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The Role of Immunological Mechanisms in Physiological Pregnancy and the Consequences of Disturbing Them during *in vitro* Fertilization

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Abstract

We observed the immunologic status of clinically healthy women and women with tuboperitoneal infertility during the planning and first trimester of pregnancy. The markers of unsuccessful *in vitro* fertilization (IVF) attempts were also identified. No pathological anomalies were noted in the clinically healthy women before and after conception. The changes in the immune system of infertile women who underwent IVF were different from the women who had physiologic pregnancy. Identification of these changes could help to guide preventive measures while preparing for IVF and during the IVF procedure.(**International Journal of Biomedicine. 2020;10(2):116-119.**)

Key Words: pregnancy • infertility • *in vitro* fertilization • immunologic status

Introduction

Studying the immunological basis of the mother-fetus interaction is one of the priorities of modern medicine. Complex immune interactions that occur during pregnancy follow the principle of direct and reverse connection.[1-3] The outcome (termination or preservation of pregnancy) depends on maintaining a balance of the complex immune interactions.[4] This problem of maintaining regulatory balance is more relevant in patients having pregnancy via *in vitro* fertilization (IVF). At the present stage of studying IVF outcomes, increasing the effectiveness of the IVF procedure is directly related to studies in the field of immunological regulation of the reproductive function of women.[5]

Immunocompetent cells (macrophages, lymphocytes) and cytokines produced by these cells (interleukines, growth factors and chemokines) play a role in folliculogenesis, ovulation, and yellow body formation and function, as well as in cyclic morphofunctional endometrial changes, fertilization and egg implantation.[6,7] Therefore, assessment of cell-mediated and humoral immunity, as well as cytokine balance, is crucial for detection of markers that may indicate an unsuccessful outcome of IVF.

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Materials and Methods

Our study was open-label, prospective and comparative. Group 1 (main group) included 70 healthy women (mean age of 25.69±0.14 years) prior to conception and in the first trimester of pregnancy. Group 2 (IVF+) included 25 women (mean age of 30.28±0.50 years) with tuboperitoneal infertility before IVF and in the first trimester of pregnancy. Group 3 (IVF-) included 45 women (mean age of 32.29±0.32 years) with tuboperitoneal infertility and an unsuccessful IVF attempt. The study was approved by the Tyumen State Medical University Ethics Committee (protocol N 68 dated 08.04.2016). Written informed consent was obtained from all participants.

The blood tests were taken twice: during the planning of pregnancy and 8 weeks after conception/IVF procedure. Blood samples were collected in accordance with the existing requirements in the morning on an empty stomach from the cubital vein in commercial heparin tubes with heparin (25 IU/ml).

The blood tests included:

•Total blood count in the blood smears, stained by the Romanovsky-Giemsa method

•Immunophenotyping of peripheral blood lymphocytes using an expanded panel of monoclonal antibodies to differentiation antigens (CD3+, CD4+, CD8+, CD16+, CD20+, CD23+, and CD38+). Lymphocytes were isolated by centrifugation on a ficoll-verographin gradient. Lymphocyte

subpopulations were determined on a FACScan (Beckton-Dickinson, USA) flow cytofluorometer equipped with an argon laser

•Determination of the concentration of cytokines (IL-1, IL-4, IL-6, IL-10, IFNɣ, and TNFα) in cell culture supernatants by flow immunofluorometry using a double-beam laser automated analyzer (Bio-Plex® Suspension Array System, Bio-Rad, USA, Bio-Rad, USA)

•Determination of the concentration of serum immunoglobulins (IgG, IgM, IgA) by the method of radial immunodiffusion (Mancini method)

Statistical analysis was performed using the Statistica 10.0 software package (Stat-Soft Inc., USA). The normality of distribution of continuous variables was tested by the Kolmogorov-Smirnov test with the Lilliefors correction and Shapiro-Wilk test. Results are presented as median (interquartile range [IQR]) (Me[25-75])). Differences of continuous variables were tested by the Mann-Whitney U-test.. The Wilcoxon criterion was used to compare the differences between the paired samples. A probability value of $P<0.05$ was considered statistically significant.

