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CORRELLATIONS BETWEEN EXPRESSION NLRP3-INFLAMMASOME AND GYNECOLOGICAL PATHOLOGY IN WOMEN WITH ENDOMETRIOSIS ASSOCIATED INFERTILITY

КОРЕЛЯЦІЯ МІЖ ЕКСПРЕСІЄЮ NLRP3-ІНФЛАСАСОМОЮ ТА ГІНЕКОЛОГІЧНОЮ ПАТОЛОГІЄЮ У ЖІНОК З ЕНДОМЕТРІОЗОМ, АСОЦІЙОВАНИМ ІЗ БЕЗПЛІДДЯМ

Резюме. Метою нашого дослідження було вивчити експресію NLRP3-інфламасоми та деякі показники в крові жінок з ендометріоз-асоційованим безпліддям за допомогою допоміжних репродуктивних технологій із включенням пробіотика.
Матеріали і методи. З метою дослідження обстежено 30 безплідних жінок. Контрольну групу склали 10 жінок з трубним безпліддям унаслідок запального процесу в анамнезі, у яких у результаті комплексного клініко-лабораторного обстеження інших захворювань не виявлено, і за станом здоров'я їх можна прирівняти до практично здорових. Жінки (віком від 21 до 42 років, середній вік – 29,75 років), які не приймали пробіотик. Основну групу склали 20 жінок із зовнішнім генітальним ендометріозом, які були включені до допоміжних репродуктивних технологій. Пацієнти основної групи приймали пробіотик по 1 таблетці 2 рази на добу з вмістом лактобактерій 10×10^9 Lactobacillus протягом 1 місяця в комплексній підготовці (лікуванні) до допоміжних репродуктивних технологій. Показники експресії NLRP3-інфламасоми визначались до та після прийому препарату. Дослідження проводили в Буковинському державному медичному університеті та Центрі репродуктивної медицини. Частота первинного безпліддя була достовірно вищою у пацієнтів основної групи. Для аналізу експресії гена NLRP3-інфламасоми та визначення відносної нормованої експресії мРНК NLRP3-інфламасоми використовували полімеразну ланцюгову реакцію зі зворотною транскрипцією в реальному часі (RT-PCR). Об'єктом для молекулярно-генетичних досліджень методом ЗТ-ПЛР була фракція мононуклеарів, виділених із цільної крові хворих на ендометріоз.
Значення p (різниця достовірності) визначали за таблицею Стьюдента-Фішера. Відмінності між контрастними середніми вважалися значущими при $p < 0,05$.
Результати та обговорення. Проаналізувавши результати наших досліджень, встановили, що в основній групі експресія NLRP3-інфламасоми складала 24,43, що достовірно вище ніж після препарату (0,70 відповідно). У контрольній групі експресія NLRP3-інфламасоми становила 0,54.
Експресія NLRP-3 інфламасоми зросла у пацієнтів до препарату більш ніж у 34 рази порівняно з пацієнтами після препарату відповідно.
Висновки. Отже, після застосування пробіотика різко знижується підвищений рівень NLRP3-інфламасоми, що свідчить про ефективність і можливість використання в програмі підготовки допоміжних репродуктивних технологій.
Ключові слова: ендометріоз, допоміжні репродуктивні технології, інфламасома.

Endometriosis is one of the most urgent problems in modern gynecology. Since this disease is observed in almost every third woman and there is a tendency to increase the frequency of cases, endometriosis remains a «subject» of special interest not only for scientists, but also for practicing doctors. According to statistics, endometriosis affects more than 10 % of women of reproductive age, which is about 176 million women worldwide. Endometriosis is a common gynecological disease. About 10 % to 15 % of women of childbearing

age exhibit endometriosis, and about 30 % of infertile women are affected by this disease. Many recent studies have suggested that immune factors play important roles in its pathogenesis. Hypothyroidism, susceptibility to vaginal candidiasis, auto-immune diseases, fibromyalgia, chronic fatigue syndrome, headaches, arthralgias and myalgias, asthma and allergies are more common comorbidities in women with endometriosis than in women without it [1].

The pathogenesis and mechanisms leading to the development of infertility continue to be studied worldwide. The same pathogenetic mechanisms that lead to the development of infertility in endometriosis can be the reason for unsuccessful attempts in IVF programs.

