Nosenko O. M., Kosyuga O. M. Peculiarities of proteome profile of eutopic endometrium in women with adenomyosis depending on fertility. Journal of Education, Health and Sport. 2019;9(3):652-662. eISSN 2391-8306. DOI http://dx.doi.org/10.5281/zenodo.3600575
http://ojs.ukw.edu.pl/index.php/johs/article/view/7666

The journal has had 7 points in Ministry of Science and Higher Education parametric evaluation. Part B item 1223 (26/01/2017).
1223 Journal of Education, Health and Sport eISSN 2391-8306 7

Received: 11.03.2019. Revised: 22.03.2019. Accepted: 29.03.2019.

PECULIARITIES OF PROTEOME PROFILE OF EUTOPIC ENDOMETRIUM IN WOMEN WITH ADENOMYOSIS DEPENDING ON FERTILITY

O. M. Nosenko, O. M. Kosyuga

Odessa National Medical University

Abstract

In recent years, several studies have reported a correlation between adenomyosis and major obstetric adverse effects. Implantation failures can be caused by changes in the expression of some molecules, referred to as "implantation markers", which are expressed by the endometrium and are required for successful interaction between the embryo and endometrium. **The purpose of the study** was to determine the features of the proteomic profile of the endometrium in women with adenomyosis, depending on their fertility. **Material and methods.** A comparative immunohistochemical (IGC) analysis of 102 eutopic endometrial specimens from women of reproductive age with adenomyosis and infertility (group I) was performed; from 20 nonpregnant women with adenomyosis who had a history of desired pregnancy and ended in childbirth and did not record episodes of miscarriages (group II), as well as from 30 conditionally gynecologically healthy women who applied for the introduction of an intrauterine system for contraception (control group C). A biopsy of the eutopic endometrium was performed on P+6 day of the menstrual cycle, during the period of the expected implantation window, with the help of aspiration pipel-curette. In immunohistochemical (IGC) determination of the proteomic profile of the endometrium HOXA10 was stained with monoclonal antibodies (MAB) to HOXA10 (sc-17159, Santa Cruz Biotechnology, USA); LIF - murine MAB against LIF (J-14F: SC-80159, Santa Cruz

Biotechnology Inc., USA); sgp130 - goat polyclonal antibodies (PAB) anti-gp130 (BAF 228, supplied 50 μg / ml, R&D Systems); IL-6 - primary rabbit antibodies (code No CXH1-066LS, supplied as rabbit IgG 20 μg / ml; Cambridge Bioscience, UK). **Results.** It was found that important factors of reproductive disorders in women with adenomyosis are: changes in the proteomic profile of the eutopic endometrium on the day of the expected implant window, such as a decrease in HOXA 10 expression in the stroma by 1.52 ($p<0.01$) and in the glands by 1.46 times ($p<0.01$), LIF in glands - in 1.12 ($p<0.01$) and sgp130 - in 2.52 ($p<0.01$), as well as increased secretion in glands of IL-6 in 1,99 ($p<0.01$) times. In fertile women with adenomyosis, less pronounced changes in the proteomic profile of the eutopic endometrium are observed, which creates the conditions for successful realization of reproductive intentions, including a decrease in expression of HOXA 10 in the stroma by 1.48 times ($p<0.01$), in the glands - in 1.12 ($p>0.05$); secretion in glands of LIF - in 1.06 ($p>0.05$) and gp130 - in 1.98 ($p<0.01$); increased IL-6 1.08 times ($p<0.05$). **Conclusions.** Changes in the proteomic profile of the endometrium play an important role in the occurrence of reproductive disorders in adenomyosis.

Key words: adenomyosis; endometrium; infertility; proteomic profile; HOXA10; Leukemia inhibitory factor; glycoprotein 130; interleukin-6.