Results

Complex evaluation of cell-mediated immunity during the planning of pregnancy showed a statistically significant rise in the count of leukocytes with a tendency to lymphopenia in Group 3, compared to Group 1 (Table 1). Analysis of the lymphocyte subpopulations in Group 2 before the IVF procedure revealed activation of the T-system immunity due to an increase in the level of CD3+T cells and CD4+T cells. Group 3 had a statistically significant drop in these cells. The level of effector CD8+cells was also significantly reduced in women with an unsuccessful IVF attempt. The highest level of CD16+natural killer (NK) cells was found in Group 2, and a statistically significant decrease was observed in Group 3. The level of B cells before pregnancy and the IVF program was also different in all groups. A statistically significant activation of B cells due to an increase of CD20+B cells and CD22+ B cells was found in Group 3 (Table 1).

In the first trimester of pregnancy (8 weeks after conception), in Group 1, we found a statistically significant decrease in the levels of CD3+T cells and CD4+T cells, and an increase in the level of CD20+cells and CD22+cells. Similar changes were found in Group 2, but the decrease in the level of CD3+T cells and CD4+T cells was more pronounced than in Group 1. A statistically significant increase in the level of CD16+NK cells and CD20+B cells was also found in Group 2. In Group 3**,** 8 weeks after an unsuccessful IVF procedure, we found a statistically significant increase in the level of CD3+T cells and a decrease in the levels of CD8+T cells and CD22+B cells (Table 1).

Cytokines are actively involved in the processes occurring in the fetoplacental complex, namely in regulating the normal development of the fetus and implementing the mechanisms of the complicated course of pregnancy.[8] Blood cytokine levels are presented in Table 2. At the stage of pregnancy planning, a statistically significant increase in the level of IL-1α was observed in Groups 2 and 3, as well as TNFα with the highest values in Group 3. The IFNγ level showed significant variability. Before conception, the IFNγ level was within normal values in Group 1 and significantly elevated in Group 2. In Group 3, the IFNγ

Table 1.