Treatment of endometriosis associated with infertility is a serious problem, because currently existing methods, which are based on surgical removal of endometriosis foci followed by the appointment of hormonal drugs, do not always demonstrate sufficient effectiveness. The success of the most effective approach – surgical treatment, to a certain extent, depends on the form and stage of the disease, the degree of infiltration of ectopias into the underlying tissue of the peritoneum and scar-dystrophic changes that accompany this disease during its development [2].

But the immunopathogenetic mechanisms of the development of endometriosis, the ways of drug influence on the clinical manifestations of endometriosis-associated infertility, and the improvement of the effectiveness of IVF methods through pathogenetically justified and optimized preparation are still not sufficiently studied [3, 4].

The possibility that effector pathways of inflammasome activation may be involved in the pathogenesis of endometriosis is discussed. It is possible that inflammation can lead to progressive destruction of tissues, which initiates the development of a chronic disease. IL-1β is a key cytokine involved in the regulation of adhesion and proliferation of endometrial cells [5, 6].

The article [7] presents the results of the study of the influence of lactobacilli on the morphology of endometriosis foci in an experimental model of endometriosis. It was established that after treatment with lactobacilli in the tissue of endometriosis foci, a complex of changes is observed, which indicate the development of significant pathological processes and their destruction.

The aim of our study was to examine the expression NLRP3-inflammasome and compare with some indices in the blood of women with endometriosis-associated infertility using assisted reproductive technologies.

Material and methods. We examined 30 women who were divided into the following groups: control group consisted of 10 women with tubal infertility due to an inflammatory process in the anamnesis, in whom, as a result of a complex clinical and laboratory examination, no other diseases were detected and in terms of their health they could be equated with practically healthy women (age from 21 to 42 years, the average age was 29.75 years) and who did not take probiotic. The main group consists from 20 women with external genital endometriosis were included in assisted reproductive technologies. Patients from main group took probiotic 1 tablet twice a day with 10×10^9 Lactobacillus during 1 month in complex preparation (treatment) before assisted reproductive technologies. The indices of NLRP3-inflammasome determined before and after preparation. The study was performed in Bukovinian State Medical University and Centre of Reproductive Medicine.

To analyze the expression of the NLPP3-inflammasome gene and determine the relative normalized expression of mRNA NLPP3-inflammasome, we used the polymerase chain reaction with reverse transcription in real time (RT-PCR). The object for molecular genetic studies by the RT-PCR method was the fraction of mononuclear cells isolated from the whole blood of patients with endometriosis.

Comparison of the two groups for a quantitative variable following a normal distribution, under the condition of equality of variances, was performed using Student's t-test.

ROC analysis was used to assess the diagnostic performance of quantitative variables in predicting a categorical outcome. The optimal cut-off value of the quantitative variable at was estimated using the Youden's J statistic.

Results and discussion. The mean age of women in control group (who did not take probiotic – 29.75±7.09 years) and in main group (who took probiotic) 30.65±2.04 (p>0,05).

We performed analysis of infertility, degree conditioning on group (Tab. 1).

Table 1

Analysis of infertility, degree conditioning on group

Variable	Categories	Group		p
		control	endometriosis	
infertility, degree	1	3 (30.0)	15 (75.0)	0.045*
	2	7 (70.0)	5 (25.0)	

* – differences are statistically significant (p <0.05)

According to the presented table, when comparing of infertility, degree, statistically significant differences were revealed depending on group (p = 0.045) *(applied method: Fisher's exact test)*.

Odds of 2 were 7.000 times less in endometriosis group than in control group, the relative difference in odds was statistically significant (OR = 0.143; 95 % CI: 0.026-0.774) (Fig. 1).

Analysis of infertility, duration was performed conditioning on group (Tab. 2).

When comparing of infertility, duration depending on group no statistically significant differences were revealed (p = 0.193) *(applied method: Student's t-test)* (Fig. 2).

Analysis of spikes was performed conditioning on group (Tab. 3).

