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MORPHOFUNCTIONAL FEATURES OF ENDOMETRIUM IN WOMEN WITH COMBINATION OF MALE AND FEMALE FACTORS

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Abstract

Treatment couples with a combination of male and female factor infertility is the most difficult and often requires precisely ART. However, ICSI efficacy currently does not exceed 35-40 %, about a third of all pregnancies are terminated early. **The purpose of the study** was to determine the morphofunctional features of endometrium in women from married couples with a combination of female and male factors of infertility. **Material and methods.** There were 100 married couples with prospective follow-up with a combination of male and female infertility factors of group A who had applied for assisted reproductive technologies (ART). All men in group A suffered from pathozoospermia. Control group C consisted of 34 couples with secondary infertility of tube genesis, who consisted of conditionally somatically healthy women with a history of childbirth, without fallopian tubes, and their men with normozoospermia. Group A was stratified each into 3 subgroups. The A1 subgroup (n = 32) included married couples who had chronic anovulation; in subgroup A2 (n = 37) - married couples who had tubal infertility; in subgroup A3 (n = 31) - married couples in which women had a combination of tubal and anovulatory factors of infertility. Material for morphological examination were endometrial pipel-biopsy specimens on day 21-22th of the menstrual cycle on the day of the expected implantation window. It were determined expression of HOXA10, Leukemia inhibitory factor (LIF), glycoprotein 130 (gp130), interleukin-6 (IL-6) in endometrium. **Results:** morphofunctional status of women's endometrium with a combination

of female and male infertility factors was characterized by an increase in estrogen-α receptor IRS in the glands and endometrial stroma, respectively, 1.86 ($p < 0.01$) and 1.37 ($p<0.01$) times, respectively. expression of P_4 receptors in endometrial glands 1.48 times ($p<0.01$) against the background of their decrease in stroma by 1.39 ($p<0.01$); increase in infiltration of endometrial stromal CD56+ - positive natural killer cells by 10.22 times ($p<0.01$) and presence of CD138+ immunopositive cell cells in 66.00 % of cases. Patients with a combination of male and female infertility factors have individual changes in endometrial morphofunctional features during the intended implantation window, one only that should be considered when determining the tactics of treating women with different male forms. **Conclusions.** Changes in the proteomic profile of the endometrium play an important role in the occurrence of reproductive disorders and are individual depending on the combination of various of female and male factors of infertility.

Key words*:* adenomyosis; endometrium; infertility; proteomic profile; estrogen-α receptor; progesterone receptor; HOXA10; Leukemia inhibitory factor; glycoprotein 130; interleukin-6.

The combination of female and male infertility factors occupies the first place in the structure of factors in the initiated cycles of assisted reproductive technologies (ART) in Ukraine and is 35.18 % [10]. Treatment couples with a combination of male and female factor infertility is the most difficult and often requires precisely ART. Given the often low sperm count in these cases, intracytoplasmic sperm injection is widely used in cycles (ICSI). However, ICSI efficacy currently does not exceed 35-40 %, about a third of all pregnancies are terminated early [8].

Improvement of pre-conceptual training, including restoration of morphofunctional status and endometrial receptivity, may be a reserve for improving the efficacy of ART in married couples with the presence of female and male infertility factors. All forms of female infertility, such as: tubal-peritoneal, endocrine and unexplained etiology, are associated with decreased endometrial receptivity, which has been proven in numerous studies. [10].

Not only does semen in contact with the female reproductive tract contribute to the survival and fertilizing ability of sperm, it also contains potent signaling agents that affect female reproductive physiology, improve the chances of fertilization and reproductive success by providing synthetic cytokines. additional glands. These agents bind to receptors on target

cells in the cervix and uterine body, activating changes in gene expression leading to functional adaptation in female tissues [4].