Adenomyosis, one of the main varieties of genital endometriosis, occupies a significant place in the structure of gynecological morbidity and presents an urgent problem of modern gynecology and obstetrics [6, 10, 15]. Genital endometriosis affects every tenth woman in reproductive age, and the prevalence of adenomyosis in patients with endometriosis ranges from 20 to 70 % [3, 9]. However, despite this, previously existing studies, due to the difficulty of clinical and instrumental diagnosis of adenomyosis, paid little attention to its impact on the course and outcomes of pregnancy, as well as the development of methods for pre-conceptual preparation and management of pregnant women with adenomyosis.

In recent years, several studies have reported a correlation between adenomyosis and major obstetric adverse effects such as miscarriage, premature rupture of the membranes, and premature birth, gestational hypertension, pre-eclampsia, preeclampsia, preeclampsia, gestational diabetes, obstetric bleeding, placental presentation [1, 2, 4, 7, 8].

An important issue is to elucidate the pathogenetic mechanisms that lead to reproductive disorders in adenomyosis. Implantation failures can be caused by changes in the expression of some molecules, referred to as "implantation markers", which are expressed by the endometrium and are required for successful interaction between the embryo and

endometrium [13]. During the implantation window, the expression of some of these markers changes in the eutopic endometrium of women with adenomyosis, suggesting that changes in the proteomic profile of the endometrium may disrupt pregnancy progression.

Therefore, **the purpose of the study** was to determine the features of the proteomic profile of the endometrium in women with adenomyosis, depending on their fertility.

Material and methods

A comparative immunohistochemical (IGC) analysis of 102 eutopic endometrial tube specimens from women of reproductive age with adenomyosis and infertility (group I) was performed; from 20 nonpregnant women with adenomyosis who had a history of desired pregnancy and ended in childbirth and did not record episodes of miscarriage (group II), as well as from 30 conditionally gynecologically healthy women who applied for the introduction of an intrauterine system for contraception (control group C).

A biopsy of the eutopic endometrium was performed on P+6 day of the menstrual cycle, during the period of the expected implantation window, with the help of aspiration pipel-curette. When evaluating the features of the proteomic profile of the eutopic endometrium, expression on the day of the expected window of implantation of the homeobox gene-10 (HOXA10), leukemia inhibitory factor (LIF), soluble glycoprotein 130 (gp130), interleukin-6 (IL-6) (6) was studied. The obtained endometrial samples were placed in neutral buffered 10 % formalin solution (pH 7.4) and fixed for 24 hours. After dehydration, the pieces were poured into paraffin. On a rotary microtome Microm HM325 with STS slice transfer system (Carl Zeiss, Germany), serial histologic sections of 4 μm thickness were made, which were then stained with hematoxylin and eosin.

For further immunohistochemical (IHC) studies, part of the serial paraffin sections were placed on an adhesive coated Super Frost Plus glass (Menzel, Germany).

IHC immunostaining of HOXA10 in the endometrium was performed using monoclonal antibodies (MAB) to HOXA10 (sc-17159, Santa Cruz Biotechnology, USA). In determining HOXA10 normal goat IgG (Santa Cruz Biotechnology, USA) was used as a negative control. Biotinylated secondary α-goat antibodies from Vector Laboratories (UK) were applied for 1 hour at 4°C. The slides were washed with PBS, incubated in avidin-biotin-peroxidase complex (Vector Laboratories, UK) for 15 min. at room temperature, washed in PBS and incubated for 5 min. in diaminobenzidine. A 20-second exposure to hematoxylin was used as the opposite. The drugs were rehydrated through ethanol for 3 min and washed with xylene.

The level of IHC immunostaining of LIF in the endometrium was determined using murine MAB against LIF (J-14F:SC-80159, Santa Cruz Biotechnology Inc., USA). ImmunoCruz™: sc-2050 or ABC: sc-2017 murine IgG staining systems were used as secondary reagents according to the manufacturer's instructions.

For IHC endometrial immunostaining of interleukin IL-6 primary rabbit antibodies (code No CXH1-066LS supplied as rabbit IgG 20 μg / ml; Cambridge Bioscience, UK) were used, which were diluted 1: 250 in 1 % BSA-PBS (bovine serum albumin + sodium phosphate buffer) and added to sections. The sections were then incubated for 60 min. at 37 °C in a humidified chamber. Rabbit IgG (20 mg / ml, DAKO Corp., Denmark) diluted to the same concentration was used as a negative control. The sections were treated identical to the immunoprotection of IL-6R described below.

