The features of endometrial receptivity in women underwent in vitro fertilization procedure

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The objective: to determine the endometrial receptivity markers in women who underwent the procedure of in vitro fertilization (IVF).

Materials and methods. The study included 191 patients who applied to the reproductive department of Caspian International Hospital from 2020 to 2022 for an examination for infertility.

The main group included 50 patients with infertility who underwent endometrial biopsy during the implantation window (7th–9th days after ovulation in the cycle preceding the IVF stage); the control group – 20 women who underwent treatment for male infertility with a positive program result.

The patients underwent IVF according to the standard method. The following expression levels were determined: leukemia inhibiting factor (LIF); vascular endothelial growth factor (VEGF); transforming growth factor-β1 (TGF-β1). The percentage expression of area indices was estimated.

Results. In the women of the main group absence of embryo transfer was in 5 (10%) cases, no pregnancy – 27 (54%) cases, presence of pregnancy – 23 (46%) cases, cycle disruption – 3 (6%) cases.

According to the results of a histological examination in women of the control group, complete secretory transformation of the endometrium was determined in 85% of cases. In the main group complete secretory transformation of the endometrium was detected in 48%, defective secretory transformation – in 42%, morphological signs of endometrial changes corresponding to the average secretory phase of the cycle – in 10% of women.

A significant decrease (p<0.001) in the expression of LIF in the histological structures (stroma) of the endometrium was revealed in the main study group compared to the control one. The most pronounced differences were observed in the expression level of TGF-β1 in the stroma (approximately 10-fold; p<0.001).

Conclusions. Thus, infertile women who underwent IVF have multidirectional changes in endometrial receptivity markers: decrease in LIF expression in the stroma and epithelium, increase in VEGF expression in the stroma, and a significant increase in TGF-β1 expression in the stroma and epithelium. These changes indicate an abnormal functional state of the endometrium and affect the results of IVF in patients (absence of oocyte transfer and failure to conceive).

Keywords: endometrial receptivity, in vitro fertilization, leukemia inhibitory factor, vascular endothelial growth factor, transforming growth factor-β1.

Особливості рецептивності ендометрія у жінок, які перенесли екстракорпоральне запліднення
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Мета дослідження: визначення маркерів рецептивності ендометрія у жінок, які перенесли процедуру екстракорпорального запліднення (ЕКЗ).

Матеріали та методи. У дослідження включено 191 пацієнтку, що звернулися до репродуктивного відділення Caspian International Hospital з 2020 по 2022 р. для обстеження з приводу безпліддя.

До основної групи увійшли 50 пацієнток із безпліддям, яким у період імплантаційного вікна (7–9-й день після овуляції у циклі, що передує етапу ЕКЗ) виконана біопсія ендометрія; до контрольної групи – 20 жінок, які перенесли лікування з приводу чоловічого безпліддя з позитивним програмним результатом.

Пацієнткам проводили ЕКЗ за стандартною методикою. Визначали такі рівні експресії: фактор інгібування лейкемії (leukemia inhibiting factor – LIF); фактор росту ендотелію судин (vascular endothelial growth factor – VEGF); трансформівний фактор росту-β1(transforming growth factor – TGF-β1). Було оцінено відсоткове вираження індексів площі.

Результати. У жінок основної групи встановлено: відсутність перенесення ембріона – 5 (10%) випадків, не настання вагітності – 27 (54%) випадків, наявність вагітності – 23 (46%) випадки, порушення циклу – 3 (6%) випадки.

За результатами гістологічного дослідження у жінок контрольної групи повна секреторна трансформація ендометрія була визначена у 85% випадків. В основній групі повна секреторна трансформація ендометрія виявлена у 48%, дефектна секреторна трансформація – у 42%, морфологічні ознаки змін ендометрія, що відповідають середній секреторній фазі циклу, – у 10% жінок.

Виявлено достовірне зниження (p<0,001) експресії LIF у гістологічних структурах (стромі) ендометрія в основній групі дослідження порівняно з групою контролю. Найбільш виражені відмінності спостерігалися за рівнем експресії TGF-β1 у стромі (приблизно у 10 разів; p<0,001).

