THE ROLE OF "MICROBIAL FACTOR" IN THE DEVELOPMENT OF ADENOMYOSIS (review)

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Abstract

Endometriosis is a multifactorial disease that affects mainly women of reproductive age. The exact pathogenesis of this disease is still a mystery. The analysis of modern etiology concepts and mechanisms of adenomyosis development was carried out. The review includes data from foreign articles published in the PubMed, UpToDate databases over the past ten years. A number of works presenting studies of the uterus microbiota and its influence on the disease development were analyzed. The possibilities of cultural and molecular genetic diagnostic methods, in particular 16S rRNA, in studying the state of the uterine cavity microbiota are described. The modern paradigm of the development and progression of adenomyosis provides for the presence of endometrium bacterial contamination which, in turn, is a trigger for cell modifications activating a vicious circle of pathology.

Keywords: *pathogenesis of adenomyosis, risk factors, endometrial microbiota.*

Adenomyosis is one of the main problems of modern gynecology, leading to significant violations of reproductive and menstrual functions, disability of patients, dysfunction of adjacent organs, decreased performance and quality of life of women [1]. All of the above determines the relevance of studying the problem and requires the search for new approaches to the diagnosis, tactics of treatment of the disease.

For the first time adenomyosis was defined in 1972 by C. Bird et al. as "benign invasion of the endometrium into the myometrium, leading to a diffuse enlargement of the uterus, which is microscopically represented by ectopic, non-neoplastic endometrial glands and a stroma surrounded by hypertrophied and hyperplastic myometrium" [2].

A search of literature sources in the databases MEDLINE (2016–2021), PubMed (2016–2021) and Science Citation Index Expanded (2016–2021) in order to identify risk factors for the development of adenomyosis showed that a genetic predisposition, inflammation, hormonal changes, extracellular matrix enzymes, and the influence of immunological factors play an important role in the onset and development of adenomyosis and explain the clinical picture of the disease.

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Throughout the history of the study of endometriosis, scientists and criticists put forward numerous theories of its origin. Currently, there are seven main theories and mechanisms for the development of endometriosis. One of the first was the embryonic (dysontogenetic) theory proposed by Recklinghausen (1896) suggesting that cells of the ectopic endometrium could develop from the cells of the Müllerian duct remaining in other tissues after migration [35].

Another theory was developed by N. S. Ivanov (1897), R. Meyer (1903), J. A. Sampson (1921), K. P. Ulezko-Stroganov (1925). It is the implantation and metaplastic concept of the endometriosis origin, according to which adenomyosis can develop as a result of metaplasia from de novo ectopic intramyometrial tissue of the endometrium [25].

Among modern theories, we pay attention to the genetic and epigenetic theory, suggesting the presence of genetic and epigenetic defects in cells, as well as the possibility of hereditary transmission of these defects [26].

The concept of Crain D. A. et al. (2008) [37] is also worth mentioning. It indicates that the development of endometriosis is based on the reprogramming of normal endometrioid cells under the influence of any external stimuli (chemicals, endocrine factors, and changes in the immune status).

To explain the reasons for the development of endometriosis, hormonal and immune theories were formed.

Discussing individual theories of the genital endometriosis pathogenesis, we should submit that none of them can reveal the main pathophysiological mechanism underlying the development of adenomyosis, namely, the penetration of the basal layer of the endometrium into the adjacent myometrium.

Noteworthy is the hypothesis put forward by Bergeron C. et al. (2006) in which adenomyosis is defined as invagination of the basal endometrium in the myometrium due to violations or absence of the uterine "connecting zone", or "transition zone" [11]. The exact trigger for intussusception is not known.

Endometrial invasion can occur during trauma at the border of the endometrium and myometrium [15].

When studying the immunological aspects of adenomyosis, we note that an important role in the disease pathogenesis is played by cytokines, mediators of intercellular interactions involved in proliferation, cell differentiation, tissue repair and remodeling, as well as in the regulation of the immune response [3–5, 8].

