Original Research Article

Vaginal microbiocinosis in women with infertility and parasite invasion

Abstract. Vaginal microbiocinosis in 47 women with infertility and parasite invasion and 35 women with infertility without parasite invasion was investigated. In women with primary infertility, ascariasis was found in 48% of examined patients, *Mycoplasma hominis* was often revealed together with worm invasion, persistence of *Ureaplasma species and urealyticum* infection in lower parts of sexual system did not depend on presence or absence of parasites. The occurence of *Esherichia colli, Klebsiella and Enterococcus faecalis* was significantly higher in vaginal content under conditions of worm invasion. Dysbiotic changes in vagina were accompanied by intestinal dysbiosis in 48% cases, whereas in patients with intestinal dysbacteriosis changes of vaginal microflora content were revealed in 90% cases.

Key-words: microbiocenosis of vagina, infertility, parasites, intestinal dysbiosis.

Introduction. Rational and effective examination of women with infertility plays an important role in obtaining positive results and bearing healthy children. Parasite invasion in women that affects reproductive function, carrying of a pregnancy and bearing of healthy posterity occupies an important place among the underinvestigated directions in the study of infertility [2, 18].

Obstetricians-gynaecologists, specialists of reproductive medicine, endocrinologists, geneticists in their practical work do not pay sufficient attention at the effect of helminthes as a factor of infertility, chronic dysbacteriosis of gastrointestinal tract and autoaggression of opportunistic pathogens on endoecology of the vagina and colon. Examination towards the presence of parasites is not included in the list of obligatory tests, which are carried upon family planning, in cases of infertility, inflammatory diseases of small pelvis organs and sexually transmitted infections [5]. The problem of infertility in women is often associated with hormonal pathologies (insufficiency of lutein phase, polycystic ovary syndrome, hyperprolactinemia, autoimmune thyroiditis, endometriosis) and chronic inflammatory disorders of urogenital system.

The results of our previous studies give evidence that in 40% of women with the inflammatory disorders of the small pelvis organs, endocervicitis, exocervicitis, recurrent colpitis eggs of ascarides and pinworms were detected [10, 18], and perinatal losses were registered in anamnesis of 67 % of women with helminthes [17].

The effectiveness of treatment of chronic inflammatory disorders in 10-25% of cases leaves much to be desired as well as relapse of opportunistic infections, such as *Gardnerella vaginalis, Staphylococcus aureus, Candida albicans, Esherichia colli Staphylococcus coagulase negative,*

Mobilincus, Klebsiella and *Enterococcus faecalis* is noted. A special place belongs to the controversial issues of effectiveness of treatment of *Mycoplasma hominis, Ureaplasma urealyticum and species*, in particular taking into account the decrease of frequency of evaluation of *Chlamydia trachomatis* in infertile women [8, 22].

Aim of the study was to examine women with primary infertility towards parasite invasion and determine the change of vaginal microbiocinosis**.**

Material and methods.

81 women, aged 23 – 40 years with primary infertility (during 1-20 years), who addressed to Lviv Municipal Centre for Family Planning and Human Reproduction were examined. Enrolled patients were devided into 2 groups: group A – women with primary infertility and verified parasite invasion (n=47); group B – women primary infertility without worm invasion (n=35). Male factor was excluded by means of evaluation of spermogram and urologist's conclusion. The control group included 40 healthy women, aged 18 – 36, who were carried out prophylactic medical examination.

All women were examined due to protocol recommendations. Examination of vaginal microbiocinosis was conducted by means of determination of the species and quantitative composition of microorganisms with the method of bacterioscopy and bacteriological examination of the vaginal content using selective differentiated diagnostic nutritional mediums due to commonly accepted schemes. Bacterial vaginosis was diagnosed according to Amsel system, bacterioscopy (positive amino-test, "key" cells in vaginal smear, pH of the vagina > 4,5) and bacteriology. Trichomoniases were verified based on microscopy of negative and methylene blue-stained smears from the content of the posterior fornix of the vagina and molecular-biologic method. Diagnosis of genital candidosis was stated due to presence of clinical signs and evaluation of fungi colonies in quantity of 10^4 colony-forming units (CFU)/ml and more. Diagnosis of chlamydiosis, trychomoniasis, herpes and cytomegalovirus was conducted due to the method of polymerase chain reaction. Genital mycoplasmosis, ureaplasmosis was confirmed due to evaluation of the causative agent in quantity of 10^4 CFU/ml and more. TORCH infections were determined using immune-enzyme test: Ig G and Ig M to toxoplasmosis, herpes type I and II, cytomegalovirus, chlamydias, Ig G to rubeolla. Parasite examination was conducted exploring feces towards the presence of pinworms' eggs, determiunation of antibodies to toxocara, ascarides and lamblias. Examination of microbiocenosis of the large intestine included the determination of species and content of microflora. The severity of detected violations were evaluated according to methodical recommendations "Diagnosis and treatment of dysbacteriosis of the large intestine" (Moscow, 1991).

