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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

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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-1β, IL-2, IL-10, Foxp3, TLR9, IL-2Rα 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-1β, IL-2, Foxp3, TLR9, IL-2Rα 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-1β (IL-1β), interleukin-2 (IL-2), interleukin-10 (IL-10) transcription factor Foxp3, toll-like receptor-9 (TLR-9), immune system cellular marker – receptor – α of interleukin-2 (IL-2Rα) [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 °C till the beginning of the study. Based on the reverse transcription-polymerase chain reaction method, a study of the presence of IL-1β, IL-2, IL-10, Foxp3, TLR9, IL-2Rα 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 guanidinetiocyanate, 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 μl. The original specific oligonucleotides and M-MuLV reverse transcriptase were used as primers for reverse transcription. The reaction was carried out at t = 40 ° C for 30 minutes, followed by inactivation of reverse transcriptase at t = 95 ° 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 (ΔΔCq method) for 2 normalizing genes (B2M, GUSB). B2M is a gene of β2-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 β-glucuronidase.

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

$[I] = 2 * (NF-Cpi)$ (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):

$NF = 1/2 (Cp_{(B2M)} + Cp_{(GUSB)})$ (2),

where Cp – 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 ± 0.30 y.o., and in the control group C - 30.09 ± 0.32 ($p > 0.05$). The average number of cases of spontaneous miscarriage after ART in the main group was 3.24 ± 0.11, the average term of pregnancy termination was 8.15 ± 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-1β, IL-2, Foxp3, TLR9, IL-2Rα 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-1ß	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-2Rα	24.2(22.1-25.5)	24.6(22.9-25.8)
IL-10	22.7(20.4-25.3)^{C}	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: ^{C} 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.

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