Recent publications increasingly discuss the role of infections in the etiology of diseases of the female genital organs [10]. This problem is most relevant in women of reproductive age. It is the reproductive age that is active in terms of sex life, pregnancy, childbirth, and the use of contraceptives.

Chen C. et al. (2017) reported the existence of various bacterial communities throughout the female reproductive tract and their influence on diseases of the uterus [16].

At the turn of the 20th century, Henry Tissier (Tissier, 1900) expressed the paradigm of the sterile uterus, which is one of the stable dogmas [18]. Sterility is maintained by cervical mucus, which provides a barrier to bacteria from entering the vagina [14, 48, 54]. However, the endocervical barrier can be disrupted, which is confirmed by the study by Kunz Getal (1997), demonstrating how radioactively labeled macrospheres reach the uterine cavity within a few minutes after their insertion into the external cervical canal [14].

The subject of research in recent years is the study of the uterus microbiota from the viewpoint of reproductology [17]. Microbiota is a collection of microorganisms living in a separate human biotope, which are in symbiosis with the host organism. Despite the fact that these symbiotic relationships have developed evolutionarily, our understanding of the physiological and pathophysiological role of the microbiota remains largely insufficient [31]. In 2007 the staff of the National Institute of Health (USA), using highly sensitive molecular genetic methods, demonstrated the importance of the physiological role of the microbiota of various biopsies in healthy female volunteers. Samples of biomaterial discharge from the vagina and aspirate from the uterine cavity were studied. To determine the species composition of the microbiota, they used the method of sequencing the 16S ribosomal RNA (rRNA) subunit, which is unique for each bacterium. The obtained data showed that such organs of the human body as the uterine cavity and placenta, which had been previously considered sterile, were colonized by their unique microflora [21, 22, 29, 31].

It should be noted that the researchers paid the most attention to the study of the vaginal biotope microbiota. Normally, the vaginal microbiota of a healthy woman is dominated by lactobacilli, although this indicator is characterized by significant variability and depends on many factors, such as age, hormonal status, age of the first sexual intercourse, pregnancy and childbirth of the menstrual cycle. In addition, sedentary lifestyles, contraceptives, and late pregnancy, common in modern life, can affect the microbiota of the female reproductive system. [7, 27, 42, 51].

Baker J. M. (2018) [14] examined the uterus microbiota in healthy women and identified the following types of bacteria: *Firmicutes, Bacteriodetes*, *Proteobacteria* and *Actinobacteria*.

Chen C. et al. (2017) [16] conducted a systematic study of microbiota samples in 95 women of reproductive age using culture methods. The material was obtained from the lower third of the vagina, posterior fornix, cervical mucus, endometrium, fallopian tubes, and peritoneal fluid. The study showed that genus *Lactobacillus* with low diversity was dominant in the lower third of the vagina and posterior fornix. These samples contained *L. crispatus*, *L. iners*, and another *Lactobacillus* spp. The obtained results are similar to those of other researchers [19, 23, 34]. Cervical mucus samples contain lower amounts of *Lactobacillus* than vaginal samples. *Lactobacillus* was not dominant in the endometrium, and bacteria such as *Pseudomonas, Acinetobacter, Vagococcus*, and *Sphingobium* made up a significant proportion of the microbiota. The content of these bacteria increased in the fallopian tubes, and the average relative abundance of *Lactobacillus* was 1.69%. *Lactobacillus* was absent in peritoneal fluid samples, but contained a diverse microbiota other than the endometrium.

Baker J. M. (2018) [14] also presents possible transmission routes of bacteria (hematogenous, oral, intestinal, canalicular, iatrogenic (during assisted reproductive technologies), intrauterine contraceptive administration. In addition, bacterial colonization of the uterus is associated with adverse reproductive health outcomes, including premature birth, chorioamnionitis, and endometritis [16, 20, 22, 24, 28, 30, 38–41, 44, 49, 50].

The microbiota of the reproductive tract is mainly studied by two methods: cultural and molecular genetics.

