

Immunological interaction of organisms of the women with infertility included in the IVF program

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It is known from scientific research that immune effector mechanisms that are under the regulatory influence of the pituitary gonadotropins and sex hormones at the local level become critical for successful implantation. In such patients, at the pre-conceptual stage and at the stage of the program of fertilization of «in vitro fertilization» (IVF), pathological mechanisms of violation of trophoblast invasion are triggered, in which activated in the early stages of pregnancy development, the immune-pathological process leads to the implementation of the mechanism of miscarriage. Since all the processes of immunological recognition, protection or detachment in married couples with unsuccessful implantation attempts within the IVF program in early pregnancy, undergo directly at the conception cycle, immunotherapy should be started immediately before pregnancy, which creates preconditions for the formation of complete trophoblast invasion.

The objective: to evaluate some of the indicators of the cytokine profile in women with infertility and clinical manifestations of subchorionic hematoma in the first trimester of pregnancy.

Patients and methods. We have performed a clinical and laboratory-instrumental examination of 80 women with infertility, treated under the conditions of IVF program, with manifestations of subchorionic hematoma. The control group included 20 patients with no reproductive function impairment. In parallel, studies and analysis of the peculiarities of vascular endothelial function and cytokine profile were performed using immunoassay.

Results. At the pre-conceptual stage against the background of accustomed miscarriage in anamnesis, an increase in 3,2 times of IL-1 β concentration in the peripheral blood of the studied groups of women ($p < 0,05$), TNF- α – in 7,3 times and a decrease in 2,6 times of the content of IL-4 against the data of the control group ($p < 0,01$) were observed. Estimation of the cytokine status in this category of patients during the nidation stage and the development of the fetal egg allowed to mark a progressive increase in 3,6 times of the IL-1 β level, as well as a significantly higher was percentage of TNF- α level in 4 times as compared to the control ($p < 0,05$), while IL-4 level remained lower in 3,0 times as for the control indices ($p < 0,05$).

Conclusions. The performed studies of the immune system in pregnant women with treated infertility showed moderate inhibition and an imbalance of immunological parameters characteristic for pregnancy with the presence of maternal suppression. The analysis of the obtained results testifies to the necessity of general immune-correction and prophylactic treatment in this category of patients. Thus, it is evident that the inadequate activation of the local protective factors of the immune system plays a major role in the pathogenetic mechanisms of miscarriage initiating, which requires the expansion of the boundaries of scientific research and is a promising direction for further research.

Key words: *infertility, miscarriage, subchorionic hematoma, auxiliary reproductive technologies, cytokines.*

It is known from scientific research that immune effector mechanisms, being under the regulatory influence of pituitary gonadotropins and sex hormones at the local level, become critical for successful implantation [2]. In such patients, at the pre-conceptual stage and at the stage of in vitro fertilization program (IVF), pathological mechanisms of trophoblast invasion disorders are triggered, the so-called “vicious circle” is formed, in which the activated immune-pathological process in the early stages of development of pregnancy leads to the implementation of the miscarriage mechanism [1, 8].

Taking into account the above-mentioned data, the specialists are united in the opinion of the necessity of fundamentally different therapeutic tactics in case of the use of assisted reproductive technologies, taking into account the methods of immunological examination and immune correction [7]. According to scientific sources, nowadays, after numerous studies and comprehensive discussion, it is considered generally known that immunological disorders, which are at the basis of early miscarriages, are caused by alloimmune and autoimmune mechanisms. In autoimmune processes a fertilized egg is affected by thrombosis of the trophoblast vessels, limitation of its invasion, as well as a result of a direct damaging effect of auto-antibodies on phospholipids of the forming placenta [5]. In alloimmune responses, the cellular immune reaction of a woman is directed against antigens of an embryo of paternal origin; this also creates barriers in the formation of protective immune-modulation [8]. Endometrium is an active place of production and the influence of cytokines. The embryo is capable of communicating with the endometrium using cytokines and cytokine receptor language. The necessary steps in implantation mechanisms are cytokine dialogue at the implantation stage and the formation of a fetal egg [3]. Alongside with this fact, it is known that among other mediators of intercellular interaction a special role belongs to the so-called growth factors, among which the TGF- β – cytokine of immunosuppressive action deserves attention [4]. The physiological level of this immunosuppressive cytokine, necessary for the normal course of pregnancy, is ensured by intensive synthesis at the level of the fetoplacental complex. TGF- β regulates the processes of trophoblast invasion, possessing immunosuppressive properties, synthesis of pro-inflammatory cytokines (IL-1, IL-6, IL-8, TNF- α), reduces their production in amnion and cells of the decidual membrane and suppresses NK-cells activated by lymphokines [4]. The influence of TGF- β on the course of pregnancy remains poorly understood, there are only some reports of changes in its content during the complicated course of pregnancy [4].