The lymphocyte subpopulations in study groups

Indicators	Group 1 (n=70)			Group 2 (n=25)			Group 3 (n=45)		
	before	*P*	after	before	*P*	after	before	*P*	after
Leukocytes, $\times10^9$/L	6.0$^{\#\#\#}$ (5.6;6.2)	0.650	6.0$^{\#\#\#}$ (5.8;6.5)	6.1$^{\wedge\wedge\wedge}$ (5.9;6.5)	0.002	7.0$^{\wedge\wedge\wedge}$ (6.7;7.5)	7.2$^{\#\#\#\wedge\wedge\wedge}$ (7.0;7.4)	0.004	7.9$^{\#\#\#\wedge\wedge\wedge}$ (7.8;7.9)
Lymphocytes,%	24.0$^{**\#\#\#}$ (23.0;25.0)	0.004	22.0$^{**\#\#}$ (22.0;23.0)	25.0$^{**\wedge\wedge\wedge}$ (24.0;26.0)	0.000	22.0$^{**\wedge\wedge\wedge}$ (21.0;23.0)	21.0$^{\#\#\#\wedge\wedge\wedge}$ (20.0;22.0)	0.000	28.0$^{\#\#\wedge\wedge\wedge}$ (27.0;28.0)
CD3+	64.2$^{***\#\#\#}$ (62.2;65.4)	0.000	57.5$^{***\#\#\#}$ (56.5;57.6)	65.7$^{***\wedge\wedge\wedge}$ (65.2;66.5)	0.000	56.7 $^{***\wedge\wedge\wedge}$ (55.9;57.6)	58.2$^{\#\#\#\wedge\wedge\wedge}$ (57.7;59.1)	0.030	60.2$^{\#\#\#\wedge\wedge\wedge}$ (58.8;61.1)
CD4+	34.3$^{*\#\#\#}$ (34.2;35.4)	0.003	29.9$^{*\#\#\#}$ (28.2;31.2)	35.8$^{*\wedge\wedge\wedge}$ (33.8;36.9)	0.000	27.0$^{*\wedge\wedge\wedge}$ (26.1;30.3)	27.9$^{\#\#\#\wedge\wedge\wedge}$ (25.9;29.0)	0.356	29.0$^{\#\#\#\wedge\wedge\wedge}$ (27.9;29.8)
CD8+	21.7$^{\#\#}$ (20.9;22.5)	0.000	27.3$^{\#\#}$ (26.8;27.8)	22,3$^{\wedge\wedge}$ (21.0;22.7)	0.061	22.4$^{\wedge\wedge}$ (22.2;23.3)	20.7$^{\#\#\wedge\wedge}$ (18.7;21.9)	0.005	18.8$^{\#\#\wedge\wedge}$ (17.9;19.0)
CD4+/CD8+	1.6$^{\#\#}$ (1.5;1.6)	0.000	1.1$^{\#\#}$ (1.09;1.2)	1.6$^{\wedge}$ (1.5;1.7)	0.001	1.2$^{\wedge}$ (1.3-1.4)	1.3$^{\#\#\wedge}$ (1.3;1.5)	0.062	1,5$^{\#\#\wedge}$ (1.2;1.3)
CD16+	15.7$^{***\#\#\#}$ (15.0;16.4)	0.031	16.5$^{***\#\#\#}$ (16.1;17.0)	17.0$^{***\wedge\wedge\wedge}$ (16.3;17.5)	0.001	19.4$^{\#\#\#\wedge\wedge\wedge}$ (18.9;19.8)	13.9$^{\#\#\#\wedge\wedge\wedge}$ (13.6;14.0)	0.060	12.1$^{\#\#\#\wedge\wedge\wedge}$ (11.7;12.5)
CD20+	16.0$^{\#\#\#}$ (15.3;16.5)	0.000	22.0$^{\#}$ (20.5;23.2)	15.5$^{*\wedge\wedge\wedge}$ (15.1;15.9)	0.000	21.0 (19.2;21.3)	21.7$^{\#\#\#\wedge\wedge\wedge}$ (20.9;22.0)	0.255	20.9$^{\#}$ (20.1;21.2)
CD22+	7.5$^{\#\#\#}$ (7.4;7.7)	0.042	8.7$^{*\#\#\#}$ (7.6;8.9)	8.8$^{\wedge\wedge\wedge}$ (8.2;9.3)	0.350	9.0$^{*\wedge\wedge\wedge}$ (8.1;9.2)	9.8$^{\#\#\#\wedge\wedge\wedge}$ (9.0;10.0)	0.001	5.9$^{\#\#\#\wedge\wedge\wedge}$ (5.8;5.9)

Footnotes:

** - statistically significant differences between Groups 1 and 2: * - P<0.05; ** - P≤0.01; *** - P≤0.000*

 # - statistically significant differences between Groups 1 and 3: # - P<0.05; ## - P≤0.01; ### - P≤0.000

^ - statistically significant differences between Groups 2 and 3: ^ - P<0.05; ^^ - P≤0.01; ^^^ - P≤0.000

level was significantly decreased against the background of high values of IL-4, IL-6 and IL-10. Groups 2 and 3 showed an increase in the IL-6 level during the preconception period, compared to Group 1 (Table 2).

In the first trimester of pregnancy, in Group 1 we found an increase in the level of IL-4 and IL-6, and a decrease in the IFNγ level. In Group 2, we observed a significant increase in the IL-1α level against the background of decreased levels of IFNγ and TNFα. At the same time, the level of IL-4 significantly increased, and the level of IL-6 did not change, but significantly exceeded the indicator of Group I. In Group 3, 8 weeks after an unsuccessful IVF procedure, we found a decrease in the IL-1α level, but it was significantly higher than in Group 1. Against this background, the levels of IFNγ and TNFα increased, and IL-4 and IL-10 decreased, while IL-6 did not change in dynamics.