Cultural methods have some limitations: the duration and complexity of the study, the need to provide microbiological laboratories with special equipment, strict requirements for the storage and transportation of biomaterials [9, 12–14, 45, 53]. Moreover, a new approach to the study of the microbiota of the reproductive tract, in particular, the uterine cavity, using molecular genetic research methods has recently appeared. Most studies of endometrial microbiota have been carried out using the next generation sequencing method (NGS sequencing), an expensive approach which is poorly adapted for practical health care system [14, 31–33]. The most suitable for every day research is the molecular genetic method (Polymerase chain reaction – PCR) in real time. Currently, the use of molecular genetic research methods allows identifying associations of difficult to cultivate and uncultured microorganisms on the surface of the endometrium in women of reproductive age [30, 46, 47, 52].

Hilier S. et al. (2013) [24] conducted a study of 136 women with chronic pelvic pain who underwent pipel biopsy of the endometrium, followed by histological examination and microbiological assessment of the endometrium using PCR. In 55 (40%) women with clinical signs of chronic pelvic pain, endometritis was histologically confirmed. A wide spectrum of bacteria was obtained from 53 endometrial samples, represented by 63 different species, including 8 species of opportunistic microorganisms. The presence of true pathogens such as *Neisseria gonorrhoeae* and/or *Chlamydia trachomatis* in endometrial specimens was associated with endometritis (29% vs. 6%, $p<0.001$). Among opportunistic microorganisms with histologically confirmed endometritis, G. vaginalis (35% versus 16%, $p=0.01$) and *A. vaginae* (22% versus 3%, $p<0.001$) were significantly more often detected.

Cicinelli E. et. al (2012) [43] assessed the uterine cavity microbiota in women of reproductive age with infertility and miscarriage by PCR. *Lactobacillus spp.* was detected in 86.1% of cases, opportunistic microorganisms were identified in 36.1% of the samples, including 22.2% in combination with lactobacilli and in 13.9% without lactobacilli.

Swidsinski et. al (2013) [36] used the FISH fluorescent hybridization probes to detect *G. vaginalis, A. vaginae, Lactobacillus, Bacteroides, Prevotella, Enterobacteriaceae,* and *Eubacteria*. The study showed that the microbiological environment of the endometrium differs from that of the vagina.

Mitchell M. et al. (2015) [29] studied uterus samples of 58 women. Material for research was obtained from the upper part of the endocervix and the body of the uterus after opening it under sterile conditions. Vaginal discharge was collected before surgery. The analysis was performed using the 16S rRNA sequencing method. Microbial contamination of the uterine cavity was detected in 55 (95%) patients, 52 of them had only 1 type of microorganisms. The most common species were: *Lactobacillus iners (L. iners)* (45% of women had it in the uterine cavity and 61% of women – in the vagina), *Prevotella spp.* (33% of patients had it in the uterine cavity, and 76% – in the vagina), *Lactobacillis crispatus (L. crispatus)* (33% – in the uterine cavity, 56% – in the vagina). *G. vaginalis, A. vaginae* and *Lactobacillus jensenii* (L. jensenii) were found in the vagina in more than 40% of women, but much less frequently in the uterine cavity (*G. vaginalis* in 19% of women, *A. vaginae* in 10% of women, and *L. jensenii* – in 20%). The uterine cavity colonization by microorganisms was significantly lower than that of the vagina. The endometrium inflammation markers did not significantly differ in women who did not have microorganisms in the uterine cavity compared to those who had only lactobacilli or microbes associated with bacterial vaginosis.

Verstraelen H. et al. (2016) [40] studied the composition of the endometrial microbiota using 16S rRNA sequencing in 19 patients with implantation failures and miscarriage. To obtain material in order to exclude contamination with the vaginal microflora, the authors used a Tao Brush cytobrush surrounded by a transparent casing that protects the sample taken from the endocervical and vaginal discharge. As a result of the study, 15 types of microorganisms were represented in all samples. 90% of patients had a similar composition of the endometrial microbiota, where *Bacteriodes xylanisolvens, B. thetaiotaomicron,* and

B. fragilis predominated. In 6 women, *L. crispatus* or *L. iners* predominated in the presence of *Bacteriodes*. The results of this study are consistent with previous evidence of dysbiotic shifts in the endometrial microbiota in the absence of a predominance of lactobacilli, and such disorders are most common in the sub fertile population.