Data of scientific studies, covering the problem of reproductive immunology, state that with the activation of immune mechanisms in the pathogenesis of local non-progressive chorion detachment and repeated unsuccessful attempts of auxiliary reproductive technologies, the chance of successful carriage without optimal therapy is quite low (about 30%) [9]. However, despite the topicality of these points, many of the problems associated with reasoning of the advisability of the use of immunomodulatory therapy methods, remain unresolved. Thus, not all

researchers see the necessity of the appointment of gestogens, corticosteroids, the discussion issues of the beginning and duration of the courses of antithrombotic therapy remain controversial, etc. [6].

Since all the processes of immunological recognition and, as a result, protection or detachment in married couples with unsuccessful implantation attempts under the IVF program in early periods of pregnancy undergo directly in the conception cycle, in connection with what immune-correcting therapy should be started immediately before pregnancy, that creates the preconditions for the formation of a complete trophoblast invasion.

The objective: of this work was to evaluate some of the indicators of the cytokine profile in women with treated infertility and clinical manifestations of subchorionic hematoma in the first trimester of pregnancy.

PATIENTS AND METHODS

To solve these problems, we have performed the detailed clinical and laboratory-instrumental examination of 80 women with infertility, treated according to the IVF program, and with manifestations of subchorionic hematoma. The control group included 20 patients without disorder of reproductive function, without somatic and gynecological pathology.

In parallel, studies and analysis of the peculiarities of vascular endothelial function and cytokine profile were performed by immunoassay. An important step of our research was to study the peculiarities of immune regulation in this category of patients by identifying the products of cytokines belonging to the two polar types of the immune response – Th1 and Th2, as well as assessing the level of TGF- β and the degree of their effect realization. These parameters were evaluated by enzyme immunoassay method using the «StatFax 303 Plus» (USA) with the help of «ProCon IL-1 β » reagent kits (Proteinovy Kontur Ltd., Russia), «Accucyte Human IL-4» (Cytimmune Sciences Inc., USA), «Biotrak FNH human ELISA system» (Amersham Pharmacia Biotech, UK). To determine the content of IL, a solid-phase enzyme-linked immunosorbent method using chromium peroxidase as an indicator enzyme was used.

All types of statistical processing are performed using the standard package «Statistica for Windows – 6.0». The reliability of the obtained results was determined using Student's criterion.

RESULTS

An analysis of the age composition of women included in assisted reproductive technology programs has shown that more often early reproductive loss was observed in patients aged 26–30 years and more – in 48,27% of cases, that is, the genesis of this complication has a close relationship with the long-term effect, and hence, a more pronounced degree of neuro-endocrine and psycho-emotional changes. In 52,5% of patients there were clinical signs of the threat of abortion with more than two episodes of bloody discharge, at the pre-conceptual stage, the diagnostic algorithm allowed the establishment in 46,25% – chronic genital infections, and in half of cases – ureaplasma infection (21,25%), in 13,75% – chlamydia, in 18,75% – a viral infection, in 7,5% – mycoplasma one. Thus, a high percentage of urogenital infection in women with infertility, whose pregnancy was complicated by the development of clinical manifestations of subchorionic hematoma and early reproductive losses, as well as a high percentage of vaginal dysbiosis, attract attention.

The evaluation of the effectiveness of ART programs in this category of patients allowed marking in 38,75% of cases more than three attempts of IVF in anamnesis, in 52,5% of observations the IVF program was performed for the first time.

