N E W S
OF THE NATIONAL ACADEMY OF SCIENCES OF THE REPUBLIC OF KAZAKHSTAN
SERIES OF BIOLOGICAL AND MEDICAL
ISSN 2224-5308
Volume 1, Number 319 (2017), 5 – 12

Yu. S. Krylova[1], V. O. Polyakova[1], A. M. Gzgzyan[1], V. N. Lokshin[2], I. M. Kvetnoy[1]

[1]FSBSI "The Research institute of Obstetrics, Gynecology and Reproductology named after D. O. Ott", Saint Petersburg, Russia,
[2]«PERSONA» the International Centre of Clinical Reproduction, Almaty, Kazakhstan.
E-mail: vyacheslavnl19@rambler.ru

ENDOMETRIOSIS GENITALIS EXTERNA, IMMUNOHISTOCHEMICAL DIAGNOSIS OF ENDOMETRIUM IMPLANTATION RECEPTIVITY

Abstract. The lack of implantation in cycles of in vitro fertilization (IVF) is one of the main problems of modern reproductive medicine. Implantation is a multifactorial process, it is difficult to imagine the existence of a single criterion, with which it is possible to determine the receptivity of the endometrium. However, the possibility of accurate diagnosis of the state of endometrium receptivity cannot be overestimated in terms of clinical relevance. Endometrial biopsy today remains the most common method to diagnose pathological conditions that lead to a significant reduction or loss of the ability of endometrial implantation.

Important one is the ability to predict the onset of implantation, since the detection of violations make it possible to avoid unnecessary emotional stress, not to carry out the support of the luteal phase and the hopeless refrain from embryo transfer, preserving them for the delayed transfer. We evaluated the possibility of using the immunohistochemical study with the assessment of key biological molecules involved in the formation of implantation window (LIF, ER, PR, Integrin, TGF-β1, VEGF), for prognosis and diagnosis of disorders of the endometrium implantation receptivity in patients with external genital endometriosis. As a result of studies there have been verified informative immunohistochemical markers that have been used to create the IVF treatment outcome prediction algorithm. With the help of the developed model it is possible to predict the group with poor outcome of treatment that can reasonably plan the tactics of treatment measures and enhances the effectiveness of IVF treatment.

Key words: IVF, endometrium implantation receptivity, receptivity, "implantation window", LIF, VEGF, Integrin αVβ3, TGFβ1, ER, PR.

Infertile couple is an important medical, social and economic problem. According to WHO, its frequency is 10-15%, and has no tendency to decrease. Lack of efficiency of restoration methods of natural human fertility has stimulated the development of new assisted reproductive technologies such as in vitro fertilization (IVF), the effectiveness of which varies from 28.5% to 32.5% [1]. Optimizing the efficiency of IVF is directly related to the decision of the key issues of reproductive medicine - the identification of the receptivity period (implantation receptivity of the endometrium) to determine the operational readiness of the endometrium to the blastocyst implantation [2]. Endometrium implantation receptivity is a complex of structural and functional characteristics of the endometrium with clear time constants that define the ability of the endometrium to provide implantation of the embryo. Implementation of endometrium receptivity is performed through genetic and molecular reactions that lead to the expression of large amounts of biologically active molecules such as cytokines, growth factors, adhesion molecules, etc. Traditionally assessment of the endometrium receptivity is performed by biopsy, allowing to identify the lack of the luteal phase [3]. However, this method does not allow to get complete information on the receptivity of the endometrium. So, the researchers admit that at the histological visualization it is not possible to identify functional subliminal disturbances of implantation susceptibility. Determination by immunohistochemistry of signaling molecules ensuring the endometrium implantation ability enables for the evaluation of operational readiness of the endometrium for implantation.

Disorder of the functional activity of the endometrium is also probable in the normal morphological state that manifests with a change in the balance of regulatory molecules, providing endometrium receptivity. Based on the detection of violations in receptivity it is possible ещ appoint the complex therapy for women with reserved ovulatory cycles promoting trophic and metabolic processes in the endometrium.

The aim of this study was verifying the most specific and significant immunohistochemical markers of endometrium receptivity for the development of IVF treatment outcome prediction algorithm at external genital endometriosis of the I-II degree.