Immunostaining of gp130 was performed with primary goat polyclonal antibodies (PAB) anti-gp130 (BAF 228, supplied 50 μg / ml, R&D Systems), which was added for 1 hour at a dilution of 1:20 in 5 % rabbit serum / 1 % - of BSA-PBS. The control antibody (I5256, goat IgG, Sigma) was added with the same concentration of protein to control slice color.

In the quantification of HOXA10 used the formula H-score = Σ Pi (i + 1), where the intensity of HOXA10 nuclear staining was 0, 1, 2 or 3 (none, weak, moderate, strong), and Pi - the percentage of colored nuclei for each intensity. When evaluating endometrial expression of LIF, sgp130, and IL-6 counted positively stained cells in three fields of view and calculated the percentage of positive cells relative to at least 1000 cellular elements of the stroma or glands.

The examination of histological specimens in light was performed on an Olympus AX70 microscope (Japan) with an Olympus DP50 digital video camera connected to a personal computer. Microphotography and morphometry were performed using ANALYSIS Pro 3.2 (SoftImaqinq, Germany).

Statistical analysis of the results of the study was carried out with the calculation of arithmetic mean (M), standard deviation error (± SE), to use the Student's t-test to compare parametric values.

Results and Discussion

The mean age of women in group I was 28.23 ± 0.28 years, group II was 29.15 ± 0.64, group CI was 27.50 ± 0.46 and was not statistically significant between the groups. The distribution of patients in the groups by mass-growth characteristics was also homogeneous:

body mass index in infertile women with adenomyosis was 22.29 ± 0.34 kg / m^2, in fertile persons with adenomyosis - 23.10 ± 0.85 kg / m^2, in the control group - 23,20 ± 0,38 kg / m^2.

The duration of infertility in group I was 5.15 ± 0.363 years.

An important factor that may be involved in impaired implantation in women with adenomyosis is the altered function of the HOXA-10 gene. This gene is part of the transcription factors containing homeobox required for embryonic development and proper growth of the endometrium in adulthood [17], and in women with adenomyosis its expression is much lower in the middle secretory phase compared to fertile control [5]. In the study, the immunostaining of HOXA10 in the eutopic endometrium was observed in both stroma and endometrial glands, it was predominant in stromal cells. In pregnant women of reproductive age with adenomyosis and reproductive disorders, the expression of HOXA10 in the stroma was 434.64 ± 9.42 % and in the glands –171.51 ± 4.36 %; in pregnant women with adenomyosis, in whom the desired pregnancy ended in childbirth and had no miscarriages, - 446,13 ± 21,88 and 222,94 ± 11,67 %, as well as in conditionally gynecologically healthy pregnant women without adenomyosis, who have a history of the desired pregnancy ended with childbirth - 660.27 ± 11.54 and 250.61 ± 6.92 % (Fig. 1).