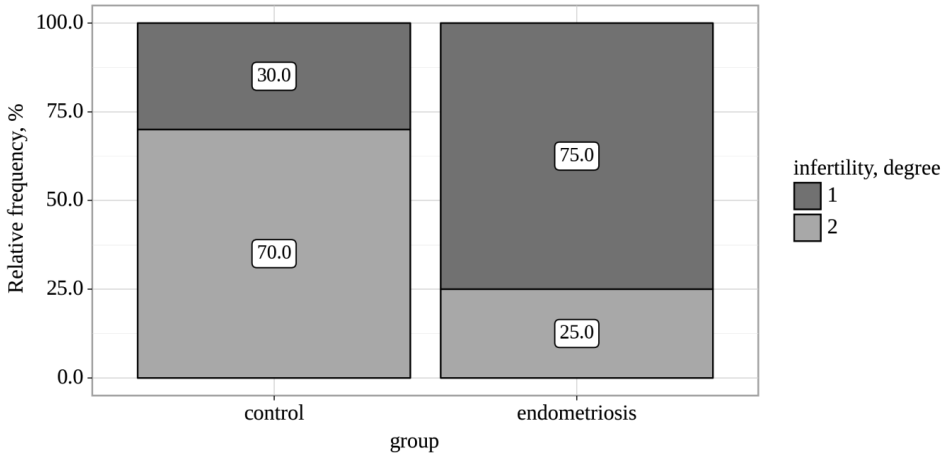

Figure 1. Analysis of infertility, degree conditioning on group

Table 2

Analysis of infertility, duration conditioning on group

Variable	Categories	infertility, duration			p
		M ± SD	95 % CI	n	
group	control	6 ± 2	5-7	10	0.193
	endometriosis	5 ± 2	4-6	20	

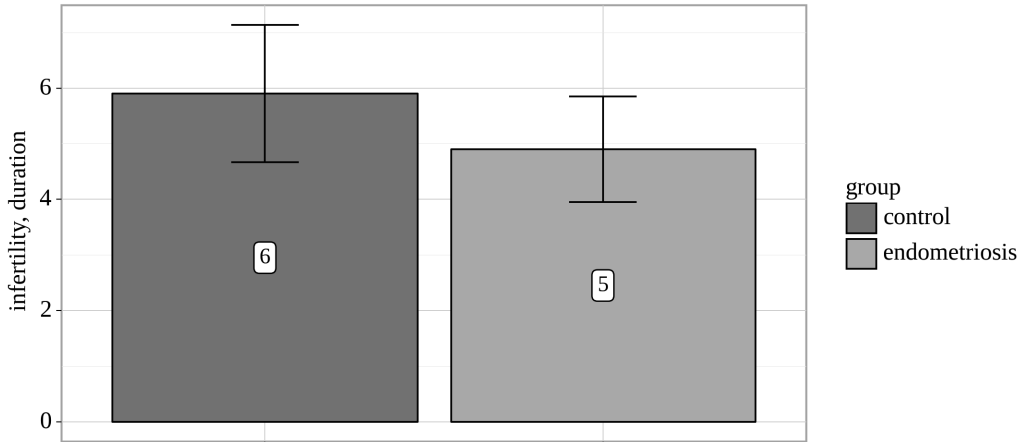

Figure 2. Analysis of infertility, duration conditioning on group

Table 3

Analysis of spikes conditioning on group

Variable	Categories	Group		p
		control	endometriosis	
spikes	no	0 (0.0)	15 (75.0)	< 0.001*
	yes	10 (100.0)	5 (25.0)	

* – differences are statistically significant (p <0.05)

Statistically significant differences were revealed when comparing of spikes depending on group (p<0.001) *(applied method: Fisher's exact test)* (Fig. 3).

Analysis of leiomyoma was performed conditioning on group (Tab. 4)

When comparing of leiomyoma depending on group there were no statistically significant differences (p = 0.657) (applied method: Fisher's exact test).

Odds of yes were 1.714 times less in endometriosis group than in control group, the relative difference in odds was not statistically significant (OR = 0.583; 95 % CI: 0.102-3.325) (Fig. 4).

Analysis of the NLRP3 before-after dynamics depending on group was performed.

Statistically significant differences were revealed when comparing the variable at the NLRP3 level before treatment period. (p < 0.001) (applied method: Fisher's exact test). The analysis performed showed that there were statistically significant differences when comparing the variable at the NLRP3 level after treatment period. (p = 0.003) (applied method: Pearson's chi-square test).

