CZU: 616.34-008.87: 312.6
DOI: https://doi.org/10.52692/cfzl2024.19

THE RELATIONSHIP OF THE INTESTINAL MICROFLORA AND REPRODUCTIVE HEALTH (REVIEW)

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Abstract. *În prezent, există o creștere pe scară largă a interesului pentru microflora intestinală și impactul acesteia asupra sănătății și bolilor umane. Acest articol prezintă sinteză narativă privind interrelația dintre microbiomul intestinal și sănătatea reproductivă. Sunt studiate atât patologiile sistemului de reproducere feminin asociate cu disbioza intestinală, cât și relația dintre microbiota intestinală și funcția de reproducere a bărbaților. Cercetările viitoare ar trebui să se orienteze pe identificarea mecanismelor moleculare care stau la baza relației cauzale dintre microbiota intestinală și sănătatea reproducerii.*

Cuvinte-cheie. *sănătatea reproductivă, microbiota intestinală, disbioza intestinală, mecanisme moleculare.*

The human intestinal microflora is an integral part of the human body and performs numerous vital functions. The total number of microorganisms inhabiting various parts of the macroorganism is approximately two orders of magnitude greater than the number of its own cells, amounting to about 10^{14}-10^{15}. The highest concentration of microorganisms is found in the gastrointestinal tract (GIT), including the oropharynx (75-78%), while the rest inhabit the urogenital tract (up to 2-3% in men and up to 9-12% in women) and the skin. In healthy individuals, the intestine contains more than 500 species of microorganisms. The total mass of the intestinal microflora ranges from 1 to 3 kilograms [32].

Currently, there is a widespread increase in interest in the intestinal microflora and its impact on human health and disease. Data has emerged indicating a connection between the gut microbiota and diseases not only of the gastrointestinal tract [31; 37], but also of the cardiovascular system

[39], obesity [26], diabetes mellitus [33], malignant neoplasms [38], allergic [34] and immune pathologies [36; 9].

The "epidemic" of reproductive pathologies and the increase in the prevalence of intestinal dysbiosis have triggered the study of the relationship between these phenomena. This article is dedicated to the systematization of information regarding this issue.

A literature review has shown that changes in the number of certain types of microorganisms in a specific biotope or the detection of species not characteristic of a given habitat serve as signals for adaptive or irreversible changes in the corresponding link of the microecological system. It is known that these changes can be interrelated and occur simultaneously in several biotopes. In this regard, the study of intestinal and vaginal microbiota as indicators of women's reproductive health deserves special attention today. Several recent studies have identified a correlation between microbial communities present during pregnancy and pregnancy complications. Both vaginal and intestinal microbiota contribute to the transmission of bifidobacteria from mother to child, and establishing the fact that vaginal and intestinal bacteria represent the same population is an important step for future research on maternal and newborn health [35].

It is known that the intestinal microbiome influences hormone levels in the host organism, including estrogen levels in women [10]. The intestinal microbiota primarily regulates estrogen levels by secreting β-glucuronidase (gmGUS), an enzyme that converts conjugated estrogen into deconjugated estrogen in the gastrointestinal tract, facilitating its binding to estrogen receptors. Another mechanism by which the intestinal microbiome can influence sex steroid hormone levels in women is the production of short-chain fatty acids (SCFAs). SCFAs are the main by-products of bacterial anaerobic fermentation of dietary fibers in the intestine. Acetate, propionate, and butyrate are SCFAs that play a role in this process. Research indicates that women with polycystic ovary syndrome (PCOS) exhibit distinct intestinal microbiota profiles compared to age-matched healthy controls. The abundance of microorganisms from the genera *Ruminococcaceae UCG-002* and *Clostridiales XIII AD3011* correlated with markers associated with PCOS, such as cystic ovarian morphology and higher testosterone levels. Moreover, patients with PCOS and prediabetes had significantly lower alpha diversity (Shannon index) and higher content of the genera Dorea and Bacteroides (*Ruminococcus torques group* and *Lachnospiraceae UCG-004*) compared