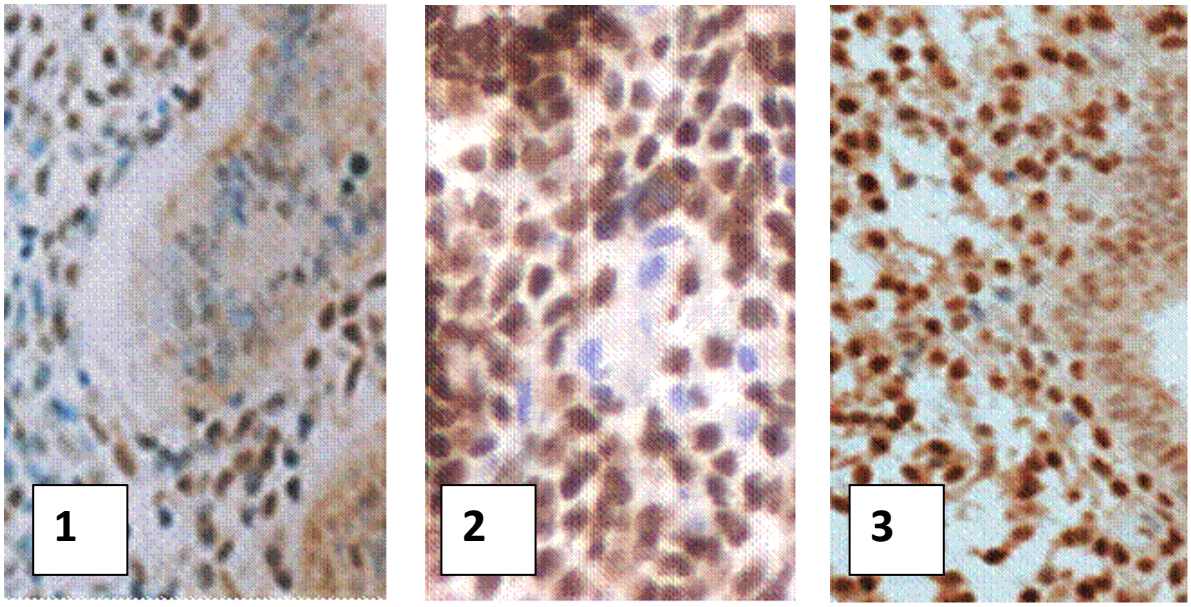

Fig. 1 HOXA10 expression in eutopic endometrium at P + 6 day: 1 - in infertile patients with adenomyosis; 2 - in fertile persons with adenomyosis; 3 - in women of the control group. IGC from MAB to HOXA10, magnification × 150.

HOXA10 expression in eutopic endometrial stroma in infertile women with 1.52 ($p<0.01$) adenomyosis and 1.88-fold in fertile women with adenomyosis was less than that in control women, whereas in the glands it was statistically significantly less by 1.46 times ($p<0.01$) only in group I, and in group II it was less by 1.12 times, but it is unbelievable.

The results obtained are in agreement with those of C.P. Fischer et al. (2011) that in women with adenomyosis, HOXA10 expression is significantly lower in the middle secretory phase compared to the fertile control [5].

LIF is associated with endometrial susceptibility and lower in women with infertility compared to healthy controls. A. Winship et al. (2015) in mouse experiments found that LIF plays an important role in trophoblast invasion in vivo and can facilitate cross-linking between trophoblast and deciduous immune cells to provide adequate remodeling of the spiral arteries [16]. Immunostaining of LIF was observed in the cytoplasm of endometrial glands with predominant localization in the apical part, in the superficial epithelium, slightly in the stroma. Only epithelial LIF expression in the endometrium was evaluated in the study. Hscore LIF in eutopic endometrium of persons with infertility and adenomyosis was 250.60 ± 3.69 %, in fertile women with adenomyosis - 264.49 ± 8.97 %, in control - 280.81 ± 3.53 % (Fig. 2).