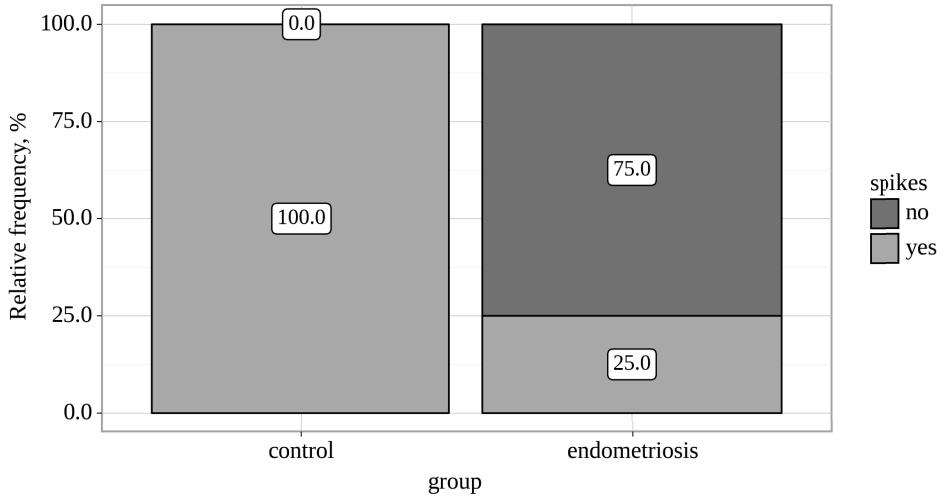

Figure 3. Analysis of spikes conditioning on group

Table 4

Analysis of leiomyoma conditioning on group

Variable	Categories	Group		p
		control	endometriosis	
leiomyoma	no	7 (70.0)	16 (80.0)	0.657
	yes	3 (30.0)	4 (20.0)	

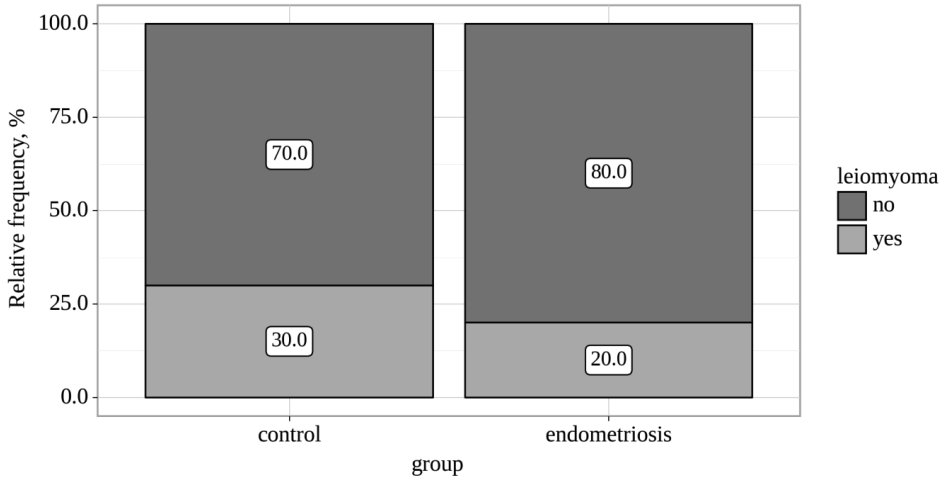

Figure 4. Analysis of leiomyoma conditioning on group

The analysis didn't show statistically significant changes in the endometriosis group (p = 0.157) (applied method: Wilcoxon test).

Endometriosis is one of the global problems of modern gynecology facing scientists. This is the most common disease in women, associated with the impossibility of realizing the reproductive function and a decrease in the quality of life of such patients. Endometriosis is estimated to occur in approximately 1 in 10 women of reproductive age in the general patient population and is diagnosed in 40-50 % of women with infertility and 70-90 % of women with chronic pelvic pain [8].

Despite the increasing number of scientific and clinical studies on various aspects of endometriosis, a significant number of questions regarding the diagnosis, frequency of occurrence, characteristics of the course and therapy remain controversial, and the cost of treatment is equal to the cost of treating Crohn's disease, type 2 diabetes and rheumatoid arthritis [9].

Thus, endometriosis is a disease that in the 21st century does not have a single point of view regarding its root cause and etiological factors. Severe clinical symptoms, the impossibility of fully using non-invasive diagnostic methods in determining the problem and the lack of complex treatment that would ensure the absence of relapse after a course of treatment force scientists and doctors to deepen their knowledge and look for methods of solving the problems of this pathology [10].