Висновки. Отже, у жінок з безпліддям, які пройшли процедуру ЕКЗ, спостерігаються різноспрямовані зміни маркерів рецептивності ендометрія: зниження експресії LIF у стромі та епітелії, підвищення експресії VEGF у стромі та значне підвищення експресії TGF-β1 у стромі та епітелії. Ці зміни свідчать про аномальний функціональний стан ендометрія і впливають на результати ЕКЗ у пацієнток (відсутність перенесення ооцитів і не настання вагітності).

Ключові слова: рецептивність ендометрія, екстракорпоральне запліднення, фактор інгібування лейкемії, фактор росту ендотелію судин, трансформівний фактор росту-β1.

Infertility and reproductive dysfunction are the pressing problem of medicine and society. It is defined as a public health priority in many regions of the world [1, 2].

Reproductive issues are quite common: up to 15–18% of couples are infertile, half of them are forced to resort to the use of assisted reproductive technologies (ART) [3]. In vitro fertilization (IVF) is the most commonly used method of infertility treatment; however, IVF does not guarantee pregnancy. According to currently available evidence, 60% of couples who sought medical help in specialized institutions must undergo a second attempt, and some require three or more IVF procedures. Up to 70–85% of IVF program cycles are ineffective and more than 8 out of 10 transferred embryos do not implant [4–6].

Timely detection and correction of disorders of female reproductive function is a strategically important task of modern medicine.

Certain types of infertility or pregnancy loss are the result of defects in uterine receptivity, which leads to loss of synchrony in the development of the embryo, endometrium and ovary. Embryonic factors represent approximately one third of reasons for IVF failure, with the remainder due to implantation problems [7].

One of the important factors in IVF failure is a lack of synchronicity between endometrial maturation and embryo development as this can lead to decreased endometrial receptivity (ER) and a lack of implantation. Endometrial receptivity is a complex process that provides the embryo with the opportunity to attach, invade, and further develop, culminating in a new individual and continuation of the species [8, 9].

The time period when the endometrium is receptive to blastocyst implantation is termed the implantation window, which corresponds to 6–10 days after the peak of luteinizing hormone in the blood, or 20–24 days of the 28-day menstrual cycle. During this period, the plasma membrane of the endometrial epithelium loses microvilli and the apical surfaces of the cells form a dome-shaped protrusion termed pinopodes [10].

Pinopodes formation during the luteal phase is a major indicator of endometrial readiness for embryo implantation, and an assessment of this condition has been proposed as one of the markers of endometrial receptivity. The appearance of pinopodia in the mid-luteal phase is associated with an increase in progesterone secretion and the expression of leukemia inhibitory factor (LIF), LIF receptor (LIF-R), integrin V3, epidermal growth factor (EGF), glutaredoxin, which suggests their direct participation in the implantation process [11].

A number of studies have been conducted to evaluate ER based on endometrial biopsy analysis, hysteroscopy, and ultrasound findings [12–14].

Nevertheless, in a recent meta-analysis, it was concluded that these approaches are characterized by a poor ability to predict clinical pregnancy [15].

The most comprehensive assessment of the condition of the endometrium and its reciprocity is possible using modern immunohistochemical research methods, which make it possible to determine the expression of growth factors, cytokines and components of the intercellular substance. The interest of researchers is concentrated on studying a wide range of potential markers of endometrial receptivity [8, 12].

Many candidate markers have been proposed, including integrins, glycodelins, LIF, HOXA genes, heparin binding factor-like epidermal growth factor, vascular endothelial growth factor (VEGF), colony stimulating factor, interleukin - 15 and others [11, 16].

However, none of them is a universal marker of endometrial receptivity and this issue still requires study.

The **purpose of the study** was to determine the endometrial receptivity markers in women underwent IVF procedure

MATERIALS AND METHODS

In total 191 patients who applied to the reproductive department of Caspian International Hospital between 2020 and 2022 for examination for infertility were observed. Of these, 146 (76.4%) were aged 20–35 years, 45 (23.6%) were aged 36–50 years. Among the infertility factor the we noted several causes of infertility: male factor, the ovarian factor, tube factor. Besides, some cases were related to unexplained reason. In a number of couples, infertility depends on both sides. For evaluation of endometrial receptivity two groups were formed: main group included 50 women with different form of infertility except male factor, 20 women who underwent male infertility caused treatment with positive program outcome were included in control group.