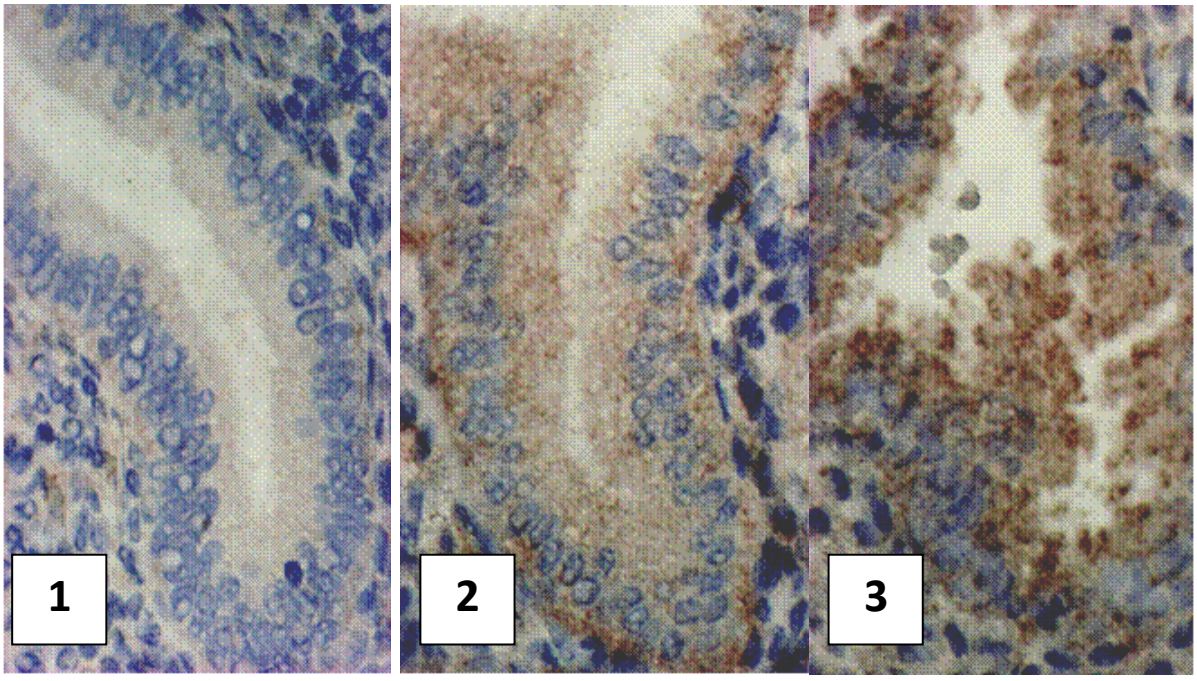

Fig. 2 Expression of LIF in eutopic endometrium at P + 6 day: 1 - in infertile patients with adenomyosis; 2 - in fertile persons with adenomyosis; 3 - in women of the control group. IHC from MAB to LIF, magnification × 300.

Expression of LIF in eutopic endometrium in infertile women with adenomyosis relative to the control group was reduced by 1.12 ($p<0.01$) times, respectively, whereas in the group of fertile patients was not significantly different ($p> 0.05$). C.F. Yen et al. (2017) also showed a significant decrease in LIF receptor expression and a decrease in subsequent signaling activation, strongly suggesting a working model of how implantation markers, including LIF, can affect the endometrium of patients with adenomyosis. These molecular changes support the reduced rate of implantation reported in patients with adenomyosis [18].

Expression in the eutopic endometrium of gp130, the LIF receptor and IL-6, was mainly expressed in the cytoplasm of gland epithelial cells and was also found in vascular endothelium. Its Hscore was equal in group I 311.52 ± 4.67 %, in group II - 397.42 ± 13.48 % and in group C - 786.27 ± 9.88 % (Fig. 3).