The mechanisms involved in activating the NLRP3-inflammasome remain unclear. Possible processes include changes in intracellular calcium concentration [11], lysosomal damage [12], mitochondrial damage [13], potassium ion efflux [14] and reactive oxygen species (ROS) production [15]. The main function of this inflammasome is to initiate assembly of the inflammatory complex, and adaptor protein of the inflammasome to connect upstream NLRP3 and downstream caspase-1. When the NLRP3-inflammasome becomes over-activated, it induces pyroptosis by generating excessive inflammatory factors and participates in the development of certain diseases.

Endometriosis is a common gynecological disease. About 10 % to 15 % of women of childbearing age exhibit endometriosis, and about 30 % of infertile women are affected by this disease [16, 17]. Many recent studies have suggested that immune factors play important roles in its pathogenesis. Hypothyroidism, susceptibility to vaginal candidiasis, autoimmune diseases, fibromyalgia, chronic fatigue syndrome, headaches, arthralgias and myalgias, asthma and allergies are more common comorbidities in women with endometriosis than in women without it. Therefore, a possible link between endometriosis and autoimmunity has been suggested [18]. Peritoneal fluid samples from women with endometriosis show defectively activated macrophages and natural killer (NK) cells, which alter the recognition and clearance of endometrial cells. Macrophages secrete different products such as growth factors, enzymes, prostaglandins, and cytokines that stimulate the adhesion of endometrial tissue to mesothelial cells, promoting the invasion of extracellular matrix creating islands of endometrial cells where they can proliferate [19]. Previous studies have shown that inflammation is an important pathophysiological basis for endometriosis [20]. The intraabdominal inflammatory environment and immune abnormalities are closely related to ectopic endometrial hyperplasia. Inflammation is a response from living tissues to infection or damage. Bullon *et al.* [18] proposed that abnormal activation of inflammasome is closely related to the occurrence of endometriosis. As an important inflammatory mediator in inflammatory responses, the NLRP3-inflammasome is an important component of inflammasomes [21]. The same study also explored whether the NLRP3- inflammasome acts in the pathogenesis of endometriosis by establishing an endometriosis model: when the NLRP3 level was reduced, the production of inflammatory cytokines was inhibited [19, 22].

But till many researches didn't completely found mechanisms of pathogenesis, noninvasive methods of diagnostics and understanding how possible suppressed NLRP3-inflammasome due to use probiotics. We study the determination of NLRP3-inflammasome level in infertile women before and after preparation with probiotics to assistant reproductive technologies.

Study of the effect of lactobacilli on the tissue of endometriotic lesions in experimental models of endometriosis. Were found that after treatment with lactobacilli in the tissue of endometriotic lesions were observed range of changes that indicate significant development of pathological processes and destruction of endometriotic lesions [7].

The level of NLRP3-inflammasome is shown in Table 5.

Table 5

The level of Nlrp3-inflammasome

Groups of women under studies	n	Expression of NLRP3-inflammasome level	p
Endometriosis	20	24,43	<0,05
Control	10	0,54	<0,05

Reduction level of expression NLRP3-inflammasome in patients of control group was marked by the fact that such patients practically healthy women.

After analyzing data presented in Table 6, we can see that the main group (women with endometriosis) consisted of patients who had our proposed preparation for assistant reproductive technologies with the inclusion of probiotics and control group who had been prepared for assistant reproductive technologies without inclusion probiotic.

Table 6

The level of Nlrp3-inflammasome before and after preparation

Group	Expression of NLRP-3 inflammasome level		p
	Before preparation (treatment)	After preparation (treatment)	
Endometriosis	24.43	0.70	<0.05
Control	0.54	-	-
p	<0.05	-	

After analyzing data presented in Table 6, we can see that the main group (women with endometriosis) consisted of patients who had our proposed preparation for assistant reproductive technologies with the inclusion of probiotics and control group who had been prepared for assistant reproductive technologies without inclusion probiotic.

In main group expression of NLRP3-inflammasome was 24.43, which is significantly higher than after preparation (0,70 accordingly). In control group expression of NLRP3-inflammasome was 0.54.

Expression of NLRP3-inflammasome increased in patients before preparation more than 34 times compare with patients after preparation accordingly.