The concentration of serum immunoglobulins is presented in Table 3. At the stage of pregnancy planning, the IgA level was decreased in Group 2 and increased in Group 3, compared to Group 1. The IgM level in Groups 2 and 3 was significantly higher than in Group 1, with the highest values being determined in Group 3. Group 3 had also the highest IgG level.

In the first trimester of pregnancy, no significant changes in the immunoglobulin levels were found in Group 1. In Group 2, we observed a statistically significant decrease in the level of IgM and IgG, while the IgA level did not change. In Group 3, a decrease in all studied immunoglobulins was detected 8 weeks after an unsuccessful IVF procedure, at that the IgM level was significantly higher than in Group 2.

Discussion

Analysis of cell-mediated immunity showed a significant difference between the 3 groups of women at the preconception stage. Group 1 had no significant immunologic abnormalities during the study. In women of Group 3, T-cell immunity depression against the background of a tendency to neutrophilic

Table 2

Blood cytokine levels in study groups

Indicators (pkg/ml)	Group 1 (n=70)			Group 2 (n=25)			Group 3 (n=45)		
	before	*P*	after	before	*P*	after	before	*P*	after
IL-1α	14.0$^{***\#\#\#}$ (13.9;14.3)	0.120	12.1$^{***\#\#\#}$ (12.0;12.2)	22.1$^{***\wedge\wedge\wedge}$ (22.0;22.2)	0.005	26.0$^{***\wedge\wedge\wedge}$ (25.9;26.2)	99.5$^{\#\#\#\wedge\wedge\wedge}$ (99.3;99.6)	0.008	96.0$^{\#\#\#\wedge\wedge\wedge}$ (95.9;96.2)
IFN γ	152.0$^{***\#\#\#}$ (149.0;156.0)	0.000	122,0$^{***\#\#\#}$ (119.0;129.0)	270.0$^{***\wedge\wedge\wedge}$ (261.0;272.0)	0.007	257.0$^{***\wedge\wedge\wedge}$ (255.0;260.0)	96.0$^{\#\#\#\wedge\wedge\wedge}$ (94,0;105,0)	0.000	165.0$^{\#\#\#\wedge\wedge\wedge}$ (163.0;166.0)
TNFα	18.1$^{***\#\#\#}$ (18.0;18.2)	0.000	26.1$^{\#\#\#}$ (26.0;26,25)	38.12$^{***\wedge\wedge\wedge}$ (36.1;42.4)	0.000	26.1$^{\wedge\wedge\wedge}$ (26.0; 26.25)	92.0$^{\#\#\#\wedge\wedge\wedge}$ (87.0;103.0)	0.046	98.4$^{\#\#\#\wedge\wedge\wedge}$ (98.2;98.5)
IL-4	43.11$^{\#}$ (43.10;43.12)	0.005	45.1$^{\#}$ (45.07;45.14)	42.11$^{\wedge\wedge}$ (42.0;42.12)	0.005	46.11$^{\wedge\wedge\wedge}$ (45.1;47.12)	59.25$^{\#\wedge\wedge}$ (56.2;60.50)	0.001	36.11$^{\#\wedge\wedge\wedge}$ (36.09;36.12)
IL-6	35,2$^{***\#\#\#}$ (35.1;35.4)	0.010	39.5$^{**\#\#\#}$ (39.0;39.8)	55.2$^{***\wedge\wedge\wedge}$ (55.0;55.4)	0.250	56.0$^{***\wedge\wedge\wedge}$ (55.7;56.3)	110.3$^{\#\#\#\wedge\wedge\wedge}$ (110.0-110.5)	0.331	109.1$^{\#\#\#\wedge\wedge\wedge}$ (103.0;113.2)
IL-10	8.8$^{\#\#}$ (7.5;9.1)	0.310	9.0$^{\#\#}$ (8.2;9.3)	9.3$^{\wedge\wedge\wedge}$ (9.0;9.6)	0.305	9.8$^{\wedge\wedge}$ (8.9;10.0)	16.5$^{\#\#\wedge\wedge\wedge}$ (15.9;17.7)	0.001	12.6$^{\#\#\wedge\wedge}$ (10.4;13.5)

Footnotes:
** - statistically significant differences between Groups 1 and 2: * - P<0.05; ** - P≤0.01; *** - P≤0.000*
 # - statistically significant differences between Groups 1 and 3: # - P<0.05; ## - P≤0.01; ### - P≤0.000
^ - statistically significant differences between Groups 2 and 3: ^ - P<0.05; ^^ - P≤0.01; ^^^ - P≤0.000

Table 3.