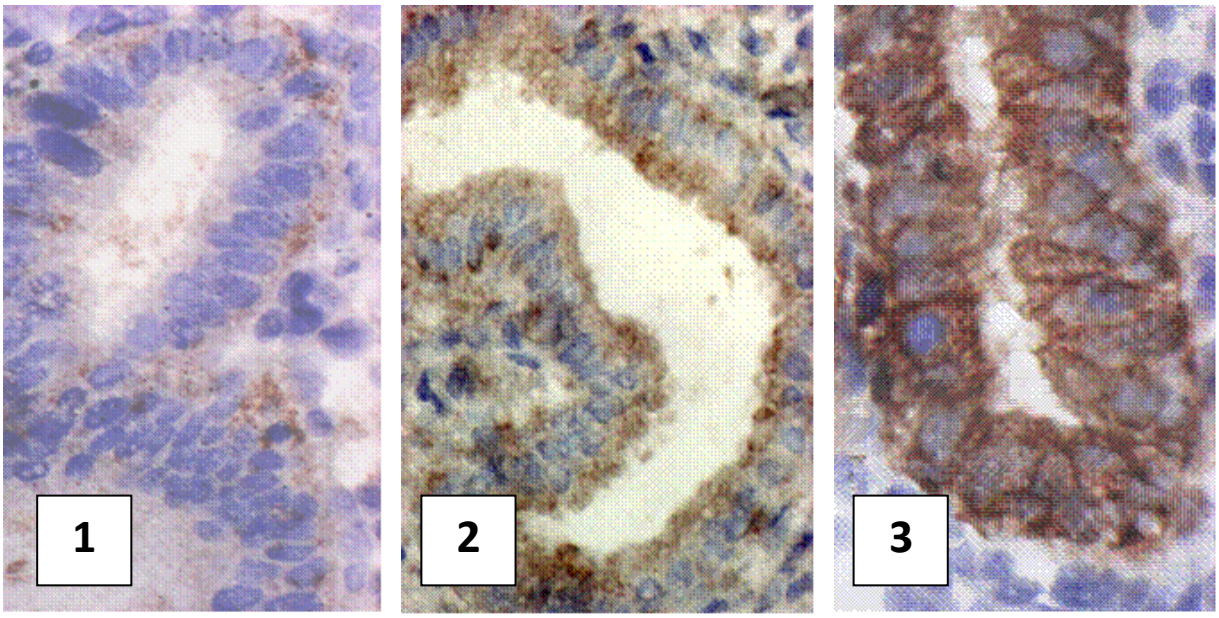

Fig. 3 Expression of gp130 in eutopic endometrium on P+6 day: 1 - in infertile patients with adenomyosis; 2 - in fertile persons with adenomyosis; 3 - in women of the control group. IHC with PAB against gp130, magnification × 300 (1, 2), × 400 (3).

Expression of gp130 in the eutopic endometrium of infertile patients with adenomyosis was reduced relative to the same indicator of the control group by 2.52 times ($p<0.01$) and in fertile patients with adenomyosis - by 1.98 ($p<0.01$). A characteristic feature was a more pronounced decrease in the expression of gp130 in women with adenomyosis and infertility at 1.28 ($p<0.01$) than in fertile individuals with adenomyosis.

According to H. Pitman et al. (2013), altered expression of IL-6 and its receptors is observed in various cell types in the placental bed (stromal myometrium, glandular epithelium, interstitial extravillus trophoblastic cells, vascular smooth muscle cells and endothelial cells) in spontaneous 14 innocence. The expression of IL-6 in the study was the most intense in the cells of the glandular and superficial epithelium, so the work evaluated the epithelial staining of IL-6.

The most pronounced IHC staining of cytoplasm of the glandular epithelium of MAB against IL-6 was observed in infertile women with adenomyosis (447.15 ± 3.47 %), which was higher than in fertile persons with adenomyosis (241.68 ± 7.37 %) in 1.85 times ($p<0.01$) and women in the control group (224.66 ± 3.47 %) - 1.99 ($p<0.01$). IL-6 expression in the eutopic endometrium was not statistically different between groups II and C (Fig. 4).