The effect of pathozoospermia on endometrial receptivity in women with infertility has been poorly understood. Studies using a mouse model have demonstrated the importance of cytokines present in sperm. In particular, the role of familial transforming growth factor-β [9] in interacting with uterine epithelial cells to induce the expression of an array of proinflammatory cytokines has been described, leading to a classic inflammatory response that ends with the entry of leukocytes into endometrial tissues [1]. Most recently, these studies were conducted in humans after intercourse [3, 6, 7]. Inflammatory phenomena that are known to occur during insemination may also have a further effect on the immunoendocrine processes important for embryo development, implantation, and trophoblast invasion [2, 5]. Thus, significant changes in the environment of the endometrial cytokines, as a result of exposure to the family fluid, may contribute to the improvement of embryo development prior to implantation and the conditions in the uterine mucosa required for the opposition, attachment, and implantation of the embryo. These early steps in establishing pregnancy are critical to the success of pregnancy, but not well understood when combining male and female infertility factors.

The purpose was to determine the morphofunctional features of endometrium in women from married couples with a combination of female and male factors of infertility.

Material and methods

There were 100 married couples with prospective follow-up with a combination of male and female infertility factors of group A who had applied for ART. All men in group A suffered from pathozoospermia. Control group C consisted of 34 couples with secondary infertility of tube genesis, who consisted of conditionally somatically healthy women with a history of childbirth, without fallopian tubes, and their men with normozoospermia. Group A was stratified each into 3 subgroups. The A1 subgroup (n = 32) included married couples who had chronic anovulation; in subgroup A2 (n = 37) - married couples who had tubal infertility; in subgroup A3 (n = 31) - married couples in which women had a combination of tubal and anovulatory factors of infertility.

Criteria for inclusion in group A: ages 22 to 38; the presence of a combination of male and female infertility factors; pathozoospermia; the need for treatment of infertility by the methods of ART; women are good respondents. Exclusion criteria from group A: genital defects, genital endometriosis, diabetes mellitus, HIV infection, thyrotoxicosis, malignancies, acute pelvic infectious process, azoospermia.

The examination of married couples included an objective examination (somatic and gynecological) according to the standard method, in compliance with the requirements of the protocol on infertility treatment, approved by the order of the Ministry of Health of Ukraine. Material for morphological examination were endometrial pipel-biopsy specimens on day 21-22th of the menstrual cycle on the day of the expected implantation window. 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 embedded in paraffin according to the standard procedure, serial histological sections were made with a thickness of 4 μm, which was then stained with hematoxylin and eosin according to the standard method.

The study of steroid hormone receptors in the glands and endometrial stroma was performed by immunohistochemical (IHC) method using test systems "Rakocytomation En Vision" (USA), horseradish peroxidase according to the firm's instructions. Mouse monoclonal antibodies (MAB) to estrogen-α receptors (clone 1D5, DAKO, Denmark), progesterone (P_4) (clone 16 & SAN27, Novocastra, UK) were used. For visualization of the histological structure of the visualization of the immunohistochemical preparation, the hematoxylin Mayer was painted over and they were taken into the Canadian balm.

For evaluating the expression of receptors of estrogen-α and that of receptor P_4 in the endometrium, the index of immunoreactive score is given by the formula: IRS = SI × PP, where the IRS is the immunoreactive score index, SI is the optical staining intensity, and PP is the percentage of positively stained nuclei.

To determine the uterine natural killer cells of CD56+, CD138+, the obtained endometrial sections were placed on an adhesive coated glass Super Frost Plus (Menzel, Germany). To "unmask" the antigens, the rehydrated sections were heat-treated in Target Retrieval Solution (DAKO, Denmark) using a Samsung CE118KFR microwave oven (South Korea). After blocking non-specific protein binding by protein block (DAKO, Denmark) and endogenous peroxidase activity peroxidase block (DAKO, Denmark) was applied primary antibodies. Used MAB to CD56+ (clone 123C3.D5, Diagnostic BioSystems, USA), to CD138+ (clone MI15, Dako, Denmark). Imaging of the primary antibodies was performed using the DAKO Advance high-sensitivity polymer detection system. DAB + (DAKO, Denmark) was used as the horseradish peroxidase substrate. The preparations were stained with Mayer's hematoxylin. The painted sections were then embedded in a semi-synthetic Permanent Mounting Medium (DAKO, Denmark). Positively stained cells were counted in three fields of view of at least 1,000 stromal cell elements. Preparation microscopy and all morphometric studies were performed on an Olympus AX70 Provis microscope (Olympus,

Japan) using the Analysis 3.2 Pro (Soft Imaging, Germany) image analysis software, as recommended by the software manufacturer.

