitative indicators of registration of these types of enterobacteria were within lg 4.4 — lg 5.2 CFU/mL. The composition of the microbial spectrum of the vagina with a significant frequency included *Candida spp.*

**Table 1.** Vaginal microbiota in patients with adenomyosis and infertility (% , lg CFU/mL)

Microorganisms	Patients with adenomyosis and infertility n = 65		Healthy women n = 30	
	%	lg CFU/mL	%	lg CFU/mL
<i>S. epidermidis</i>	23.4	5.4 ± 0.05 ◊	20.0	3.3 ± 0.04
<i>S. epidermidis ( heme +)</i>	17.2	4.6 ± 0.05 ◊	6.7	2.5 ± 0.02
<i>S. aureus</i>	12.5	4.0 ± 0.04 ◊	3.3	2.0 ± 0.02
<i>E. faecalis</i>	15.6	4.4 ± 0.05 ◊	13.3	3.5 ± 0.04
<i>S. agalactiae</i>	14.1	4.2 ± 0.024 ◊	6.7	2.5 ± 0.02
<i>Corynebacterium spp.</i>	18.8	3.8 ± 0.02	10.0	3.3 ± 0.04
<i>E. coli</i>	25.0	5.2 ± 0.05 ◊	13.3	3.2 ± 0.04
<i>E.coli ( heme +)</i>	14.1	4.4 ± 0.05 ◊	6.7	3.0 ± 0.03
<i>Klebsiella spp.</i>	15.6	4.8 ± 0.04 ◊	3.3	2.0 ± 0.02
<i>Enterobacter spp.</i>	12.2	4.6 ± 0.05 ◊	6.7	3.2 ± 0.04
<i>Candida spp.</i>	25.0	4.8 ± 0.05 ◊	10.0	3.3 ± 0.04
<i>Lactobacillus spp.</i>	100.0	3.4 ± 0.02 ◊	100.0	6.2 ± 0.08
<i>Bifidumbacterium spp.</i>	10.9	3.4 ± 0.02 ◊	13.3	4.0 ± 0.04
<i>Bacteroides spp.</i>	17.2	4.6 ± 0.04 ◊	43.3	5.2 ± 0.06
<i>Peptostreptococcus spp.</i>	29.7	5.2 ± 0.06 ◊	20.0	4.0 ± 0.04
<i>Fusobacterium spp.</i>	26.6	4.8 ± 0.05	13.3	4.2 ± 0.04
<i>Prevotella spp.</i>	18.8	4.2 ± 0.04 ◊	40.0	5.0 ± 0.06
<i>Veilonella spp.</i>	21.9	4.8 ± 0.02	10.0	4.2 ± 0.04
<i>Atopobium vaginae</i>	28.1	5.2 ± 0.05 ◊	6.7	4.0 ± 0.04
<i>Clostridium spp.</i>	15.6	4.2 ± 0.05 ◊	13.3	3.8 ± 0.04

◊ — the difference statistically probable between indicators in patients and healthy people women (p < 0.05)

In 78.1% of women with adenomyosis and infertility, a deficiency of protective vaginal microorganisms was found. The quantitative registration level of *Lactobacillus spp.* was  $\lg 3.4$  CFU/mL.

The analysis of the anaerobic composition of the vaginal microbiota shows that the microbiocenosis of the vagina in sick women is characterized by an increase in the frequency of vaginal insemination by anaerobic bacteria — *Peptostreptococcus spp.*, *Fusobacterium spp.* Less frequently, the anaerobic microbial spectrum included *Bacteroides spp.*, *Prevotella spp.* Also, patients have a significant increase in vaginal seeding of vaginal — associated microorganisms: *Veilonella spp.* (21.9%) and *Atopobium vaginae* (28%).

In 81.3% of women with adenomyosis and infertility, associative forms of bacterial contamination of the vagina with significant content in the composition of associations of obligate anaerobes were found. Two-component associations of microflora were registered in 20.3% of patients, three-component — in 54.7%, four-component — in 15.6%, monocultures — in 9.4%. Associations of *Firmicutes* with enterobacteria, anaerobes with *Staphylococcus* were detected with the greatest frequency *spp.* and *E. coli*, *Enterococcus spp.* with *Candida spp.* and *E. coli*. Representatives of normal microflora — *Lactobacillus spp.* in the composition of associations had an insignificant specific weight. Analysis of the anaerobic spectrum of vaginal microflora in patients revealed excessive proliferation of *Gardnerella vaginalis* (31.3%).

Analysis of the results of the intestinal microbiota study in women with adenomyosis and infertility revealed dysbiotic changes of the II degree (59.4%) and III degrees (40.6%) in the majority of the examined. A significant decrease in *Bifidobacterium content spp.* was observed in 64.1% of cases, *lactobacillus* deficiency *spp.* registered in 70.3% of cases. A significant decrease in the content of *E. coli* was also noted (43.8%). Quantitative indicators of intestinal contamination with protective microflora did not reach the norm: *Bifidobacterium spp.* —  $\lg 3.37$  CFU/mL, *Lactobacillus spp.* —  $\lg 4.2$  CFU/mL, *E. coli* —  $\lg 6.3$  CFU/mL.