Consequently, after using probiotic, the increased level of NLRP3-inflammasome sharply decreases, indicating the effectiveness and the possibility of use in the program for assisted reproductive technologies preparation.

Conclusion. The very high expression of m-RNA NLRP3-inflammasome depicts that pathogenesis of endometriosis closely related with inflammation.

Using of probiotic in complex preparation before assisted reproductive technologies significantly improve well-being of patients and sharply reduce expression of m-RNA NLRP-3 inflammasome. We recommend our preparation with probiotic in practice.

References

1. Bulun SE, Monsavais D, Pavone ME, Dyson M, Xue Q, Attar E, et al. Role of estrogen receptor-β in endometriosis. Semin Reprod Med 2012;30:39-45. doi: 10.1055/s-0031-1299596.

2. Hryshchenko VI, Shcherbyna MO. Akusherstvo i Hinekolohija. Kn.2: Hinekologiia: pidruchnyk. Kyiv: VSV «Medytsyna»; 2011. 376 s. [in Ukrainian].

3. Likhachov VK. Hinekolohiia: kerivnytstvo dlia likariv. Vinnytsia: Nova Knyha; 2018. 688 s. [in Ukrainian].

4. The American Fertility Society. The American Fertility Society classifications of adnexal adhesions, distal tubal occlusion, tubal occlusion secondary to tubal ligation, tubal pregnancies, Mullerian anomalies and intrauterine adhesions. Fertil. Steril. 1988;49(6):944-55. DOI:10.1016/s0015-0282(16)59942-7.

5. MOZ Ukrayiny. Nakaz № 353 vid 13.04.2016 Pro zatverdzhennya ta vprovadzhennya medyko-tekhnolohichnykh dokumentiv zi standartyzatsiyi medychnoyi dopomohy pry anomal'nykh matkovykh krovotechakh [Internet]. Kyyiv: MOZU; 2016 [onovleno 2016 kvit. 13; tsytovano 2023 sich. 24]. Dostupno na: https://zakononline.com.ua/documents/show/63582___63582. [in Ukrainian].

6. Ulrich U, Buchweitz O, Greb R, Keckstein J, I von Leffern, Oppelt P at all. National German Guideline (S2K). Guideline for Diagnosis and Treatment of Endometriosis, AWMF Registry No 015-045. Geburtsh Frauenheilk. 2014; 74:1104-18. doi:10.1055/s-0034-1383187.

7. Itoh H, Sashihara T, Hosono A, Kaminogawa S, Uchida M. Lactobacillus gasseri OLL2809 inhibits development of ectopic endometrial cell in peritoneal cavity via activation of NK cells in a murine endometriosis model. Cytotechnology. 2011 Mar;63(2):205-10. doi: 10.1007/s10616-011-9343-z.

8. Esteves SC, Yarali H, Vuong LN, Carvalho JF, Özbek İY, Polat M, et al. Cumulative delivery rate per aspiration IVF/ICSI cycle in POSEIDON patients: a real-world evidence study of 9073 patients. Hum Reprod. 2021 Jul 19;36(8):2157-2169. doi: 10.1093/humrep/deab152.

9. Zondervan KT, Becker CM, Missmer SA. Endometriosis. N Engl J Med. 2020 Mar 26;382(13):1244-1256. doi: 10.1056/NEJMra1810764.