In statistical processing of the study materials, the parameters of the general population were estimated according to the sample data; determined the mean M and the standard deviation error SE; the eligibility of the hypotheses was determined by statistical criteria: the t-test was used to compare the mean of the independent samples and the related (dependent) samples; χ^2-criterion - for analysis of conjugation of signs, comparison of frequencies of events; correlation analysis - to study stochastic dependence between indicators. The odds ratio (OR) was determined by the odds ratio of the event in the comparison groups, and 95 % confidence interval (CI) was calculated.

Results and Discussion

The mean age of the patients in group I was 30.67 ± 0.30 years, in the control - 31.12±0.58. Women aged 22-25 years met respectively in 8.92 and 7.00 % of cases, 26-30 years - in 42.00 %, 31-35 years - in 45.00 %, 36-38 years - in 6,00 %. The mean body mass index in group A was 24.47 ± 0.39 versus 22.75±0.50 kg/m^2 in group C, p<0.03. In patients of subgroup A1 with the presence of anovulatory and male factors infertility mass and body mass index (70,22±1,09 kg and 25,92±0,38 kg/m^2), as well as subgroup A3 with the combination of anovulatory, tubular and male factor infertility (67.94±1.67 kg and 25.17±0.59 kg/m^2) statistically significantly exceeded the corresponding values in patients with tubal and male factor of infertility in the A2 subgroup (61.81±2.01 kg and 22,63±0.79 kg/m^2) and control women (60.41±1.32 kg and 22.75±0.50 kg/m^2).

When applying to the ART clinic, 78.00 % of the group A subjects had primary infertility, 22.00 % had secondary infertility. The highest incidence of primary infertility occurred in women with anovulatory and male infertility factors - in 93.75 % of cases. The incidence of primary infertility in subgroup A1 was higher than that in subgroup A2 (65.57 %) by 1.43 times (p<0.01; OR 7.20; 95 % CI 1.47-35.25) and above in the subgroup A3 (74.19 %) - by 1.26 (p<0.03; OR 5.22; 95 % CI 1.01-26.95). The mean duration of infertility was 4.27±0.29 years in group A, 3.16±0.34 years in subgroup A1 (p_{a1-a2}<0,02, p_{a1-a3}<0,05), A2 – 6,30±0,51 (p_{a2-a3}<0,04), subgroup A3 – 3,94±0,33 years.

When studying the morphofunctional features of endometrium in women with a combination of female and male infertility factors, the expression of steroid hormone receptors, natural killer cells of CD56+ and a marker of inflammation of CD138+ were evaluated. The study showed that the IRS of estrogen-α receptors in the glands and endometrial stroma of women in group A (77.72±4.0 and 84.40±3.29 conventional units)

exceeded that in group C (41.89±0,75 and 61.48±0.87 conventional units) respectively 1.86 ($p<0.01$) and 1.1.37 ($p<0.01$) times (Table 1).

Table 1

Average IRS of steroid receptors (in conventional units) in endometrium, M±SE, in conventional units

Group	IRS estrogens-α receptors		IRS P_4 receptors	
	in the glands	in the stroma	in the glands	in the stroma
A, n=100	77,72±4,0 [c]	84,40±3,29 [c]	117,56±3,91 [c]	109,60±3,54 [c]
A1, n=32	86,29±7,48 [a2,c]	87,97±4,20 [c]	131,76±7,18 [a2,c]	101,31±5,67 [a2,c]
A2, n=37	65,02±6,36 [a1,a3,c]	78,61±5,83 [c]	102,81±5,44 [a1,a3,c]	124,46±6,21 [a1,a3,c]
A3, n=31	84,04±7,07 [a1,c]	87,63±4,20 [c]	120,51±7,18 [a1,a2,c]	100,43±5,49 [a1,a2,c]
C, n=34	41,89±0,75	61,48±0,87	79,61±1,11	152,56±1,57

Note. [c, a1, a2, a3] – statistically significant difference with indicators of groups C and subgroups A1, A2, A3 ($p<0,05$).