to patients with PCOS with normal glucose tolerance [18]. It has been reported that the number of bacteria producing gamma-aminobutyric acid, including *Parabacteroides distasonis* and *Bacteroides fragilis*, was increased in patients with PCOS, while *Escherichia coli* showed a positive correlation with serum luteinizing hormone (LH) levels and the LH/follicle-stimulating hormone (FSH) ratio [16]. Finally, Qi et al. (2021) found that the level of *Bacteroides vulgatus* was elevated in the intestinal microbiota of people with PCOS [20].

Recent data suggest that endometriosis is associated with dysbiosis of intestinal microorganisms, although findings from different studies vary widely. For example, one study found that patients with stage three or four (moderate to severe) endometriosis had more microorganisms from the genera *Shigella* and *Escherichia* in their colon microbiota than healthy participants [3]. Conversely, another study found no differences between the intestinal microbiomes of patients with endometriosis and a healthy control group during the proliferative and secretory phases of the menstrual cycle [19]. However, this study did not specify the stages of endometriosis. A larger study conducted on human stool samples showed that both alpha diversity (microbial diversity within a single sample) and beta diversity (a measure of similarity or dissimilarity between two communities), as well as the *Firmicutes* to *Bacteroidetes* ratio, were higher in the stool samples of control groups (n=198) than in those of patients with endometriosis [25]. The study by Svensson et al. (2021) also reported significant differences in 12 bacteria belonging to the classes *Bacilli*, *Bacteroidia*, *Clostridia*, *Coriobacteriia*, and *Gammaproteobacteria* between stool samples from patients with endometriosis (n=66) and those from a similar healthy control group (n=198). Two bacteria from the class *Bacteroidia* (*Bacteroides* and *Parabacteroides*) and two from the class *Clostridia* (*Oscillospira* and *Coprococcus*) were found in higher quantities in patients with endometriosis, whereas two species of bacteria from the class *Bacteroidia* (*Paraprevotella* and one unidentified) and the class *Clostridia* (*Lachnospira* and one unidentified) were found in lower quantities in the stool samples of patients with endometriosis. This study appears to contradict another study in which patients with stage three or four endometriosis had lower alpha diversity of gut microbiota and a higher *Firmicutes* to *Bacteroidetes* ratio than healthy control groups [21].

Microbiological studies of the intestinal microflora in healthy women and those with chronic endometritis have shown that the number

of representatives of the normal microflora – lactobacilli and bifidobacteria – is significantly reduced in patients with chronic endometritis: by 81.1% and 64.2%, respectively. Alongside the reduction in bifidobacteria (the average value corresponding to 10^4 CFU/mL with reference values of 10^9-10^{10} CFU/mL), there was a decrease in *Fusobacterium* and *Peptostreptococcus*, and an increase in the growth of *Clostridium* and *Candida albicans*. Fungal flora of more than 10^5 CFU/mL was present in 13.7% of observations ($p<0.001$). Only 39.6% of patients with chronic endometritis had sufficient *E. coli* of the typical type in their intestines (reference values – 10^7-10^8 CFU/g), compared to 51.5% of practically healthy women [30]. The differences in the results of these studies indicate the need for larger-scale studies evaluating potential factors that may influence the development (e.g., stage of endometriosis, age, race/ethnicity, other health conditions, medications, and diet).