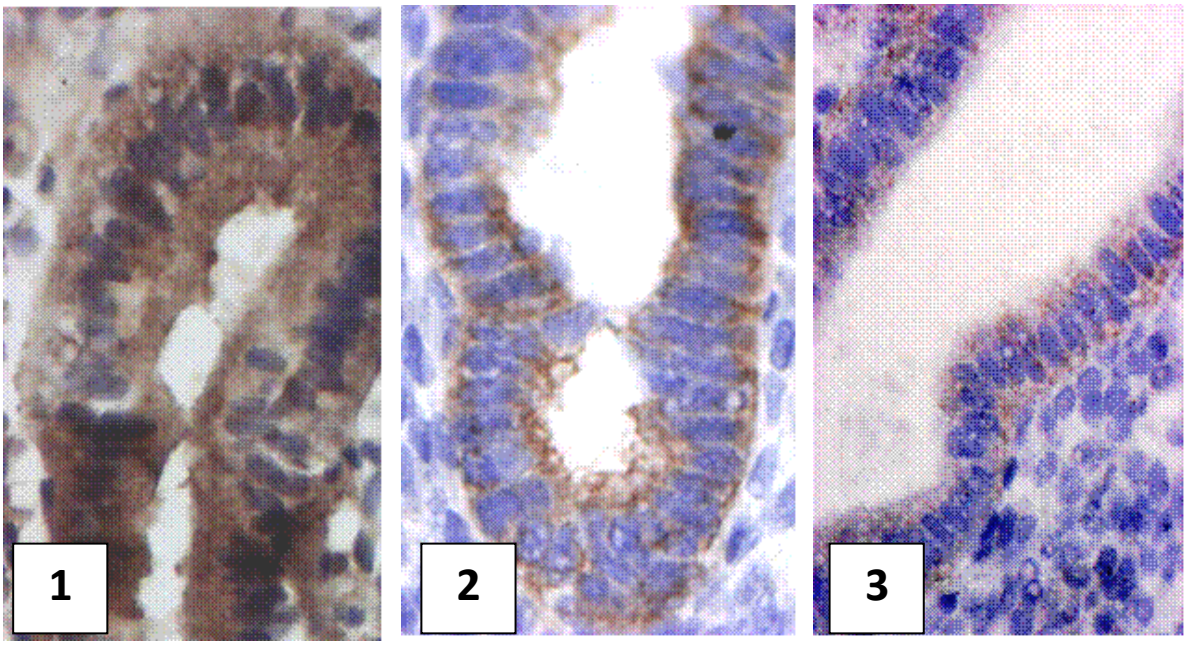

Fig. 4 Expression of IL-6 in eutopic endometrium on P + 6 day: 1 - in infertile patients with adenomyosis; 2 - in fertile persons with adenomyosis; 3 - in women of the control group. IHC with MAB against IL-6, magnification ×400 (1, 2), ×300 (3).

Conclusions

One factor in reproductive disorders in women with adenomyosis and infertility is the change in the proteomic profile of the eutopic endometrium on the day of the expected implant window, which includes a decrease in HOXA 10 expression compared to stroma control of 1.52 ($p<0.01$) and glands in 1,46-fold ($p<0.01$); a decrease in LIF expression glands of 1.12

($p<0.01$) and gp130 - 2.52-fold ($p<0.01$), and an increase in IL-6 gland secretion of 1.99 ($p<0,01$) times.

In fertile women with adenomyosis, less pronounced changes in the proteomic profile of the eutopic endometrium are observed, which creates the conditions for successful realization of reproductive intentions, including a decrease in expression of HOXA 10 in the stroma by 1.48 times ($p<0.01$), in the glands - in 1.12 ($p>0.05$); secretion in glands of LIF - in 1.06 ($p>0.05$) and gp130 - in 1.98 ($p<0.01$); increased IL-6 1.08 times ($p<0.05$).

Changes in the proteomic profile of the endometrium play an important role in the occurrence of reproductive disorders in adenomyosis.

References

1. Berlac JF, Hartwell D, Skovlund CW, Langhoff-Roos J, Lidegaard O. Endometriosis increases the risk of obstetrical and neonatal complications. Acta Obstet Gynecol Scand. 2017;96(6):751-760. doi: 10.1111/aogs.13111.

2. Conti N, Cevenini G, Vannuccini S, Orlandini C, Valensise H, Gervasi MT, et al. Women with endometriosis at first pregnancy have an increased risk of adverse obstetric outcome. J Matern Fetal Neonatal Med. 2015;28(15):1795-8. doi: 10.3109/14767058.2014.968843.

3. Di Donato N, Montanari G, Benfenati A, Leonardi D, Bertoldo V, Monti G, et al. Prevalence of adenomyosis in women undergoing surgery for endometriosis. Eur J Obstet Gynecol Reprod Biol. 2014; 181: 289-293. doi: 10.1016/j.ejogrb.2014.08.016.

4. Exacoustos C, Lauriola I, Lazzeri L, De Felice G, Zupi E. Complications during pregnancy and delivery in women with untreated rectovaginal deep infiltrating endometriosis. Fertil Steril. 2016; 106: 1129-1135. e1. doi: 10.1016/j.fertnstert.2016.06.024.