Regarding the expression of P4 receptors in the endometrium of patients with a combination of female and male infertility factors, in the glands, it exceeded that of women in the control group by 1.48 times (117.56±3.91 vs. 79.61±1.11 conventional units, $p<0.01$), and in the stroma, on the contrary, it was less than 1.39 (109.60±3.54 vs. 152.56±1.57 conventional units, $p<0.01$) (see Table 1).

The IRS of estrogen-α receptors in the glands of women with tubal and male infertility factors in the A2 group (65.02±6.36 units) was significantly less than that in individuals with the combination of anovulatory and male infertility factors in the A1 group (86,29±7,48 conventional units) in 1,33 times, as well as in comparison with the similar content in women with combination of anovulatory tubular and male factors of infertility in group A3 (84,04±7,07 conventional units) - 1.29 times.

Progesterone receptor IRS in glands in women with combination tubal and male infertility factors in group A2 (102.81±5.44 conventional units) was significantly lower than in subjects with combination of anovulatory and male infertility factors in group A1 (131,76±7,18 conventional units), respectively, 1.28 times, as well as in comparison with similar content in women with combination of anovulatory tubular and male factors of infertility in group A3 (120,51±7,18 conventional units) - 1.17 times. At the same time, the P_4

receptor IRS is in patients with endometrial stroma. with the combination of tubal and male factors of infertility in the A2 group (124.46±6.21 conventional units) was significantly higher than in persons with the combination of anovulatory and male factors in the A1 group (101.31±5.67 conventional units) by 1.23 times, as well as in comparison with women with combination of anovulatory tubular and male factors of infertility in group A3 (100.43±5.49 conventional units) – by 1.24 times.

Expression of estrogen-α and P_4 receptors in the glands and in the stroma varied in women with a combination of female and male infertility factors, regardless of the subgroup from expressed (Fig. 1.1, Fig. 2.1), moderate (Fig. 1.2, Fig. 2.2) and to weak (Fig. 1.3 and Fig. 2.3). That is, the profile of steroid receptor expression in the glands and endometrial stroma was specific for each woman, which is unique to her, and should be taken into account when determining treatment tactics.

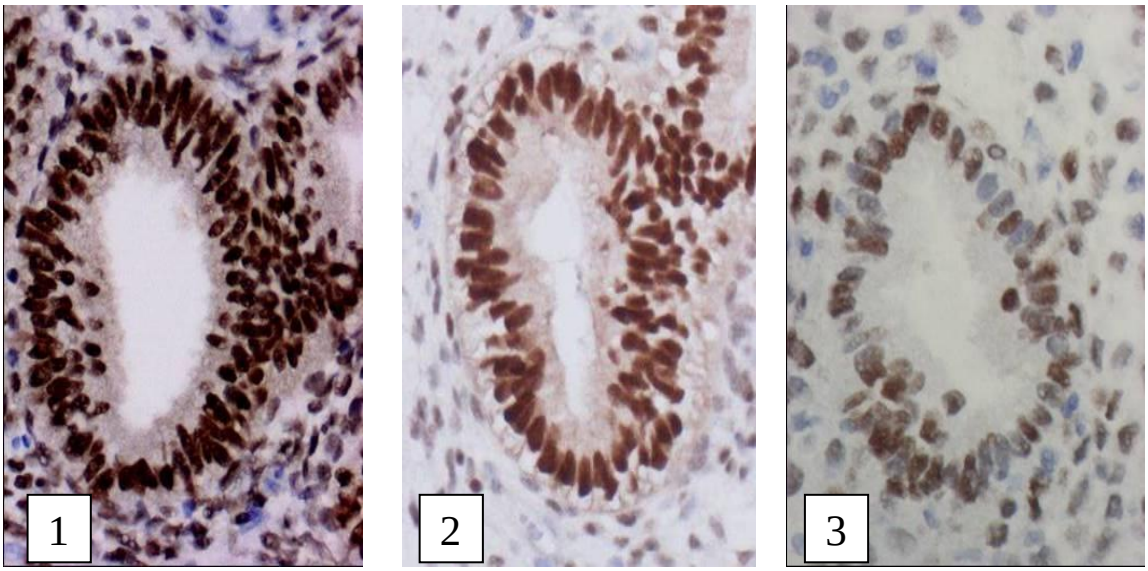

Fig. 1 Estrogen receptor-α expression in endometrial women with a combination of female and male infertility factors: 1 - expressed, 2 - moderate, 3 - weak. IHC from MAB to P_4.×250.