Wang Z. et al. (2019) found that the microbial composition of the intestine differed significantly between stool samples from patients with cervical cancer and those from a healthy control group. Specifically, cancer patients had more *Bacteroides* and fewer *Firmicutes* than the healthy controls [27]. Another study showed that *Prevotella*, *Porphyromonas*, and *Dialister* were significantly more prevalent, while *Bacteroides*, *Alistipes*, and *Lachnospiraceae* were less prevalent in the intestinal microbiomes of patients with cervical cancer compared to those of a similar healthy control group [24]. Similarly, Kang GU reported that *Prevotella* was more abundant in stool samples from patients with early-stage cervical cancer than in those from healthy controls [13]. The intestinal microbiota composition of patients with ovarian cancer was also studied, revealing that patients with primary platinum-resistant disease had lower phylogenetic diversity than those who were platinum-sensitive [12].

Although the exact mechanism remains unclear, it is certain that the intestinal microbiota is closely associated with tryptophan and serotonin (5-HT) metabolism. 5-HT is present in the midpiece of human spermatozoa [11]. 5-HT can activate transmembrane adenylyl cyclase (tmAC) and open membrane Ca^{2+} channels (CatSper), leading to Ca^{2+} influx and activation of protein kinase A (PKA), which is involved in regulating tyrosine phosphorylation and Ser/Thr protein phosphorylation in sperm. This, in turn, leads to improved sperm quality [4; 14]. Studies are needed to determine whether 5-HT secreted by the intestinal

microbiota can penetrate the testes through the bloodstream and play a direct role in spermatogenesis [23].

Studies have shown that intestinal microbiota can alter the content and proportion of polyunsaturated fatty acids (PUFAs) [2], one of the important biological effects of which is to enhance sperm activity [5]. For example, *Bacillus proteus* and *Lactobacillus plantarum* reduce PUFA levels in the intestine by converting linoleic acid (LA) to stearic acid. Additionally, intestinal bacteria can regulate the accumulation of lipid droplets [6], affecting the absorption of PUFAs, which influence sperm membrane stability, sperm motility, acrosome reaction, and sex hormone synthesis, as well as enhance the anti-inflammatory and antioxidant capabilities of testicular cells [7]. Intestinal microbiota and its metabolites can promote glycolysis to maintain sperm energy supply and improve sperm motility. For instance, leucine supplementation can enhance the average sperm bending speed [17]. 5-HT can lower circulating melatonin levels [15], which plays an important role in male reproduction, including promoting lactate production by Sertoli cells by activating certain glycolysis-related enzymes and increasing sperm energy supply. Additionally, *Bacteroides*, *Streptococcaceae*, and *Akkermansia* are believed to improve energy supply to spermatid cells by enhancing glycolysis [29]. When intestinal barrier permeability changes, microorganism-associated molecular structures such as lipoproteins and peptidoglycans can enter the bloodstream, reach the testes through the testicular artery, and bind to receptors on the cell membrane of testicular cells, ultimately causing cellular oxidative stress, local inflammation, and disruption of testicular structure.

There is cross-talk between the intestinal microbiota and the endocrine system. The abundance of *Adlercreutzia*, *Ruminococcus*, *Dorea*, *Clostridium*, and *Parabacteroides* is significantly correlated with testosterone levels [28]. The numbers of *Acinetobacter*, *Dorea*, *Ruminococcus*, *Megamonas*, and *Lactobacillus* are also notably associated with testosterone levels [22; 1]. A study has shown that *Lactobacillus* and *Bifidobacterium* improve sexual behavior by enhancing the activity of the dopaminergic system [8].

The presented data convincingly demonstrate that disruptions in the intestinal microbiome are associated with several diseases of the female reproductive tract, commonly including endometriosis, PCOS, gynecological cancers, and infertility. It is also undeniable that the intestinal microbiota, by influencing levels of tryptophan, serotonin,

melatonin, LPS, and PUFAs, can affect male reproductive function. However, there is a significant gap in knowledge regarding the mechanisms through which specific bacterial species or groups of species can influence reproductive system function. Collectively, the aforementioned studies have shown that reproductive health is linked to changes in the intestinal microbiome, but causal relationships have not been established. While much remains to be discovered, the existing data suggest that the influence of the intestinal microbiome is a lever for impacting human reproductive health.

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