The patients underwent IVF according to standard clinical procedures. Ovulation induction was conducted by administering recombinant follicle-stimulating hormone (225 IU daily), starting from the third day of the menstrual cycle. After observing two follicles reaching a minimum mean diameter of 17 mm, human chorionic gonadotropin (3300–10,000 IU) was administered. Oocyte collection was carried out 36 hours after the administration of human chorionic gonadotropin with the introduction of progesterone on the day of oocyte collection. Pregnancy was verified 4–5 weeks after embryo transfer using ultrasound (Samsung SonoAce, South Korea).

The ovulation was confirmed by ultrasound with determining the luteinizing hormone peak in urine. The endometrium samples obtained by biopsy were standardly treated to get paraffin blocks. Histological analysis was made in accordance with criteria of M. Mazur and R. J. Kurman [17].

Endometrium biopsy was performed in 50 of patients within implantation window (7th-9th day after ovulation in the cycle preceding the IVF stage) using suction curette Pipelle de Cornier (Jiangsu Suyun Medical Materials Co., Ltd., China). Histological examination and IHC of endometrial samples were carried out at the histological laboratory of Azerbaijan Medical University. Paraffin blocks were formed from endometrial samples by the histoprocessor (Leica ASP200, Germany), sections with a thickness of 3–5 µm were subsequently made using a Microm HM340E microtome (Thermo Scientific, USA).

Sections were stained with eosin and hematoxylin for histological examination. The immunohistochemical method of staining the biomaterial was used to assess the expression of sex steroid receptors in the glands and endometrial

stroma using the polymer EnVision method using an imaging system (DakoCytomation, Denmark). LIF, VEGF, TGF-β1 expression on the membranes of surface epithelial cells of the endometrial glands and stroma were determined by counting the number of stained cells in a field of view. The percent area expression of indices was assessed.

The Number Cruncher Statistical System program was used to evaluate the data. The mean, standard error (m), and frequency of the trait (absolute number and percentage) were determined. Compliance of quantitative data with normal distribution was checked using the Shapiro–Wilk test and graphical studies. Comparison of groups with a normal distribution was carried out the Student's t-test, groups without a normal distribution were evaluated using the Mann–Whitney U-test. Differences were considered significant at p<0.05.

RESULTS AND DISCUSSION

Based on the results of in vitro fertilization, among the 191 patients the following outcomes were obtained: no transfer – 8 cases (4.2%), absence of pregnancy – 91 cases (47.6%), presence of pregnancy – 82 cases (42.9%), cycle disruption – 10 cases (5.2%).

According to the results of a histological study of endometrial samples obtained on days 7–9 after ovulation, in women of the control group, full secretory transformation of the endometrium was determined in 85% of cases. In the main group, full-fledged secretory transformation of the endometrium was detected in 48%, defective secretory transformations in the endometrium – in 42%, morphological signs of endometrial changes corresponding to the middle secretory phase of the cycle – in 10% of women.

When analyzing the outcomes in women of main group who were studied immunohistochemically (n=50) the data were as following: no transfer – 5 cases (10%), absence of pregnancy – 27 cases (54%), presence of pregnancy – 23 cases (46%), cycle disruption – 3 cases (6%).

The levels of endometrial receptivity markers' expression are presented in Table.

A significant decrease (p<0.001) in LIF expression was detected in the histological structures (stroma) of the endometrium in the main study group compared to control group. The expression of LIF in epithelium was also decreased, but in less degree.

The indicators of endometrial receptivity in women underwent IVF procedure, M±m

Indicators (PAE,%)	Main group (n=50)	Control group (n=20)	p
LİF / str	13,0± 1,4	23.6 ± 1.2	<0.001
LİF / ep	14,4±1,5	18.4± 1.3	<0.05
VEGF / str	9,1±1,2	7.9 ± 1.6	<0.05
VEGF / ep	3,6±1,3	3.9 ± 1.3	>0.05
TGFβ1 / str	55,7±4,5	4.8 ± 0.5	<0.001
TGFβ1 / ep	16,3±2,1	10.6 ± 1.38	<0.01

Notes: PAE – percent area expression (%); /str – expression of indicator in stroma; /ep – expression of indicator in epithelium; p-coefficient of significance.