Analysis of the immune reactivity of the endometrium during the proposed implantation window showed that infiltration of the endometrial stroma of CD56+ - positive natural killer cells in patients with a combination of female and male infertility factors (55.27±1.93,4) was higher than similar to (5,41±0,40 ‰) in control 10.22 times ($p<0.01$) (Table 2).

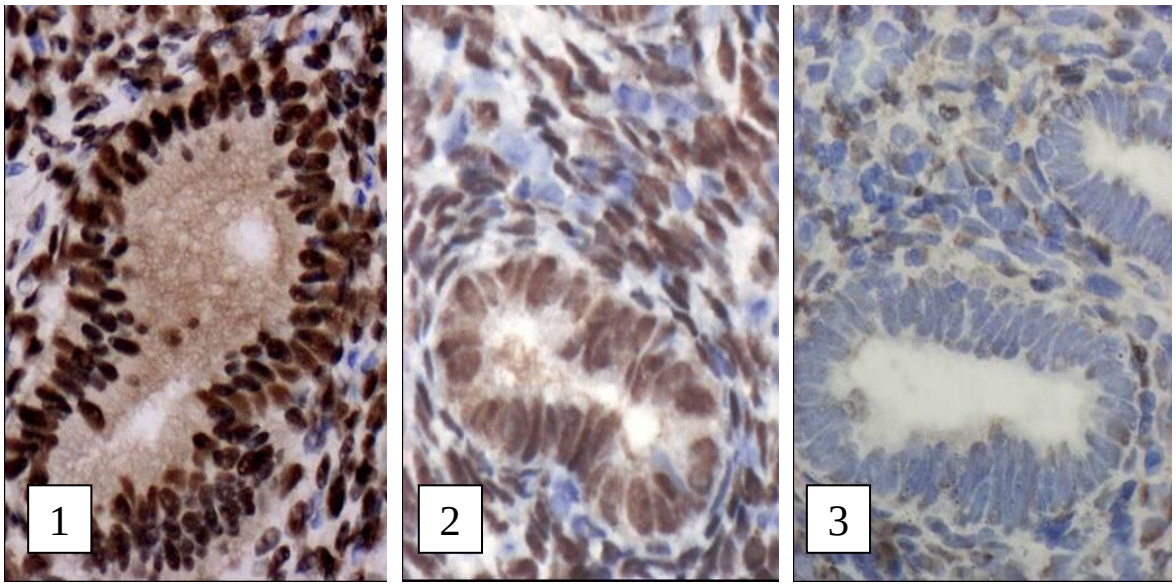

Fig. 2 P_4 receptor expression in endometrial women with a combination of female and male infertility factors: 1 - expressed, 2 - moderate, 3 - weak. IHC from MAB to P_4. ×250.

Table 2

The number of immunopositive cells to CD56+ in the stroma of the endometrium during the intended implantation window, M±SE, ‰

Group	Number of immunopositive cells up to CD56 +
A, n=100	55,27±1,93 ^{c}
A1, n=32	44,04±3,96 ^{a2,a3,c}
A2, n=37	54,17±2,34 ^{a1,a3,c}
A3, n=31	68,13±2,29 ^{a1,a2,c}
C, n=34	5,41±0,40

Note. $^{c,\ a1,\ a2,\ a3}$ – statistically significant difference with indicators of group C and subgroups A1, A2, A3 ($p<0,05$).