5. Fischer CP, Kayisili U, Taylor HS. HOXA10 expression is decreased in endometrium of women with adenomyosis. Fertil Steril. 2011 Mar 1;95(3):1133-6. doi: 10.1016/j.fertnstert.2010.09.060.

6. Gladchuk IZ, Rozhkovskaya NN, Garbuzenko ND, Stamova NA. Age characteristics of uterine motility in infertile women with adenomyosis, uterine fibroids and their combination. The health of woman. 2016;3(109):149–152.

7. Glavind MT, Forman A, Arendt LH, Nielsen K, Henriksen TB. Endometriosis and pregnancy complications: a Danish cohort study. Fertil Steril. 2017;107(1):160-166. doi: 10.1016/j.fertnstert.2016.09.020.

8. Harada T, Taniguchi F, Onishi K, Kurozawa Y, Hayashi K, Harada T, et al. Obstetrical complications in women with endometriosis: a cohort study in Japan. PLoS One. 2016; 11(12):e0168476. doi: 10.1371/journal.pone.0168476.

9. Hashimoto A, Iriyama T, Sayama S, Nakayama T, Komatsu A, Miyauchi A, et al. Adenomyosis and adverse perinatal outcomes: increased risk of second trimester miscarriage, preeclampsia, and placental malposition. J Matern Fetal Neonatal Med. 2018;31(3):364-369. doi: 10.1080/14767058.2017.1285895.

10. Kaminsky BB, Prokopovich EV. An alternative to hormonal and non-steroidal anti-inflammatory drugs in the treatment of secondary dysmenorrhea caused by adenomyosis. The health of woman. 2018; 4: 66-70.

11. Kutsenko II, Kravtsova EI, Simovonik AN, Rudeeva OA. Ultrasound diagnosis of adenomyosis I degree of spread. Modern problems of science and education. 2017: 6. URL: http://www.science-education.ru/en/article/ view? Id = 27350.

12. Mannini L, Sorbi F, Noci I, Ghizzoni V, Perelli F, Di Tommaso M, et al. New adverse obstetrics outcomes associated with endometriosis: a retrospective cohort study. Arch Gynecol Obstet. 2017; 295: 141-151.

13. Mikolajczyk M, Wirstlein P, Skrzypczak J. Leukaemia inhibitory factor and interleukin-11 levels in uterine flushings of infertile patients with endometriosis. Hum Reprod. 2006 Dec;21(12):3054-8.

14. Pitman H, Innes BA, Robson SC, Bulmer JN, Lash GE. Altered expression of interleukin-6, interleukin-8 and their receptors in decidua of women with sporadic miscarriage. Hum Reprod. 2013;28(8):2075-86. doi: 10.1093/humrep/det233.

15. Vdovichenko JuP, Gnip IP, Vorobiy VD. Optimization of diagnostics and endoscopic treatment of adenomyosis and disorders of reproductive function. Woman's health. 2015; 6:149–151.

16. Winship A, Correia J, Zhang JG Nicola NA, Dimitriadis E. Leukemia Inhibitory Factor (LIF) Inhibition during Mid-Gestation Impairs Trophoblast Invasion and Spiral Artery Remodeling during Pregnancy in Mice. PLoS One. 2015;10(10):e0129110. doi: 10.1371/journal.pone.0129110.

17. Xu B, Geerts D, Bu Z, Ai J, Jin L, Li Y, et al. Regulation of endometrial receptivity by the highly expressed HOXA9, HOXA11 and HOXA10 HOX-class homeobox genes. Hum Reprod. 2014;29(4):781-90. doi: 10.1093/humrep/deu004.

18. Yen CF, Liao SK, Huang SJ, Tabak S, Arcuri F, Lee CL, et al. Decreased Endometrial Expression of Leukemia Inhibitory Factor Receptor Disrupts the STAT3

Signaling in Adenomyosis During the Implantation Window. Reprod Sci. 2017;24(8):1176-1186. doi: 10.1177/1933719116681515.