Statistical procession of obtained results was performed with the use of computer programs "Statistica 6.0" and “Excel 5.0”. The difference was supposed to be statistically significant at P<0,05.

Results of studies and discussion.

Considering the results of our studies towards parasite invasion due to the examination of feces towards the presence of worm eggs and pinworms in the smear, obtaining positive titres of antibodies to at least one of the studied parasites: lamblia, toxocara, ascaride, in 58% of examined women with primary infertility parasites were detected. In 14% of examined women with primary infertility ascarides eggs were found in feces, enterobiosis was detected in 13% of patients. Among parasites in 41% of patients increased level of Ig G to ascarides was found, in 18% - to toxocara, in 6% - to lamblias (Tab.1).

Table 1.

Frequency of IgG to *Ascaris lumbricoides*, *Giardia lamblia*, *Toxocara canis* detection and eggs of parasites in the stool in women with primary infertility and parasitic invasion.

Investigated parameters	IgG to *Ascaris lumbricoides* (n=80)	IgG to *Giardia lamblia* (n=78)	IgG to *Toxocara canis* (n=77)	Eggs of *Pinworm* the stool (n=70)	Eggs of *Ascaris lumbricoides* the stool (n=70)
Frequency of findings	31 (41%)	5 (6%)	14 (18%)	11 (13%)	10 (14%)

It should be noted that in women of the control group (without complaints and gynecologic pathology) worm invasion was also found, but its frequency was significant lower (10%).

Evaluation of ascaridosis in women with primary infertility using combined methods of investigation of feces for worm eggs and determination of IgG to ascarides showed positive result in 39 women from 82 examined patients, what made 48%.

Significant frequency of ascaride invasion should be mentioned in patients of group A. Thus, in 21% patients eggs of ascarides were found in feces and high titre of IgG to ascarides made 70%. Combined presence of eggs of ascarides in feces and high titre of antibodies we noted only in 5 of 47 patients with primary infertility what makes 10%.

Analysis of complaints of patients of group A confirmed the disturbances of functioning of gastrointestinal tract: chronic constipation was found in 45% of examined women, whereas in group B only in 15% and in control group in 5% of examined females. In anamnesis of patients of group A frequency of chronic gastritis and biliary dyskinesia was two-fold higher.

Analysis of investigations in women with primary infertility found high rate of *Ureaplasma urealyticum and species* invasion - in 47 (57%) women, less frequently *Mycoplasma hominis* occurred - in 10 (13%) and *Chlamydia trachomatis* - in 3 (4%), from 82 examined patients.

These infections were found on the background of pronounced vaginal dysbiosis in 60 (74 %) examined women who presented *Mycoplasma hominis* and *Ureaplasma urealyticum and species* in titre 10^4 and more CFU / ml. The patients had typical complaints on significant homogenous vaginal discharge of milky white colour with an acute unpleasant smelln and discomfort in the area of external genital organs.

Analyzing indices of titres of immunoglobulins to TORCH infections in women with primary infertility we noted the following: 29% of patients had high titre of IgG to chlamydia, in 48% of women protective immunity to toxoplasma was revealed; 68% of patients had herpes infection, 63% of patients had positive titre of Ig G to CMV (Tab.2). Special TORCH-infections dependently from the presence or absence of parasites were not seen.

Table 2.

Frequency of TORCH infections evaluation in women with primary infertility and parasite invasion

Studied indices	Ig G toxoplasmosis (n=68)	Ig G herpes type I and type II (n=71)	Ig G cytomegalovirus (n=67)	Ig G rubella (n=59)	Ig G chlamydia (n=33)	Ig A chlamydia (n=47)
Frequency of findings	33 (48%)	48 (68%)	42 (63%)	48 (81%)	14 (42%)	9 (19%)

Evaluating vaginal microbiocinosis in patients with infertility it should be stated that in 44% patients decompensated dysbiosis of vagina was diagnosed, what manifested as acute decrease (up to full absence) of Lactobacillus spp. We also found the increase of the quantity of excreted opportunistic and pathogenic microorganisms up to 10^7 - 10^{11} CFU/ml on the background of the increase of the quantity of microorganisms in microbial associations (from 2 to 4 opportunistic and

pathogenic agents) in 25% of women. Thus, in 27 from 61 of examined women we found in significant quantity microorganisms that were absent in patients with vaginal normocinosis (Mobilincus spp., Enterococcus face., Streptococcus spp., Gardnerella vag): Gardnerella vaginalis in 9 patients, Candida albicans - in 5, Enterococcus faecalis - in 11, Esherichia colli – in 6, Klebsiella — in 3 (Tab.3).

