INTRODUCTION

The usual miscarriage occurs in 1–5% of pregnancies [3], defined as the occurrence of two or more pregnancy loss before the 20th week of pregnancy. After supplementary reproductive technologies (ART), more than 50% of pregnant women are faced with the problem of the recurrent pregnancy loss (RPL), especially in the first trimester [2]. RPL is due to several factors, including chromosomal, anatomical, endocrinologic, infectious and autoimmune anomalies [1, 14].

ANALYSIS OF LITERATURE DATA

In recent years, numerous studies have found that the imbalance in the maternal-fetal interface plays a role in the pathogenesis of RPL, and the interaction between the array of cytokines is believed to contribute to the ability of the maternal immune system to transfer genetically incompatible fetuses. Decidual tissue, an important component of the maternal immune system to transfer genetically incompatible fetuses, contains decidual stromal cells that secrete cytokines, which are believed to contribute to the ability of the maternal immune system to transfer genetically incompatible fetuses.

EXPRESSION OF THE mRNA OF THE INFLAMMATORY COMPONENT OF THE IMMUNE RESPONSE IN WOMEN WITH RECURRENT PREGNANCY LOSS IN THE PROGRAMS OF ASSISTED REPRODUCTIVE TECHNOLOGIES

The usual miscarriage occurs in 1–5% of pregnancies [3], defined as the occurrence of two or more pregnancy loss before the 20th week of pregnancy. After supplementary reproductive technologies (ART), more than 50% of pregnant women are faced with the problem of the recurrent pregnancy loss (RPL), especially in the first trimester [2]. RPL is due to several factors, including chromosomal, anatomical, endocrinologic, infectious and autoimmune anomalies [1, 14].
and decidual immune cells, including T cells, uterine killer cells and macrophages [8]. Decidual cells that develop from the endometrium are regulated by steroid hormones of the ovaries after implantation of the blastocyst. Decidua tissue is essential for implantation of the germ cell and for the development of embryos. In addition, it has power functions of the blastocyst, regulating the endocrine environment, regulating the trophoblast invasion and protecting the embryo from rejection of the mother, and therefore plays an important role in pregnancy. In the maternal-fetal interface, the balance of local proinflammatory and anti-inflammatory cytokines is important for successful pregnancy. Therefore, changes in the pattern of expression of cytokines in the endometrium at the time of the alleged implantation window can lead to inflammation of the microenvironment of the endometrium, which leads to spontaneous abortion [9, 14, 15].

The most informative markers for inflammation in the endometrium among cytokines are interleukin-1β (IL-1β), interleukin-2 (IL-2), interleukin-10 (IL-10), transcription factor Foxp3, toll-like receptor-9 (TLR-9), cellular marker of the immune system – receptor-α interleukin-2 (IL-2Ra) [1].

The purpose of the study was to identify the peculiarities of the expression of the mRNA genes of the inflammatory component of the immune response during the expected implantation window in women with RPL in assisted reproductive technologies programs.

**MATERIAL AND METHODS OF THE STUDY**

Under supervision it were 240 patients of the H group with RPL in the programs of ART and 100 conditionally healthy fertile women in the control group K with the presence in history of at least one childbirth in time and the absence of episodes of miscarriage. All patients were residents of the South-Western region of Ukraine. All women had a paipel-biopsy of the endometrium during the expected implantation window. Samples were frozen at t = -70 °C. until the study.

The study of the expression of IL-1β, IL-2, IL-10, transcription factor Foxp3, TLR9, and IL-2Ra cytokine genes was performed on the basis of the reverse transcription polymerase chain reaction (RT-PCR) method [1].

In the determination of the transcriptional profile of the immune response genes for the isolation of nucleic acids, the “Test NK” (Russian Federation) sets were used. The resulting cells were lysed in 4 M solution of guanidiniothiocyanate, the nucleic acids were precipitated with isopropanol in the presence of a co-sediment, followed by washing with ethanol and acetone. Due to the presence of endometrial tissue in samples, the phenol-chloroform extraction method was used [13]. The reverse transcription reaction (synthesis of complementary DNA from the resulting RNA) was performed in a volume of 40 μl. Primary specific oligonucleotides and reverse transcriptase M-MuLV were used as back-transcription primers. The reaction was carried out at t = 40 °C. for 30 minutes followed by inactivation of the reverse transcriptase at t = 95 °C. for 5 minutes. The amplification was carried out in real time with the measurement of fluorescence level along the FAM channel at each cycle at annealing temperature of the primers. The reaction was put in two replicates for each point. The normalization was performed by comparing the threshold cycles (Cp) for the identified cytokines (ΔΔCq method) with 2 normalizing genes (B2M, GUSB). B2M is a gene of β2-microglobulin, a component of the light chain of the main complex of histocompatibility of class I (MHC I), presented on all nuclear cells of the human body (except red blood cells). GUSB is a gene that produces an enzyme called β-glucuronidase.