Material and Methods. The study material amounted to 95 cases of endometrial biopsy of women treated by IVF and following transferring the embryo into the uterus, on the basis of FSBSI "Ott RI OGR". To study the following groups were formed. Group I included 25 women with EGE of I-II degree and a positive outcome of the treatment program (pregnancy is confirmed echografically), II group consisted of 30 women with EGE of I-II degree, whose pregnancy has not occurred. Group III, the control group, included 40 women who were treated due to male factor of infertility, and the positive outcome of the program. The study included women with infertility in age from 24 to 35 years old, the level of follicle-stimulating hormone in the blood, defined on 3-5 days of the menstrual cycle (d.m.c.) of all surveyed women did not exceed 11 IU / L, at ultrasound investigation on 3-5 d.m.c determined from 7 to 12 antral follicles in the ovaries maximum echographic cut. All women received standard protocol IVF or IVF with intracytoplasmic sperm injection (ICSI) with the use of gonadotropin-releasing hormone antagonists. In order to support the luteal phase of the cycle the micronized progesterone was used. It was conducted transfer of only morphological qualitative embryos on the fourth or fifth day of cultivation. The average dose of recombinant gonadotropins, the number of obtained oocytes and the transferred embryos did not differ in clinical study groups. Pregnancy diagnostics was performed by determination of human chorionic gonadotropin in the blood on the 14 day after embryo transfer into the uterine cavity and ultrasonically on the 21 day. Forming a study group, we standardized as much as possible all the indicators, so that would be the only variable in the study was the endometrium implantation receptivity.

Endometrial biopsy was performed in the period of the intended implantation window on 7-9 day after ovulation in the cycle, prior to IVF cycle, using Pipelle de Cornier suction curette ("Jiangsu Suyun Medical Materials Co., Ltd.). Ovulation was confirmed by ultrasound and by determining the peak of luteinizing hormone in urine. The resulting endometrial samples were teated according to standard procedure to obtain the paraffin blocks. Histologic examination was conducted according to the criteria proposed by Mazur and Kurman [5], on the basis of which it was selected the samples containing middle stage secretion phase endometrium no evidence of inflammation and fibrosis. In case of doubt, the additional color on the Van Gieson method was used to identify chronic inflammation, leukocyte subpopulations CD20, CD138 were determined by immunohistochemistry.

Immunohistochemistry (IHC) was performed on deparaffinized and dehydrated sections of 4-6 um of thick with an avidin-biotin immunoperoxidase method. Expression levels were determined: leukemia of inhibiting factor (LIF) (Anti-LIF, Abcam (ab135629), 1:100); vascular endothelial growth factor type A (VEGF-A) (Anti-VEGF-A, Abcam (ab28775), 1:50); transforming growth factor beta 1 (TGF-β1) (Anti-TGF-β1, Novocastra, 1:40); estrogen receptor type alpha (ER) (Anti-ER, DAKO (1D5), 1:35); progesterone receptor, type A (PR) (Anti-PR, DAKO (636), 1:35); integrin alpha five beta three (Integrin αVβ3) (Anti- Integrin αVβ3, Abcam (ab7166), 1:250).

To quantify the immunohistochemical reaction there were obtained images using a Nikon DXM 1200 camera and "AST 1" software, version 2.12, fixing of the image was carried out on the x40 magnification. To evaluate the results of immunohistochemical staining it was performed morphometric study of micrographs using the system of computer analysis of microscopic images of Morphology 5.2. (VideoTest, Russia). In each case, all of the material was analyzed, except the field of vision, containing dyeing defects and artifacts.

The results of the reactions to the antigens having nuclear localization (ER, PR), was evaluated by counting system «histochemical score» (HS). The maximum value of HS is 300 points.

The intensity of reactions with antigens, localized in the cytoplasm and on cell membranes (LIF, VEGF-A, Integrin αVβ3, TGF-b1) was evaluated by two parameters - the relative area of expression and the optical density. The relative area of expression was calculated as the ratio of the area occupied by the immunopositive cells to the total cell area in the field of vision and expressed in percentage. The optical