CD56+ endometrial stroma infiltration was lowest in women with a combination of anovulatory and male infertility factors, and highest in women with a combination of anovulatory, tubal, and male infertility factors. The number of immunopositive cells to CD56+ in group A1 (44.04±3.96 ‰) was significantly lower than in subjects with combination of tubular and male infertility factors in group A2 (54.17±2.34 ‰) by 1.23 times, as well as in comparison with similar content in women with combination of anovulatory, tubal and male factors of infertility in group A3 (68.13±2.29 ‰) - in 1,55 times. The immune

reactivity of the endometrial stroma varied within each group and subgroup from elevated (Fig. 3.1), moderate (Fig. 3.2) to weak (Fig. 3.3).

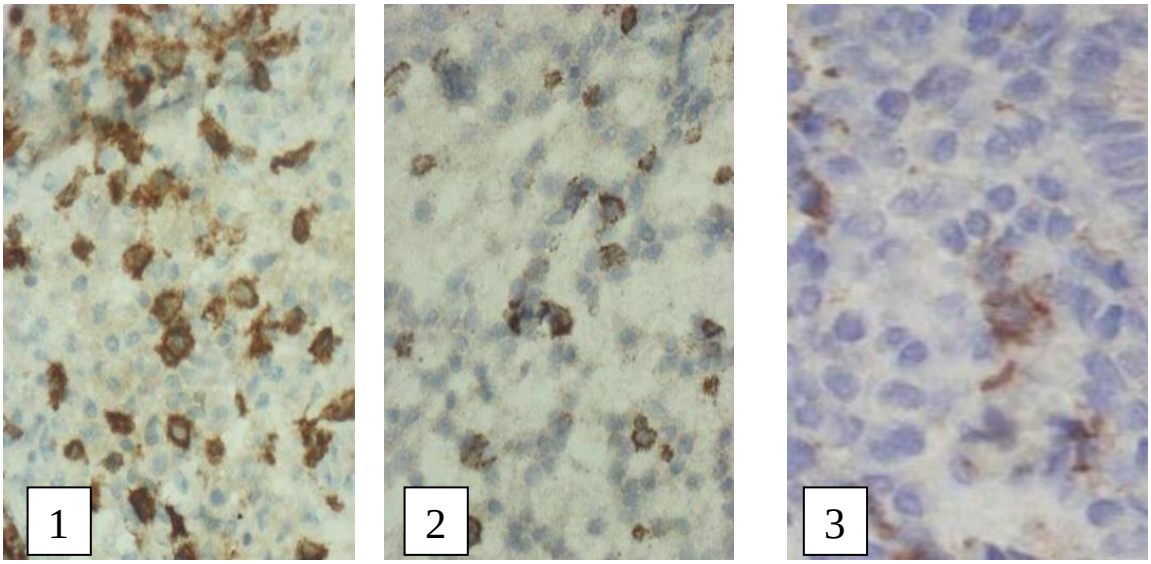

Fig. 3 Expression of CD56+ in endometrial stroma in patients with a combination of female and male infertility factors: 1 - expressed, 2 - moderate, 3 - weak. IGC from MAB to CD56+, × 300.

Chronic inflammation in the endometrium was indicated by the presence of plasmocytes - CD138+-immunopositive cells. The number of cases of their presence in women of group A was 66,00 % (Table 4).

Table 4

Presence and nature of CD138 + stroma infiltration in the endometrium during the intended implantation window, n(%)

Group	The number of immunopositive cells to CD138+	The absence of immunopositive cells to CD138+	Focal infiltration	Diffuse infiltration
A, n=100	66(66,00) ^{c}	34(34,00) ^{c}	35(35,00) ^{c}	31(31,00) ^{c}
A1, n=32	18(56,25) ^{c,a2}	14(43,75) ^{c,a2}	12(37,50) ^{c}	6(18,75) ^{c}
A2, n=37	29(78,38) ^{c,a1}	8(21,62) ^{c,a1}	15(40,54) ^{c}	14(37,84) ^{c}
A3, n=31	19(61,29) ^{c}	12(38,71) ^{c}	8(25,81) ^{c}	11(35,48) ^{c}
K, n=34	0(0,00)	34(34,00)	0(0,00)	0(0,00)

Note. $^{c,\ a1,\ a2,\ a3}$ – statistically significant difference with indicators of group C and subgroups A1, A2, A3 ($p<0,05$).