As our studies have shown, at the preconceptional stage in this category of patients there is an increase of the concentration of IL-1 β in the peripheral blood (up to 96,32 \pm 1.66 pg/ml)

($p<0,05$) – in 3,2 times, TNF- α – in 7,3 times and a decrease in 2,6 times of the content of IL-4 (up to 5,12 \pm 0,22 pg/ml) compared with the control group data ($p<0,01$). Normally, trophoblast and fetoplacental macrophages synthesize TNF- α and IL-1 β , which affect the functional activity of the endothelium, as well as their production in the villous trophoblast increases in hypoxia. It can be assumed that an increase of TNF- α production leads to a change in the function of the endothelium, an increase of endothelin-1 production, and a decrease in the synthesis of nitric oxide in this category of patients. In addition, erythropoietin, which is one of the transcription regulators under hypoxia, is synthesized in the placental cells [8]. As it is well-known, the embryo, having passed the epithelium and completing the invasion, secretes its own IL-1 β and possibly a number of other cytokines, induces receptors for IL-1 β in surrounding stroma, which provides the implantation process [8]. Thus, a significantly high level of IL-1 β can be a marker of impaired processes of implantation and placentation, as well as serve as a criterion for premature detachment of chorion and the formation of subchorionic hematomas.

According to literature data, the difference in TNF- α and IL-1 β level changes is in favor of a prolonged ischemia of the chorion and placenta, leading to the formation of primary placental insufficiency [8]. In addition, it is known that during physiological pregnancy, the content of IL-1 β in the blood varies depending on the terms of placentation [3]. In this research the study of its concentration in women from the group of risk as for the unsuccessful IVF attempts, an imbalance of pro- and anti-inflammatory interleukins was detected at preconceptional stages, IL-4 was depressively reduced in 61,25% of women, and in patients with successful attempt of implantation and transplantation of embryo – was within the control group's indexes.

The evaluation of the cytokine status in this category of patients during the nidation stage and the fetal egg development allowed marking a dynamically progressive increase of IL-1 β level in 3,8 times, as well as a significantly higher concentration of TNF- α – in 4,0 times compared to control ($p<0,05$). While the level of IL-4 remained low, it was 3,0 times lower against the control data ($p<0,05$). Similar results were obtained regarding the low level of TGF- β in peripheral blood at the monitoring stage of the first trimester of pregnancy, especially notional these indexes were in patients with recurrent episodes of subchorionic hematoma and their progressive course. The obtained results indicate an additional activation of Th1 and relative selective Th2-type suppression of the immune response in this category of patients at the preconception stage. The study of Th1 cytokine levels and their natural antagonists, synthesized by the Th2-type, determined that in the dynamics of the first trimester of pregnancy in patients with clinical manifestations of subchorionic hematoma included in the IVF program, there is a more pronounced prevalence of pro-inflammatory cytokines, especially of TNF- α level over the regulatory cytokines. It is important that in women from the group of risk, there was a significant increase of TNF- α and IL-4 depression characterized by immunosuppressive activity that provides implantation of the embryo. Thus, high levels of IL-1 β and TNF- α may be markers for impaired processes of implantation and placentation, while lowering of IL-4 level is a prognostic unfavorable factor, whereas in women with successful IVF attempt and embryo transfer, the elevated level of IL-4 is accompanied by high indicator of the effectiveness of IVF program and prolongation of pregnancy.

This suggests that in such a group of pregnant women there is an imbalance of not only hormonal homeostasis and neuro-protective defense, but also in the immune chain of regulation of the activity of adaptational processes of the organism. In pregnant women included in the IVF program, these data increase during the first trimester of gestation, which suggests that in the

diagnosis and complex treatment of such a category of pregnant women it is necessary to correct the medicinal therapy differentially. There were performed studies of the immune system and the current literature data show moderate inhibition and imbalance of immunological parameters characteristic of pregnancy with the presence of maternal suppression in pregnant women with treated infertility. With the progression of pregnancy progressive growth of the immune deficiency was noted – with violation of immune-regulation processes and with the transition of quantitative immunodeficiency to a qualitatively new condition – the change of immune-regulatory processes, as a result of which the process of maternal immune-suppression was violated. The analysis of the obtained results testifies to the necessity of general immune-correction and prophylactic treatment in this category of patients.

