

Golovatyuk K. P., Nosenko O. M., Makshayeva E. T., Trofimov D. Yu., Donnikov A. E., Kolin V. V. Molecular-genetic aspects of the endometrium state on the day of the tentative implantation window in women with recurrent miscarriage in the programs of assisted reproductive technologies. *Journal of Education, Health and Sport*. 2017;7(9):621-626. eISSN 2391-8306. DOI <http://dx.doi.org/10.5281/zenodo.1068361>  
<http://ojs.ukw.edu.pl/index.php/johs/article/view/5076>

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

© The Authors 2017;

This article is published with open access at Licensee Open Journal Systems of Kazimierz Wielki University in Bydgoszcz, Poland

Open Access. This article is distributed under the terms of the Creative Commons Attribution Noncommercial License which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and source are credited. This is an open access article licensed under the terms of the Creative Commons Attribution Non Commercial License

(<http://creativecommons.org/licenses/by-nc/4.0/>) which permits unrestricted, non commercial use, distribution and reproduction in any medium, provided the work is properly cited.

This is an open access article licensed under the terms of the Creative Commons Attribution Non Commercial License (<http://creativecommons.org/licenses/by-nc/4.0/>) which permits unrestricted, non commercial use, distribution and reproduction in any medium, provided the work is properly cited.

The authors declare that there is no conflict of interests regarding the publication of this paper.

Received: 15.08.2017. Revised: 20.09.2017. Accepted: 29.09.2017.

## MOLECULAR-GENETIC ASPECTS OF THE ENDOMETRIUM STATE ON THE DAY OF THE TENTATIVE IMPLANTATION WINDOW IN WOMEN WITH RECURRENT MISCARRIAGE IN THE PROGRAMS OF ASSISTED REPRODUCTIVE TECHNOLOGIES

<sup>1</sup>K. P. Golovatyuk, <sup>2</sup>O. M. Nosenko, <sup>1</sup>E. T. Makshayeva, <sup>3</sup>D. Yu. Trofimov,  
<sup>4</sup>A. E. Donnikov, <sup>3</sup>V. V. Kolin

<sup>1</sup>Limited Liability Company Medical Center for Reproductive Health "Gameta",  
Odessa, Ukraine;

<sup>2</sup>Odessa National Medical University, Ukraine;

<sup>3</sup>Limited Liability Company "NPF DNA-Technology", Moscow, Russia;

<sup>4</sup>FGBU Scientific Center for Obstetrics, Gynecology and Perinatology,  
named after acad. V.I. Kulakov Ministry of Health care and Social Development of  
Russia, Moscow, Russia;

e-mail: nosenko.olena@gmail.com

### Abstract

More than 50% of pregnant women after the programs of assisted reproductive technologies (ART) face the problem of recurrent miscarriage (RMC), especially in the first trimester. Significant role in the development of RMC has infectious factor and chronic inflammation in the endometrium. **The aim:** to reveal the peculiarities of immune response mRNA genes of the inflammatory component expression in the period of the tentative implantation window (TIW) in women with RMC in ART programs. **Material and methods.** The main group consisted of 240 patients with RMC in ART programs; the control group included 100 conditionally healthy fertile women. On the ground of PCR reverse

transcription, the mRNA of the IL-1 $\beta$ , IL-2, IL-10, Foxp3, TLR9, IL-2R $\alpha$  cytokine genes was examined in endometrial samples obtained with the help of biopsy on the TIW day. **Results.** Analysis of the transcriptional profile of the immune response genes in the endometrium on TIW day revealed that the relative level of mRNA expression of the IL-1 $\beta$ , IL-2, Foxp3, TLR9, IL-2R $\alpha$  genes did not differ significantly in the main and control groups. Statistically significant decrease in mRNA expression of IL-10 gene was observed in women with RPL. **Conclusions.** A feature of mRNA expression of the inflammatory component of the immune response in TIW period in women with RMC in ART programs is a decrease in the expression level of the IL-10 gene mRNA, which may be one of the reasons for the unfavorable outcomes of the onset pregnancy.

**Key words: recurrent miscarriage, assisted reproductive technologies, immune response, reverse transcription-polymerase chain reaction, tentative implantation window.**

More than 50% of pregnant women after the programs of assisted reproductive technologies (ART) face the problem of recurrent miscarriage (RMC), especially in the first trimester [11]. RMC is a genetically heterogeneous state resulted from the coexistence of two regulatory factors: maternal and fetal [13, 15]. The fetus expresses the antigens inherited from both parents, and the survival of the half-allotransplant fetus before the term is one of the most complex processes associated with pregnancy. The mother's immune system fulfills its important role in successful pregnancy, controls fertilization, implantation, placentation, development and maintenance of pregnancy itself [4, 6, 12]. The study of immune cells, immune mediators / cytokines in the endometrium and genes encoding them, opens a new pathway in understanding the maternal causes of RMC [9, 15].