Table 3.

Vaginal microbiosis in women with primary infertility

Microorganisms	Lactobacterium (n=61)	Gardnerella (n=61)	Enterococcus (n=61)	Staphylococcus aureus, Staphylococcus hemolyticus (n=61)	Candida (n=61)
% of evaluation	24 (39%)	9 (15%)	11 (18%)	12 (20%)	5 (8%)

Thus, in women with primary infertility disturbances of vaginal microbiosis are frequently diagnosed. First of all this is characterized by frequent finding of opportunistic flora and Candida fungi and decreased number of Lactobacterium. Occurrence of Ureaplasma was accompanied by decrease of the quantity of Lactobacterium. It is known that absence or acute decrease of H_2O_2-producing lactobacillus in vagina induces the risk of bacterial vaginosis development [13, 15, 21].

Considering parasite invasion, we studied in details persistence of infections according to groups A and B dependently from the presence of worm invasion (Tab.4).

Table 4.

Vaginal microbiosis in women with primary infertility dependently from parasite invasion

Found infections	Groups	
	group A (with presence of parasites) (n=47)	group B (without parasites found) (n=34)
Ureoplasmosis	29 (59.6 %)	18 (53 %)
Mycoplasmosis	8 (17 %)*	2 (5.9 %)
Chlamydiosis	2 (4 %)	1 (3 %)
Ig G to chlamydias	7 (15 %)	7 (20 %)
Ig A to chlamydias	4 (8.5 %)	5 (14 %)
Bacteriology:		
Esherichia coli 10^3-10^5	17%*	7.7%

Candida albicans 10^3-10^5	7%	11.5 %
Gardnerella vaginalis 10^4-10^5	20%	15.4 %
Enterococcus faecalis 10^4-10^6	30%*	11.5 %
Klebsiella pneumonie, oxytoca	17 %*	3.8 %
Staphylococcus of different groups	20%	11.5 %

- the difference is statistically significant between indices, obtained in women of group A and B.

Thus, parasite invasion does not affect the frequency of persistence of ureaplasma infection in lower parts of genital system. *Mycoplasma hominis* was significantly more often found in women with primary infertility and worm invasions. Occurrence of Esherichia colli, Klebsiella and Enterococcus faecalis was significantly higher at presence of worm invasion, what confirms the pathogenic influence of parasite invasion on vaginal microbiocinosis.

Balanced proportion and optimal quantity of aerobic and anaerobic microorganisms in the intestine provides a number of the most important functions: inhibits the growth and proliferation of opportunistic microbes, is involved in synthetic, digestive and desintoxificating functions of the colon; stimulates the synthesis of biologically active compounds, that affect the function of cardio-vascular system, hematopoietic organs etc; maintains high level of lizocym, immunoglobulins, interferon and other components of the immune system [3, 7]. Microecology of the vagina is closely interrelated with the condition of colonic flora. Dominating bacteria of vaginal microenvironment are *Lactobacillus spp*., the concentration of which is proportional to their content in colon. Normal microflora of the vagina provides so called colonizing resistance of genital tract. Thanks to specific adhesion, a biolayer is being formed on epithelial cells, that is composed of microcolonies of lactobacilli, surrounded by the products of their metabolism [6, 14].

Changes of the microbiocinosis of the large intestine affect endoecology of the vagina as well as presence of worms. In our studies dysbiosis of the large intestine occurred in half of the examined women. It is known that dysbiosis of the large intestine causes decrease of the quantity of lacto- and bifidobacteria in the vagina [14]. This phenomenon is accompanied by accessive growth of streptococci and staphylococci, appearance of gardnerella, enterococci, mobiluncus with specific clinical signs – a specific “fish” smell and dense milky vaginal discharge appear as well as itching, hyperemia and discomfort in the vagina and external genitals and persistence of this process results in the development of the chronic inflammatory disorders of small pelvis organs [16, 20].