CONCLUSIONS

Thus, a change in the balance of pro-inflammatory cytokines can induce structural and functional changes in endothelial cells. In addition, the increased production of pro-inflammatory cytokines on the background of insufficient expression of anti-inflammatory IL-4 and TGF- β , which has immunosuppressive

Иммунологические взаимодействия организма у женщин с бесплодием, включенных в программу IVF Н.В. Литвин

Из научных исследований известно, что иммунные эффекторны механизмы, находясь под регуляторным влиянием гипофизарных гонадотропинов и половых гормонов на локальном уровне, становятся критическими для успешной имплантации. У таких пациенток еще на прекоцепционном этапе и на этапе программы оплодотворения *in vitro fertilization (IVF)* запускаются патологические механизмы нарушения инвазии трофобласта, при которых активированный на ранних этапах развития беременности иммунопатологический процесс приводит к реализации механизма выкидыша. Все процессы иммунологического распознавания, защиты или отторжения у супружеских пар с неудачными попытками имплантации в рамках программы IVF на ранних сроках беременности проходят непосредственно в цикле зачатия, поэтому иммунокорригирующая терапия должна быть начата непосредственно к наступлению беременности, что создает предпосылки к формированию полноценной инвазии трофобласта.

Цель исследования: оценка некоторых показателей цитокинового профиля у женщин с бесплодием и клиническими проявлениями ретрохориальной гематомы в I триместре беременности.

Материалы и методы. Проведено клиническое и лабораторно-инструментальное обследование 80 женщин с бесплодием, леченным в рамках программы IVF, с проявлениями ретрохориальной гематомы. В группу контроля вошли 20 пациенток без нарушения репродуктивной функции. Параллельно было проведено исследование и анализ особенностей функции эндотелия сосудов и цитокинового профиля методом иммуноферментного анализа.

Результаты. На прекоцепционном этапе на фоне привычного невынашивания в анамнезе наблюдается рост в 3,2 раза в периферической крови женщин исследуемых групп концентрации IL-1 β ($p < 0,05$), TNF- α в 7,3 раза и снижение в 2,6 раза содержания IL-4 против данных группы контроля ($p < 0,01$). Оценка цитокинового статуса у данной категории пациенток на этапе nidации и развития плодного яйца позволила отметить прогрессирующее повышение в 3,8 раза уровня IL-1 β , а также достоверно выше был процентный уровень TNF- α – в 4,0 раза по сравнению с контролем ($p < 0,05$). В то время как уровень IL-4 оставался ниже в 3,0 раза по сравнению с показателями контроля ($p < 0,05$).

Заключение. Проведенные исследования иммунной системы у беременных с леченым бесплодием показали умеренное угнетение и дисбаланс иммунологических показателей, характерных для беременности с наличием процесса материнской супрессии. Анализ полученных результатов свидетельствует о необходимости проведения общей иммунокоррекции и профилактического лечения у данной категории пациенток. Таким образом, очевидным является факт, что в инициации патогенетических механизмов невынашивания основную роль играет недостаточная активация местных защитных факторов иммунной системы, что требует расширения границ научного поиска и является перспективным направлением дальнейших исследований.

Ключевые слова: бесплодие, невынашивание беременности, ретрохориальная гематома, вспомогательные репродуктивные технологии, цитокины.

effect, can lead to an inadequate inflammatory response from the mother's organism and, as a consequence, to a violation of the invasive capacity of the trophoblast.

The obtained data confirm that in the patients with treated infertility and subchorionic hematomas in the first trimester of pregnancy, the increase of the level of pro-inflammatory cytokines – IL-1 β , TNF- α with the simultaneous decrease of the level of anti-inflammatory IL-4 and TGF- β is observed. This suggests that in such a group of pregnant women there is an imbalance of not only in the metabolic and hormonal homeostasis, but also in the immune-regulatory link of implantation mechanisms, and in pregnant women with signs of subchorionic hematoma these indices grow even more pronounced. The obtained by us results demonstrate a significant violation of local immune responses conditioned by the imbalance of cytokine production, that in future may initiate the implementation of miscarriage mechanisms.

It is obvious that the inadequate activation of the local protective factors of the immune system plays an important role in the initiating of pathogenetic mechanisms of miscarriage, which requires the expansion of the boundaries of scientific research and is a promising direction for further research.