Franasiak J. M. et al. (2016) [22] studied 33 patients admitted for embryo transfer into the uterine cavity. Analysis of the uterine cavity microbiota was performed using the 16S rRNA sequencing method. As a result, 35 samples of biomaterial were received: 33 samples were obtained from patients and 2 control samples containing *Escherichia coli*. Pregnancy occurred in 18 women, and did not occur in 15 patients. In total, the presence of 278 different genes of microorganisms was registered in the samples under study. The uterine cavity microbiota during embryo transfer in both groups was characterized by the predominance of lactobacilli.

Moreno I. et al. (2016) [30] carried out a comparative analysis of the microbiota of paired samples of endometrial aspirate and vaginal discharge in 13 fertile women. As a result, *Lactobacillus* was identified in 71.1%, *Gardnerella* was detected in 12.6%, *Bifidobacterium* – in 3.7%, and *Prevotella* – in 0.9% of women. Patients, depending on the microbial composition of the endometrium, were divided into categories with a predominance of *Lactobacillus* (more than 90%) and without a predominance of *Lactobacillus* (more than 10% of bacteria other than *Lactobacillus*, such as A. *vaginae, G. vaginalis,* species of the *genera Clostridium, Megasphaera, Parvimonas, Prevotella, Sphingomonas* or *Sneathela*). 18 out of 26 women showed stable microbiota profiles, 12 of them were assigned to the *Lactobacillus*-dominated group, 6 women – to the *Lactobacillus*-free group. Thus, the composition of the bacterial community in most healthy fertile women was relatively stable.

The endometrial microbiome in infertile patients was assessed in a study conducted by Tao X. et al. (2017) [38]. The study included samples of endometrial microbiota obtained from 70 patients who underwent the *in vitro* fertilization (IVF) program. 33 samples contained more than 90% of lactobacilli and 50 samples contained 70% of lactobacilli. In addition to lactobacilli, opportunistic pathogens were identified: *Corynebacterium spp.* was detected in 40 women, *Bifidobacterium spp.* was identified in 15 patients, *Staphylococcus spp*. was in 38, and *Streptococcus spp*. was in 38 women.

Thus, molecular genetic research methods allow assessing the relationship between the endometrial microbiota and the frequency of embryo implantation in the IVF program. The uterus microbiota study is extremely important in reproductology.

Noteworthy are reports of differences between microbiome profiles in healthy women and women with endometrial polyps and chronic endometritis. Fang et. al [20] examined women in three groups: group I included healthy women, group II consisted of women with endometrial polyps, and group III had patients with endometrial polyps on the background of chronic endometritis. As a result, the found statistically significant content of *Firmicutes, Lactobacillus, Gardnerella, Bifidobacterium, Streptococcus,* and *Alteromonas* in the vagina and uterus samples of groups II and III compared with the group of healthy women. The detection of *Lactobacillus* more than 3.0 times in the uterine microbiome of patients in groups II and III, compared with healthy controls, may indicate the growth of vaginal bacteria.

The surveyed women without uterine leiomyoma had a higher number of *Lactobacillus spp.* in vaginal and cervical secretions, while women with uterine leiomyoma had abundant *L. iners* in cervical mucus [6].

Thus, there are more and more new data indicating that the microbiota of the female genital tract is important for women's health. Today the outdated concept of uterine sterility can be argued about, although the determination of the true uterus microbiota in normal conditions and in adenomyosis requires further detailed research.

DECLARATIONS:

Statement of Ethics

The authors have no ethical conflicts to disclosure.

Consent for publication

All authors give their consent to publication.

Disclosure Statement

The authors have no potential conflicts of interest to disclosure, including specific financial interests, relationships, and/or affiliations relevant to the subject matter or materials included.

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