Significant role in the development of reproductive disorders, in particular, RMC has infectious factor and endometrium chronic inflammation [2, 7]. The most informative markers of inflammation in chronic endometritis among cytokines are interleukin-1 $\beta$  (IL-1 $\beta$ ), interleukin-2 (IL-2), interleukin-10 (IL-10) transcription factor Foxp3, toll-like receptor-9 (TLR-9), immune system cellular marker – receptor –  $\alpha$  of interleukin-2 (IL-2R $\alpha$ ) [2].

**The aim:** to reveal the features of immune response mRNA genes of the inflammatory component expression in the period of the tentative implantation window (TIW) in women with RMC in the programs of ART.

## Material and methods

The main group consisted of 240 patients with RMC included into ART. The control group consisted of 100 conditionally healthy women with at least one terminal labours in their history and without episodes of miscarriage. All the patients under examination were residents of the South-Western region of Ukraine. All women underwent endometrial pipelle biopsy during TIW. Samples were frozen at  $t = -70\text{ }^{\circ}\text{C}$  till the beginning of the study. Based on the reverse transcription-polymerase chain reaction method, a study of the presence of IL-1 $\beta$ , IL-2, IL-10, Foxp3, TLR9, IL-2R $\alpha$  cytokine genes in the endometrial samples taken was made.

When determining the transcriptional profile of the immune response genes for the isolation of nucleic acids, the "Sample NK" kits (Russia) were used. The cells obtained were lysed in 4 M solution of guanidinetiocyante, the nucleic acids were precipitated with isopropanol in the presence of a coprecipant followed by washing with ethanol and acetone. As endometrial tissue were present in the samples, the phenol-chloroform extraction method was used [13]. The reverse transcription reaction (synthesis of complementary DNA from RNA obtained) was carried out in volume of 40  $\mu\text{l}$ . The original specific oligonucleotides and M-MuLV reverse transcriptase were used as primers for reverse transcription. The reaction was carried out at  $t = 40\text{ }^{\circ}\text{C}$  for 30 minutes, followed by inactivation of reverse transcriptase at  $t = 95\text{ }^{\circ}\text{C}$  for 5 minutes. The amplification was carried out in real time with fluorescence level measurement on the FAM channel on each cycle at the annealing temperature of the primers. The reaction was set in duplicate for each point.

The normalization was performed by comparing the threshold cycles (Cp) for the detected cytokines ( $\Delta\Delta\text{Cq}$  method) for 2 normalizing genes (B2M, GUSB). B2M is a gene of  $\beta$ 2-microglobulin, a component of the light chain of the main complex of histocompatibility class I (MHC I), presented on all nucleated cells of the human body (except red blood cells). GUSB is a gene that provides the production of an enzyme called  $\beta$ -glucuronidase.

The relative level of mRNA expression of the genes under study was calculated by the formula (1):

$$[I] = 2 * (\text{NF}-\text{Cpi}) \quad (1),$$

where [I] is the relative level of mRNA representation of the gene under study,

Cpi is the value of the threshold cycle of the corresponding gene in the sample, determined automatically by the software of the device;

NF is the factor of normalization which was calculated by the formula (2):

$$\text{NF} = 1/2 (\text{Cp}_{(\text{B2M})} + \text{Cp}_{(\text{GUSB})}) \quad (2),$$

where  $C_p$  – are the values of threshold cycles of the corresponding reference genes in the sample, determined automatically by the software of the device.

The statistical processing of the data obtained was carried out with EXCEL program. The quantitative indices obtained were represented as Me (L-H), where Me is the median, L is the lower quartile, and H is the upper quartile. To compare the two groups by the quantitative characteristics, the Mann-Whitney U test was used. The difference between the groups was considered statistically significant at  $p < 0.05$ .

### Results and their discussion

The average age of the main group women was  $29.80 \pm 0.30$  y.o., and in the control group C -  $30.09 \pm 0.32$  ( $p > 0.05$ ). The average number of cases of spontaneous miscarriage after ART in the main group was  $3.24 \pm 0.11$ , the average term of pregnancy termination was  $8.15 \pm 0.65$  weeks.

The groups were homogeneous in terms of anthropometric characteristics, somatic and infectious anamnesis.

Analysis of endometrial transcriptional profile of the immune response genes on TIW day revealed that the relative level of mRNA expression of the IL-1 $\beta$ , IL-2, Foxp3, TLR9, IL-2R $\alpha$  genes was not statistically significantly different in the patients of the main and control groups .).