Имунологічні взаємодії організму у жінок із безплідністю, включених у програму IVF Н.В. Литвин

Із наукових досліджень відомо, що імунні ефекторні механізми, знаходячись під регуляторним впливом гіпофізарних гонадотропінів та статевих гормонів на локальному рівні, стають критичними для успішної імплантації. У таких пацієнток ще на прекоцепційному етапі та на етапі програми запліднення *in vitro fertilization (IVF)* запускаються патологічні механізми порушення інвазії трофобласта, при яких активований на ранніх етапах розвитку вагітності імуннопатологічний процес призводить до реалізації механізму викидня. Усі процеси імуннологічного розпізнавання, захисту або відторгнення у подружніх пар із невдалими спробами програми IVF на ранніх термінах вагітності проходять безпосередньо у циклі зачаття, тому імунотропінальна терапія повинна бути розпочата безпосередньо до настання вагітності, що створює передумови для формування повноцінної інвазії трофобласта.

Мета дослідження: оцінювання деяких показників цитокинового профілю у жінок із безплідністю та клінічними проявами ретрохоріальної гематоми у I триместрі вагітності.

Матеріали та методи. Проведено клінічне та лабораторно-інструментальне обстеження 80 жінок із безплідністю, лікованою у рамках програми IVF, із проявами ретрохоріальної гематоми. У групу контролю увійшли 20 пацієнток без порушення репродуктивної функції. Паралельно було проведено дослідження та аналіз особливостей функції ендотелію судин та цитокинового профілю методом імунотропінального аналізу.

Результати. На прекоцепційному етапі на тлі звичного невиношування в анамнезі спостерігається зростання у 3,2 разу у периферійній крові жінок досліджуваних груп концентрації IL-1 β ($p < 0,05$), TNF- α у 7,3 разу та зниження у 2,6 разу вмісту IL-4 проти даних групи контролю ($p < 0,01$). Оцінювання цитокинового статусу у даної категорії пацієнток на етапі nidації та розвитку плідного яйця дозволило відзначити прогресуюче підвищення у 3,8 разу рівня IL-1 β , а також достовірно вищий був відсотковий рівень TNF- α – у 4,0 разу порівняно з контролем ($p < 0,05$). У той час як рівень IL-4 залишався нижчим у 3,0 разу від показників контролю ($p < 0,05$).

Заключення. Проведені дослідження імунної системи у вагітних з лікованою безплідністю засвідчили помірне пригнічення та дисбаланс імуннологічних показників, характерних для вагітності з наявністю процесу материнської супресії. Аналіз отриманих результатів свідчить про необхідність проведення загальної імунотропінальної та профілактичного лікування у даної категорії пацієнток. Отже, в ініціації патогенетичних механізмів невиношування основну роль відіграє недостатня активізація місцевих захисних факторів імунної системи, що вимагає розширення меж наукового пошуку та є перспективним напрямком подальших досліджень.

Ключові слова: безплідність, невиношування вагітності, ретрохоріальна гематома, допоміжні репродуктивні технології, цитокини.

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НОВОСТИ МЕДИЦИНЫ

ОТКАЗ ОТ УПОТРЕБЛЕНИЯ ТРАНСЖИРОВ
ПОНИЗИТ РИСК ИНСУЛЬТА

Исследователи из Йельского университета обнаружили, что отказ от употребления продуктов с высоким содержанием трансжиров может снизить риск возникновения инсульта и сердечного приступа.

Трансжирные кислоты или трансжиры содержатся в таких продуктах, как чипсы, крекеры, жареные продукты и хлебобулочные изделия.

Такие выводы ученые сделали после изучения статистики госпитализации людей в штате Нью-Йорк с 2002 по 2013 годы. В тех округах штата Нью-Йорка, где население в течение трех последних лет сократило употребление

трансжиров, число обращений в медицинские учреждения из-за случаев сердечного приступа и инсульта в среднем сократилось на 6,2 процента (по сравнению с соседними регионами).

"Сведение к минимуму или исключение из рациона трансжиров может значительно снизить риск возникновения инсульта и сердечного приступа... Запрет трансжиров на государственном уровне может стать настоящей победой для миллионов людей", - заявил один из авторов исследования.

Трансжирные кислоты или трансжиры содержатся в таких продуктах, как чипсы, крекеры,

жареные продукты и хлебобулочные изделия. Употребление продуктов с высоким содержанием трансжиров повышает риск сердечно-сосудистых заболеваний, которые являются основной причиной смерти во всем мире. В последние годы в Нью-Йорке и других американских городах принимаются меры по сокращению трансжиров в ресторанах и закусочных. Управление по контролю над продуктами и лекарствами США (FDA) заявило о решении исключить трансжиры из продуктов питания полностью к 2018 году.

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