Due to results of bacteriologic examination of feces, 50% of 38 examined women were revealed dysbacteriosis of the colon what manifested by the increase of the content of *Klebsiella pneumonie* and *Klebsiella oxytoca*, *Enterococcus faecalis*, *Candida, Esherichia coli with hemolytic properties*, decrease of the content of *Lactobacilli* and *Esherichia coli*. Frequency and severity of dysbiotic changes in patients with and without parasite invasion were different. In patients with parasite invasion dysbacteriosis in most cases was accompanied by the changes of vaginal microbiocinosis (42%). This is mediated by the fact that different kinds of bacteria colonize both in gastrointestinal tract and in vagina, which is why rectum plays an important role as a source of microorganisms, colonizing vagina [14, 19].

Lactobacteria are known to be one of the main mechanisms of the protection of geniatal ways from pathogenic and opportunistic microflora. Qualitative analysis of biocinosis of the large intestine in women with primary infertility and parasite invasion indicates the high rate of plating of *Esherichia coli with hemolytic properties* from the colon– 17%, klebsiellas – 17%, staphylococcus aureus – 17%. In patients of group B frequency of determination of opportunistic enterobacteria is significantly lower. Thus, klebsiella was found in 5.9 % of examined women, *Esherichia coli with hemolytic properties* in 3%, *Staphylococcus aureus* – in 3% of cases. Fungi Candida contaminated colon in 16% of examined women of group A, what was significantly higher compared to patients of group B (5.9%). Association of different kinds of opportunistic bacteria (*Klebsiella* and *Staphylococcus aureus*, *Esherichia coli with hemolytical properties* and Candida) was registered in patients of group A with a frequency of 30% and in patients of group B in 14% of cases.

Results of conducted studies showed that disturbances of microbiocinosis of the vagina and colon in most of women with primary infertility are of combined character . Dysbiotic changes in the vagina were accompanied by intestinal dysbiosis in 48% of cases, whereas 90% of women with dysbacteriosis of the colon had changes in 20% of patients. Our data corresponds with the data of our scientists that numerous kinds of vaginal microbiocinoses directly depend on the state of microorganisms in the colon [4, 12, 14, 19]. Structure and level of dysbiotic alterations of genital tract in women with primary infertility depend on the presence of parasites. The most significant dysbalance between the indices of opportunistic flora and lactobacilli is being formed in women with ascarides invasion. In our findings 27% of women with infertility had eggs of the parasites in feces and high level of IgG to ascarides was revealed in 39% of the examined patients. As it was mentioned above, appearance of parasites in the vagina can cause bacterial vaginosis, inflammatory processes and become the factor of infertility development [1, 18].

Ascarides are the most aggressive parasites invading female reproductive system. Differently from enterobiosis, which mainly causes pathologic changes of the lower parts of genital system,

ascaridosis makes predispositions for the development of acute and chronic appendicitis, intussusceotion, anemia, hypovitaminosis B. Surgery due to appendicitis increases the rate of peritoneal and tubular infertility and the risk of extrauterine pregnancy [9, 11]. Chronic disorders of the intestine increase the risk of synechia development, in particular in the right ileal region with the involvement of fimbria of the right ovarian tube, the latter results in its immobilization, causing inability to capture an egg by the permeate but nonworking tube.

Hence, in women with primary infertility under conditions of parasite invasion vaginal microbiocinosis is altered, growth of opportunistic flora increases, what in its turn cause the development of chronic inflammatory changes of genital system with relevant consequences. Considering high frequency of evaluation of parasites, women with primary infertility with disturbances of microbiosis of vagina and intestine should be recommended examination towards parasites as well as determination of titre of antibodies Ig G to toxocara, ascarides and lamblia.

Conclusions

1. In women with primary infertility ascaridosis was revealed in 48% of examined patients, what states about its aggressive effect on reproductive health of females.
2. Mycoplasmosis was significantly more often detected in women with worm invasions, persistence of Ureaplasma infection in lower part of the genital system does not depend on the presence or absence of parasites and is present in 53-59% of women with primary infertility.
3. Occurrence of *Esherichia colli, Klebsiella* and *Enterococcus faecalis* was significantly higher in vaginal content under conditions of worm invasion.
4. Dysbiotic changes of vagina were accompanied by intestinal dysbiosis in 48% cases, whereas in patients with colonic dysbacteriosis in 90% of women changes in vaginal microflora were found.

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References

1. T.A. Basova, G.P. Gladilin and I.E. Rogozhina, “Parasitosis in women with cervicitis, living in the city of Saratov”, Basic Research, no. 9, pp. 11-14, 2011.
2. K. I. Bodnya, L.V. Holtobina, Handbook of Ascariasis, Harkow, Ukraine, UA, 2004.
3. V.M. Bondarenko, N.M. Grachev and T.Z. Matsulevich, Intestinal dysbiosis in adults, Moscow, Russia, RU, 2003.