Table

### The relative level of mRNA expression of the studied genes,

#### Me (L-H)

Gene	Main group, n=240	Control group, n=100
IL-1 $\beta$	15.3 (13.8-16.8)	17.0(13.0-19.4)
IL -2	30.5(27.8-32.3)	30.1(28.2-32.3)
IL-2R $\alpha$	24.2(22.1-25.5)	24.6(22.9-25.8)
IL-10	22.7(20.4-25.3) <sup>C</sup>	24.8(22.0-26.6)
Foxp3	23.1(21.5-24.1)	23.0(20.3-24.2)
TLR-9	22.4(20.6-24.1)	22.9(21.1_24.5)

**Note:** <sup>C</sup> is a statistically significant difference with the C group score ( $p < 0.05$ ).

It was found that HM women had statistically significant changes in the transcriptional profile during TIW period. The latter are associated with the decrease of IL-10 mRNA gene - 22.7 (20.4-25.3) versus 24.8 (22.0 -26.6).

According to literature data, the pleiotropic cytokine IL-10 is produced by activated Th2-cells, B-cells, monocytes and macrophages, which play a central role in supporting maternal tolerance to the fetus [10]. IL-10 inhibits the secretion of pro-inflammatory Th1-type cytokines by lymphocytes and activated macrophages [5, 8]. IL-10 functions as a vital bridge that connects immunity, placental angiogenesis, inflammation and hypoxia in the interface” mother-fetus” [3]. Women with a decrease in IL-10 expression may be sensitive to inflammatory processes and are at increased risk of gestational disorders, which results in an unfavorable outcome of pregnancy. In other words, a decrease in IL-10 production contributes to worsening of the placentation with an increased incidence of spontaneous abortions [1].

### **Conclusions**

A feature of mRNA expression of the immune response inflammatory component in the period of the tentative implantation window in women with habitual miscarriage in assisted reproductive technology programs is a decrease in the expression of the IL-10 gene mRNA level, which may be the cause of unfavorable outcomes of the pregnancy onset.

### **References:**

1. Babbage SJ, Arkwright PD, Vince GS, et al. Cytokine promoter gene polymorphisms and idiopathic recurrent pregnancy loss. *J Reprod Immunol.* 2001; 1: 21–7.
2. Burmenskaya OV. Molecular-genetic markers of the immune response in inflammatory diseases of the female reproductive system: author's abstract ... diss. Doctor of Biological Sciences: 03.03.03. Moscow, 2014. 48 pp.
3. Ghazaey S, Keify F, Mirzaei F, et al. Chromosomal analysis of couples with repeated spontaneous abortions in northeastern Iran. *Int J Fertil Steril.* 2015; 9(1): 47-54.
4. Guerin LR, Prins JR, Robertson SA. Regulatory T-cells and immune tolerance in pregnancy: a new target for infertility treatment? *Hum Reprod Update.* 2009;15:517–535.
5. Mashkina EV, Kovalenko KA, Miktadova AV, et al. Analysis of IL-10 gene expression in decidual and chorionic tissues during miscarriage. *Modern problems of science and education.* 2013; 5.
6. Munoz-Suano A, Hamilton AB, Betz AG, Shelter G. The immune system during pregnancy. *Immunol Rev.* 2011;241:20–38.
7. Nosenko OM, Sayenko AI, Azyyatulova EM, et al. Immunohistochemical determination of syndecan-1 in the diagnosis of chronic endometritis in patients with unsuccessful attempts of extracorporal fertilization. Problems, achievements and prospects of development of medical-biological sciences and practical health care. *Proceedings of the Crimean State Medical University named after. SI Georgievsky.* 2010; 149 (III): 125-128.

8. Parveen F, Shukla A, Agarwal S. Cytokine gene polymorphisms in northern Indian women with recurrent miscarriages. *Fertil Steril*. 2013; 99; 433-440.
9. Prins JR, Gomez-Lopez N, Robertson SA. Interleukin-6 in pregnancy and gestational disorders. *J Reprod Immunol*. 2012;95:1–14.
10. Qaddourah RH, Magdoud K, Saldanha FL et al. IL-10 gene promoter and intron polymorphisms and changes in IL-10 secretion in women with idiopathic recurrent miscarriage. *Hum Reprod*. 2014; 29(5): 1025-34.
11. Rasti Z, Nasiri M, Kohan L. The IL-6 -634C/G polymorphism: a candidate genetic marker for the prediction of idiopathic recurrent pregnancy loss. *Int J Reprod Biomed (Yazd)*. 2016;14(2):103-8.
12. Robertson SA. Immune regulation of conception and embryo implantation-all about quality control? *J Reprod Immunol*. 2010;85:51–57.
13. Rull K, Nagirnaja L, Laan M. Genetics of recurrent miscarriage:challenges, current knowledge, future directions. *Front Genet*. 2012;3:34. doi: 10.3389/fgene.2012.00034.
14. Sambrook J, Fritschi EF, Maniatis T. *Molecular cloning: a laboratory manual*, Cold Spring Harbor Laboratory Press, New York, 1989. 10
15. Xu X, Du C, Li H, Du J, Yan X, Peng L, et al. Association of VEGF Genetic Polymorphisms with Recurrent Spontaneous Abortion Risk: A Systematic Review and Meta-Analysis. *PLoS One*. 2015;10. doi: 10.1371/journal.pone.0123696.