Most often, endometrial infiltration of CD138+ was recorded in the tubal infertility subgroup (78.38 %), whereas in the subunit with anovulatory infertility it was less common 1.39 times (56.25 %, OR 2.82, 95 % CI 1.00-8,04). Diffuse endometrial infiltration by plasmocytes was recorded in the A1 subgroup in 18.75 % of cases, in the A2 subgroup - in 34.27 %, in the subgroup A3 - in 35.48 %, and the focal respectively in 37.50 %, 40.54 %, 25.81 %. In each patient with the presence of a combination of female and male infertility, the presence and severity of inflammatory changes in the endometrium was special (Fig. 4.1-4.3).

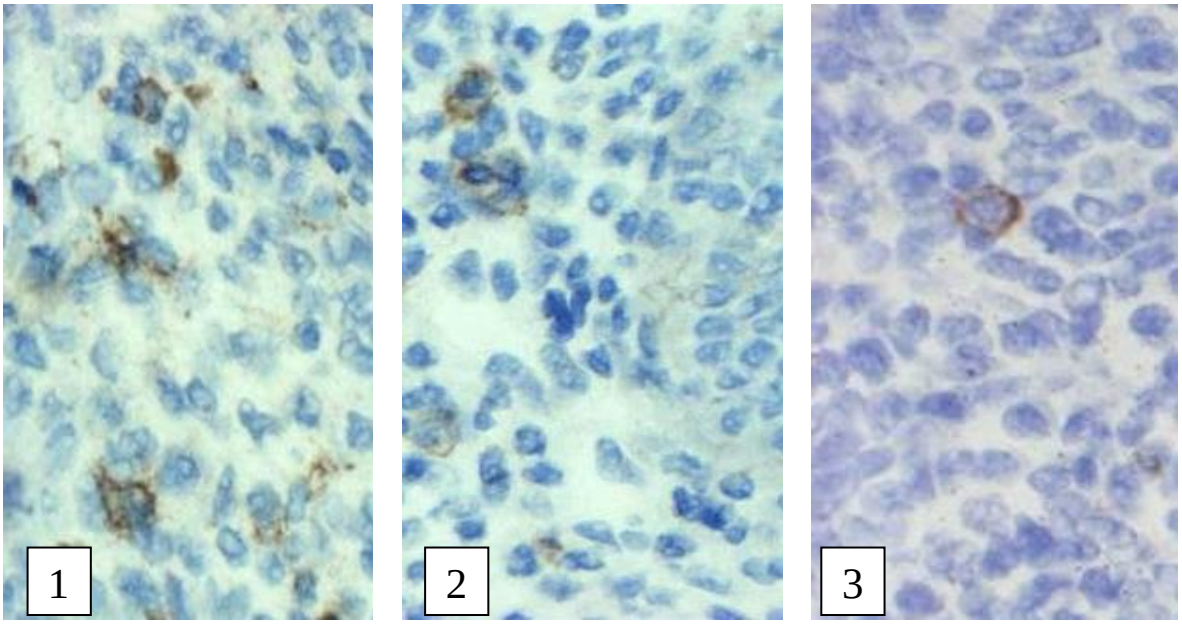

Fig. 4 Plasma cell endometrial infiltration in women with a combination of female and male infertility factors: 1 - severe, 2 - moderate, 3 - weak. IHC from MAB to CD138+. × 300.

Conclusions

Morphofunctional status of women's endometrium with a combination of female and male infertility factors was characterized by an increase in estrogen-α receptor IRS in the glands and endometrial stroma, respectively, 1.86 ($p < 0.01$) and 1.37 ($p < 0.01$) times, respectively. expression of P_4 receptors in endometrial glands 1.48 times ($p < 0.01$) against the background of their decrease in stroma by 1.39 ($p<0.01$); increase in infiltration of endometrial stromal CD56+ - positive natural killer cells by 10.22 times ($p<0.01$) and presence of CD138+ immunopositive cell cells in 66.00 % of cases. Patients with a combination of male and female infertility factors have individual changes in endometrial morphofunctional features during the intended implantation window, one only that should be considered when determining the tactics of treating women with different male forms.

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