In contrast with LIF, the expression of VEGF/str was higher than control (p<0.05). However, there were not significant differences in VEGF/ep expression between main and control groups (p>0.05).

The most pronounced differences were found out in the level of TGF-β1 expression in stroma (approximately, in 10 times, p<0.001). TGF-β1 expression in epithelium was higher than in control group, the differences were also significant (<0.01).

Thus, according to our results women with infertility which depends on female related causes had multidirectional changes of endometrial receptivity markers. These changes demonstrate abnormal functional condition of endometrium and expressed in the outcomes of IVF in our patients. So, the women with most expressed differences from control groups data had the negative outcomes such as no transfer and absence of pregnancy.

The clinical significance of a reliable diagnosis of endometrial receptivity cannot be overestimated. It is believed that endometrial biopsy is still the most common method for diagnosing abnormalities leading to a serious decrease or even complete loss of the implantation capacity of the endometrium [10, 17].

In the various studies the role of endometrium receptivity markers was assessed [13, 18–20]. But despite the large number of proposed markers, there is presently no single generally accepted universal marker(s) for assessing the ability of the endometrium to ensure embryo implantation [21].

The expression of LIF is most pronounced in the cells of the surface epithelium of the endometrium; glandular epithelial cells synthesize LIF less actively; in small quantities this marker is noted in the endometrial stroma throughout the menstrual cycle. The level of LIF changes throughout the entire cycle, with maximum expression noted during the "implantation window" [18].

In the one of previous works (E. Dimitriadis et al.) was showed that the concentration of LIF in uterine lavage and endometrial tissue samples is lower in patients with unexplained infertility, as well as in patients with unsuccessful IVF attempts compared to fertile women [22].

In turn, P. Serafini et al. revealed that infertile women with strong LIF expression in the mid-luteal phase were 6 times more likely to achieve pregnancy than those with reduced LIF expression [23].

Some authors noted that the most specific and significant endometrium receptivity markers for IVF treatment outcome prediction are TGF-β1 and LIF [24, 25].

It is known that TGF-β1, as one of the TGF-β isoforms is equal in intensity in stromal and epithelial cells. However, the precise cellular distribution varied according to the species studied, the follicular stage, and most likely, the detection method used [26]. In our study, TGF-β1 was chosen as a marker secreted in high level from the stroma, which might reflect uterine activity.

The VEGF is an important angiogenesis regulator, a mitogen for vessel endothelial cells. VEGF most significantly promotes the permeability of blood vessels and the proliferative activity of endothelial cells, suppresses apoptosis, stimulates the release of nitric oxide and prostacyclin from endothelial cells, which promotes vasodilation.

In the early stages of implantation, VEGF is involved in coordinating the processes of trophoblast differentiation, migration and invasion [27].

Some studies have explored the association between VEGF polymorphisms and the occurrence of embryo implantation failure. These results suggest polymorphisms of the VEGF gene could impact fertilization rate, embryo implantation rate, and pregnancy rate [28].

R. Bansal et al. reported an increased serum VEGF level in women with recurrent implantation failure compared to fertile controls [29], whereas X. Chen et al. in the other studies demonstrated decreased VEGF expression in all regions of the endometrium at the time of embryo implantation [30]. In our work we revealed the higher expression of VEGF in stroma in women with female infertility, while the epithelium level did not differ significantly. The patients with increased expression had the higher percentage of negative outcomes. Our results are close to X. Guo et al. data: they reported that there is a correlation between the changes of VEGF expression and reproductive failure, including recurrent implantation failure and recurrent miscarriage [31].

CONCLUSIONS

Thus, women with infertility who underwent IVF procedure has multidirectional changes of endometrial receptivity markers: decrease in LIF expression in stroma (p<0.001) and epithelium (p<0.05), increase of the expression of VEGF in stroma (p<0.05) and significant increase of TGF-β1 expression in stroma (p<0.001) and epithelium (p<0.01). These changes demonstrate abnormal functional condition of endometrium and expressed in the outcomes of IVF in our patients, such as absence of oocytes transfer and absence of pregnancy.

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