10. Zaporozhan VM, Tatarchuk TF, Kaminskyi VV, Boichuk AV, Bulavenko OV, Vdovychenko YuP, et. al. Natsionalnyi konsensus shchodo vedennia patsiientok iz endometriozom. Reproduktyvna endokrynolohiia. 2015 Ver;4(24):7-12. [in Ukrainian].
11. Rossol M, Pierer M, Raulien N, Quandt D, Meusch U, Rothe K, et al. Extracellular Ca2+ is a danger signal activating the NLRP3 inflammasome through G protein-coupled calcium sensing receptors. Nat Commun. 2012;3:1329. doi: 10.1038/ncomms2339.
12. Hornung V, Latz E. Critical functions of priming and lsosomal damage for NLRP3 activation. Eur J Immunol. 2010;40:620-3. doi: 10.1002/eji.200940185.
13. Lyu JJ, Mehta JL, Li Y, Ye L, Sun SN, Sun HS, et al. Mitochondrial autophagy and NLRP3 inflammasome in pulmonary tissues from severe combined immunodeficient mice after cardiac arrest and cardiopulmonary resuscitation. Chin Med J. 2018;131:1174-84. doi: 10.4103/0366-6999.231519.
14. Pétrilli V, Papin S, Dostert C, Mayor A, Martinon F, Tschopp J. Activation of the NALP3 inflammasome is triggered by low intracellular potassium concentration. Cell Death Differ. 2007;14:1583-9. doi: 10.1038/sj.cdd.4402195.
15. Zhou R, Yazdi AS, Menu P, Tschopp J. A role for mitochondria in NLRP3 inflammasome activation. Nature. 2011;469:221-225. doi: 10.1038/nature09663.
16. Cramer DW, Missmer SA. The epidemiology of endometriosis. Ann NY Acad Sci. 2002;955:11-22. doi: 10.1111/j.1749-6632.2002.tb02761.x.
17. Practice Committee of the American Society for Reproductive Medicine. Endometriosis and infertility. Fertil Steril 2004;81:1441-1446. doi: 10.1016/j.fertnstert.2004.01.019.
18. Bullon P, Navarro JM. Inflammasome as a key pathogenic mechanism in endometriosis. Curr Drug Targets. 2017;18:997-1002. doi: 10.2174/1389450117666160709013850.
19. Leopakorn S, Huang KG. Polypoid endometriosis of urinary bladder. Gynecol Minim Invasive Ther. 2018;7:86-7. doi: 10.4103/GMIT.GMIT_18_18.
20. Yilmaz BD, Bulun SE. Endometriosis and nuclear receptors. Hum Reprod Update. 2019;25:473-85. doi: 10.1093/humupd/dmz005.
21. Mezzasoma L, Antognelli C, Talesa VN. A novel role for brain natriuretic peptide: inhibition of IL-1β secretion via downregulation of NF-kB/Erk 1/2 and NALP3/ASC/Caspase-1 activation in human THP-1 monocyte. Mediators Inflamm. 2017;2017: e5858315.doi: 10.1155/2017/5858315.
22. Ahn SH, Khalaj K, Young SL, Lessey BA, Koti M, Tayade C. Immune-inflammation gene signatures in endometriosis patients. Fertil Steril. 2016;106:1420-31. doi: 10.1016/j.fertnstert.2016.07.005.

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Abstract. The aim of our study was to examine the expression NLRP3-inflammasome and some indices in the blood of women with endometriosis-associated infertility using assisted reproductive technologies with included probiotic.

Material and methods: For the purpose of the research we examined 30 infertile women. Control group consisted of 10 women with tubal infertility due to an inflammatory process in the anamnesis, in whom, as a result of a complex clinical and laboratory examination, no other diseases were detected and in terms of their health they could be equated with practically healthy women (age from 21 to 42 years, the average age was 29.75 years) and who did not take probiotic. The main group consists from 20 women with external genital endometriosis were included in assisted reproductive technologies. Patients from main group took probiotic 1 tablet twice a day with 10×10^9 Lactobacillus during 1 month in complex preparation (treatment) before assisted reproductive technologies. The indices of NLRP3-inflammasome determined before and after preparation. The study was performed in Bukovinian State Medical University and Centre of Reproductive Medicine. The primary infertility incidence was significantly higher in patients from main group. To analyze the expression of the NLPP3-inflammasome gene and determine the relative normalized expression of mRNA NLPP3-inflammasome, we used the polymerase chain reaction with reverse transcription in real time (RT-PCR). The object for molecular genetic studies by the RT-PCR method was the fraction of mononuclear cells isolated from the whole blood of patients with endometriosis.

The value of p (authenticity difference) was determined by Student's table-Fischer. Differences between contrasting averages were considered significant at $p < 0.05$.
Results and discussion: Analyzed the results of our research stated that in main group expression of NLRP3-inflammasome was 24.43, which is significantly higher than after preparation (0,70 accordingly). In control group expression of NLRP-3-inflammasome was 0.54.
Expression of NLRP3-inflammasome increased in patients before preparation more than 34 times compare with patients after preparation accordingly.
Conclusions. Consequently, after using probiotic, the increased level of NLRP3-inflammasome sharply decreases, indicating the effectiveness and the possibility of use in the program for assisted reproductive technologies preparation.
Key words: endometriosis, assisted reproductive technologies, inflammasome.

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