The relative expression level of the mRNA of the genes studied was calculated using the formula (1):

\[
[I] = 2^\left(-\frac{\Delta Cq}{\Delta Cq_{reference}}\right)
\]

where \([I]\) is the relative level of mRNA representation of the investigated gene, \(C_{pi}\) is the value of the threshold cycle of the corresponding gene under study in the sample, which is determined automatically by the software of the device; \(NF\) is the normalization factor calculated by the formula (2):

\[
NF = \frac{1}{2} (\frac{Cp_{B2M}}{2}) + \frac{Cp_{GUSB}}{Cp_{reference}}
\]

where \(Cp\) is the value of the threshold cycles of the reference reference genes in the sample, which are determined automatically by the software of the device.

Statistical processing of data was performed using the EXCEL program. The average value of M and the SE standard deviation error were determined. Mann-Whitney’s U-criterion is used to map two groups quantitatively. The difference between the groups was considered statistically significant at \(p < 0.05\).

**RESULTS OF THE STUDY AND DISCUSSION**

The average age of the examined women of the group H was 29.80 ± 0.30 years, the group K – 30.09 ± 0.32 \((p >0.05)\). The average number of cases of involuntary termination of pregnancy after conduction of ART in group N was 3.24 ± 0.11, the average term of termination of pregnancy was 8.15 ± 0.65 weeks.

In the analysis of the investigated transcriptional profile of the immune response gene in the endometrium on the day of the proposed implantation window, it was found that the relative level of expression of IL-1β, IL-2, Foxp3, TLR9 and IL-2Ra genes did not differ statistically significantly among patients in the main and control groups (Table).

<table>
<thead>
<tr>
<th>Genes</th>
<th>Group H ((n = 240))</th>
<th>Group K ((n = 100))</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β</td>
<td>15.35 ± 0.22</td>
<td>15.99 ± 0.19</td>
</tr>
<tr>
<td>IL-2</td>
<td>29.53 ± 0.30</td>
<td>29.65 ± 0.18</td>
</tr>
<tr>
<td>IL-2Ra</td>
<td>24.23 ± 0.17</td>
<td>23.74 ± 0.17</td>
</tr>
<tr>
<td>IL-10</td>
<td>22.67 ± 0.22</td>
<td>23.84 ± 0.15</td>
</tr>
<tr>
<td>Foxp3</td>
<td>22.26 ± 0.22</td>
<td>21.13 ± 0.33</td>
</tr>
<tr>
<td>TLR-9</td>
<td>22.34 ± 0.17</td>
<td>22.43 ± 0.15</td>
</tr>
</tbody>
</table>

\(^1\) is a significant difference with the indicator of the group K, \(p < 0.05\)

As can be seen from the table, in women with RPL during the predicted window of implantation, changes in the transcriptional profile were observed due to a statistically significant decrease in the expression level of IL-10 mRNA – 22.67 ± 0.27 vs 23.84 ± 0.15.

IL-10 is a Th2-cytokine and is known to selectively suppress the Th1-mediated cellular response, inhibit the production of inflammatory cytokines [4], and also mediates inhibitory effects of Treg-cells. Treg-cells are essential for maintaining an immunological response to autoantigens and to suppress
excessive immune responses [10–12], including inflammation that has a detrimental effect on the human body. Treg-cells secrete an IL-10 cytokine that can inhibit the secretion of various inflammatory cytokines and inhibit the activation of Th1 and Th17 cells [7]. In particular, IL-10 carries a signal-effect via the IL-10 receptor, which results in inhibition of expression of the Th17-cytokine protein and retinoic acid-dependent orphan receptor, which reduces the amount of IL-17 produced and prevents exaggerated inflammatory and immune responses [6]. That is, IL-10 functions as a vital bridge that connects immunity, placental angiogenesis, inflammation and hypoxia in the maternal and fetal interface [5]. The decrease in the expression level of IL-10 mRNA in our study confirms its role in the development of inflammation in RPL. The literature also shows deficiencies in the number and/or function of Treg-cells in cases of miscarriage in RPL [13, 16].

CONCLUSION

RPL in the treatment of infertile women in ART programs is closely related to changes in the transcription profile of the endometrium during the expected window of implantation and with a decrease in the expression level of the mRNA of the IL-10 gene.

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Мета дослідження. Виявити особливості експресії мРНК генів запального компоненту імунної відповіді в період передбачуваного вікна імплантації у жінок зі звичним невиношуванням вагітності в програмах ДРТ.

Матеріал і методи. Обстежено 240 пацієнток, з їхнім звичним невиношуванням. Серед них 120 жінок з усіх територій України, що також поділялися на основну та контрольну групи. Їх вік складався від 20 до 40 років.

Мета дослідження. Виявити особливості експресії мРНК генів запального компоненту імунної відповіді в період передбачуваного вікна імплантації у жінок зі звичним невиношуванням вагітності в програмах ДРТ.

Матеріал і методи. Обстежено 240 жінок з приватним невиношуванням вагітності в програмах ДРТ.

Висновки. Статистично значимої різниці в кількості пацієнтів з приватним невиношуванням у контрольній та основній групах не виявлено.

Ключові слова: безпліддя, звичне невиношування, ДРТ, ендометрій, вікна імплантації, IL-1β, IL-2, IL-10, Foxp3, TLR9, IL-2Rα.

Заголовок: Експресія мРНК генів запального компоненту імунної відповіді в період передбачуваного вікна імплантації у жінок зі звичним невиношуванням вагітності в програмах ДРТ

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