4. I.A. Zhabchenko, T.S. Chernenko and YV Nevyshna, Features vaginal microbiota in pregnant women and their correction in case of landslides dysbiotic, Women's Health, no. 4, pp. 87-89, 2011.

5. "On organization of outpatient obstetric care in Ukraine." № 417 MOH of Ukraine, 2011.

6. P.N. Veropotvelyan, V.G. Ginzburg and N.P. Veropotvelyan, New Treatment Methods bacterial vaginosis and dysbacteriosis intestine, Reproductive women health, vol. 31, no.2, pp. 92-96, 2007.

7. A.I. Parfenov, G.A. Osipov and P.A. Bogomolov, Intestinal dysbiosis, Consilium, vol. 1, no. 2, pp. 20-26, 2002.

8. A.M. Savicheva, Etiologic diagnosis and therapy of reproductive infections, The difficult patient. vol. 5, no. 1, pp. 1-7, 2007.

9. A.A. Gardeners, K.I. Panchenko and N. Denisov, Severe complications nematosis, Bulletin of Surgery, vol. 169, no. 6, pp. 87- 89, 2010.

10. V.O. Sklyarova, The role of various related factors in the development of recurrent candidosis vaginitis, Women health, vol. 44, no. 8, pp. 223-226, 2009.

11. G.A. Fedorchenko, Helminthiasis in practice obstetrician, Medical aspects of women's health, vol. 33, no. 5-6, pp. 30-34, 2010.

12. D.S. Yankovsky, R.A. Moiseenko and G.S. Dyment, Place of dysbiosis in human pathology, Contemporary Pediatrics, vol. 1, no. 29, pp. 154- 167, 2010.

13. J.M. Fettweis, M.G. Serrano, P.H. Girerd, K.K. Jefferson and G.A. Buck, A new era of the vaginal microbiome: advances using next-generation, Chem. Biodivers, vol. 9, no. 5, pp. 965-976, 2012.

14. M.A. Antonio, L.K. Rabe and S.L. Hillier, Colonization of the rectum by Lactobacillus species and decreased risk of bacterial vaginosis, J. Infect, Dis., vol. 192, no. 3, pp. 394–398, 2005.

15. R.H. Beigi, H.C. Wiesenfeld and S.L. Hillier, Factors associated with absence of H2O2-producing Lactobacillus among women with bacterial vaginosis, J. Infect. Dis, vol. 191, no. 6, pp. 924-929, 2005.

16. R.M. Brotman, Vaginal microbiome and sexually transmitted infections: an epidemiologic perspective, J. Clin. Invest, vol. 12, no. 12, pp. 4610-4617, 2011.

17. A. Gimez-Delgado, R. Rivera-Cedillo, Prevalence and risk factors associated with intestinal parasitoses in pregnant women and their relation to the infant's birth weight, Ginecol Obstet Mex, vol. 70, pp. 338-343, 2002.

18. V. Sklyarova, Helminthiasis as impact factor of gynecological disorders, Indian. J. Sex. Transm. Dis, vol. 31, pp. 58-60, 2010.

19. N.A. El Aila, I. Tency, B. Saerens, E. De Backer, P Cools, G.L. dos Santos Santiago, H. Verstraelen, R. Verhelst, M. Temmerman and M. Vaneechoutte. Strong correspondence in bacterial loads between the vagina and rectum of pregnant women, Res. Microbiol, vol. 162, no. 5, pp. 506-513, 2011.

20. P.Gajer, R.M. Brotman, G. Bai, J. Sakamoto, U.M. Schütte, X. Zhong, S.S. Koenig, L. Fu, Z.S. Ma, X. Zhou, Z. Abdo, L.J. Forney and J. Ravel, Temporal dynamics of the human vaginal microbiota, Sci. Transl. Med, vol. 4, no. 132, pp. 132ra52, 2012.

21. B.A.Wilson, D.J. Creedon and K.E. Nelson, The vaginal microbiome in health and disease, Trends Endjcrinol. Metab, vol. 22, no. 10, pp. 389-393, 2011.

22. S.S. Witkin, I.M. Linhares and P. Giraldo, Bacterial flora of the female genital tract: function and immune regulation, Best. Pract. Res. Clin. Obstet. Gynaecol, vol. 21, no. 3, pp. 347-354, 2007.