Suppression of the growth of normal intestinal microbiota was accompanied by excessive reproduction of certain opportunistic bacteria. Active proliferation of representatives of the phylum was noted *Firmicutes*. Yes, the frequency of sowing *Staphylococcus epidermidis* heme + was 37.5%, *S. aureus* 15.6%, *Streptococcus agalactiae* 18.8%, *Enterococcus faecalis* 21.9%, *Streptococcus pyogenes* — 12.5%.

It is known that an increase in the seeding level of microorganisms with hemolytic or other pathogenic properties can contribute to the destruction of the intestinal mucosa and the spread of these microorganisms by hematogenous means in various organs of the macroorganism.

All examined women with adenomyosis and infertility had intestinal microflora in associations. Three-component bacterial associations were found in 28.1% of cases, four-component — in 46.9%, five-component — in 25.0%. Associations of *Firmicutes* with enterobacteria, anaerobes with *E. coli*, *Enterococcus spp.* with *Candida spp.* and *Klebsiella spp.*

Bacterial associations with a significant frequency included *Candida spp.* (43.8%), the concentration of which exceeded the norm —  $\lg 5.0$  CFU/mL.

Significant violations were observed in the composition of microorganisms of the *Enterobacteriaceae* family. Compared to the norm, *E. coli* was sown with greater frequency, with weak enzymatic properties — 21.9% and hemolyzing strains — 17.2%. A tendency to increase *Klebsiella spp.* seeding rates was also revealed. Quantitative indicators of intestinal insemination by representatives of opportunistic bacteria exceeded the norm  $> \lg 6.0$  CFU/mL.

Analysis of the composition of anaerobic intestinal microorganisms in women with adenomyosis and infertility revealed an increase in the seeding level of Gram-positive coccal bacteria. An increase in the seeding rate of *Peptostreptococcus spp.* was observed — 40.6% and *Peptococcus spp.* — 26.6%. The quantitative level of cultivation of these anaerobic microorganisms exceeded the norm ( $\lg 5.6$  —  $\lg 6.2$  CFU/mL).

In contrast to the detected increase in the seeding level of Gram-positive anaerobic cocci, the frequency of intestinal contamination by representatives of the *Bacteroidetes* family *spp.* and *Prevotella spp.* was reduced. The frequency of registration of Gram-negative anaerobic bacilli was: *Bacteroides spp.* — 32.8%, *Prevotella spp.* — 25%. Quantitative indicators of intestinal *Bacteroides contamination spp.* and *Prevotella spp.* did not reach the norm ( $\lg 3.4$  CFU and  $\lg 3.8$  CFU/mL, respectively).

## Discussion

The results of the studies show that in patients with adenomyosis and infertility, the state of the microbiota of the vagina and intestines is associated with an increase in the level of colonization of the epithelium by representatives of the phylum *Firmicutes*, family *Enterobacteriaceae* and *Candida spp.*, which are able to produce enzymes and other metabolites, which leads to a violation of the integrity of the mucous membrane and the formation of an inflammatory process.

An imbalance between individual components of the anaerobic spectrum of intestinal microbiota can have a negative impact on the body as a whole [21, 22]. Conditionally pathogenic anaerobic bacteria produce a significant amount of endogenous toxins, enzymes of

aggression, metabolites that play the role of virulence factors. An imbalance between representatives of anaerobic intestinal bacteria can be accompanied by a violation of the synthesis of short-chain fatty acids, which provide the trophic and energetic functions of the epithelium, cytoprotection, ion homeostasis, and contribute to the normalization of the immune system [19].

Taking into account the existing data on the possibility of the spread of intestinal microorganisms due to the destruction of the mucous membrane to other biotopes of the body, we conducted a comparative analysis of the taxonomic properties of bacteria of the genital tract and intestines [27]. Biochemical, morphological, and serological characteristics of microorganisms isolated simultaneously from the intestine and genital tract were studied.

Certain representatives of intestinal and vaginal microbiota had the same properties to break down sugars, amino acids, the ability to hemolysis, lecithinase activity, plasma coagulation, sensitivity to antibiotics.

Complex violation of vaginal and intestinal contamination by aerobes, obligate anaerobes, and other infectious agents in women with hyperproliferative syndrome indicates the complexity of its etiopathogenesis and the need to use differentiated approaches to correct the dysbiotic changes detected.

## Conclusions

In patients with adenomyosis and infertility, a pathological microbiota of the genital tract is formed, which is manifested by bacterial vaginosis or nonspecific vaginitis, an increase in the structure of the vaginal microbiota of representatives of potentially pathogenic aerobic and vaginosis — associated bacteria, and a deficiency of lactobacilli.

In patients with adenomyosis and infertility, associative forms of bacterial and fungal contamination of the genital tract are registered in vaginal cultures.

Microbiota in patients with infertility and adenomyosis is characterized by the formation of multicomponent associations, which with a significant frequency contain bacteria with pathogenic or altered biological properties, as well as *Candida spp.*

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