The concentration of serum immunoglobulins in study groups

Indicators (g/L)	Group 1 (n=70)			Group 2 (n=25)			Group 3 (n=45)		
	before	*P*	after	before	*P*	after	before	*P*	after
Ig A, g/L	1.55$^{***\#\#\#}$ (1.54;1.55)	0.786	1.55$^{***\#\#\#}$ (1.49;1.57)	1.11$^{***\wedge\wedge\wedge}$ (1.10;1.12)	0.444	1.2$^{***\wedge\wedge\wedge}$ (1.15;1.25)	1.89$^{\#\#\#\wedge\wedge\wedge}$ (1.87;1.91)	0.001	1.0$^{\#\#\#\wedge\wedge\wedge}$ (0.98;1.05)
Ig M, g/L	1.19$^{***\#\#\#}$ (1.17;1.20)	0.395	1.19$^{***\#\#\#}$ (1.17;1.19)	1.27$^{***\wedge\wedge\wedge}$ (1.25;1.28)	0.001	0.97$^{***\wedge\wedge\wedge}$ (0.96;0.97)	17.29$^{\#\#\#\wedge\wedge\wedge}$ (17.28;17.31)	0.000	1.64$^{\#\#\#\wedge\wedge\wedge}$ (1.64;1.65)
Ig G, g/L	15,95$^{\#\#\#}$ (14.98;15.98)	0.151	16.97$^{***\#\#\#}$ (16.95;16.98)	16.0$^{\wedge\wedge\wedge}$ (16.56;16.06)	0.000	11.05$^{***\wedge\wedge\wedge}$ (11.05;11.07)	42.2$^{\#\#\#\wedge\wedge\wedge}$ (42.00;42.30)	0.000	9.28$^{\#\#\#\wedge\wedge\wedge}$ (9.27;9.30)

Footnotes:
** - statistically significant differences between Groups 1 and 2: * - P<0.05;** - P≤0.01; *** - P≤0.000*
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leukocytosis could indicate an ineffective suppression of latent chronic inflammation.[9] On the contrary, an increase in the level of cells with cytotoxic and killer activity in Group 2 patients indicated a tension in the immune response that impeded the maintenance of active inflammation.[10,11] In the first trimester of pregnancy, women of Group 1 exhibited a characteristic immunological rearrangement aimed at preventing fetal rejection. In Group 2, the changes had a similar orientation; however, a more pronounced inhibition of T-cell immunity was observed, which could be a consequence of the induction of superovulation. In Group 3, 8 weeks after an unsuccessful IVF procedure, on the contrary, we observed activation in T-cell immunity along with inhibition of B-cell immunity.

The study of cytokine status at the stage of pregravid preparation revealed an increase in the concentration of pro-inflammatory cytokines (IL-1α, TNFα, and IFNγ) in patients of Group 2. In Group 3, along with high values of IL-1α and TNFα, the level of IFNγ was significantly reduced. This cytokine imbalance in women of Group 3 was probably due to a statistically significant increase in the level of IL-4, IL-6, and IL-10. Secreted cytokines cause the loss of ability of Th1 cells and cytotoxic T cell to proliferate and to produce IFNγ. The balance between Th1 cells and Th2 cells and the cytokines produced by them is shifted towards Th2, which leads to the implementation of humoral reactions.

In the first trimester of pregnancy, in Group 1 the cytokine balance shifted towards immunosuppressive Th-2 cytokines, which are able to inhibit the activity of cellular immunity and stimulate the production of progesterone and chorionic gonadotropin. The increase in IL-1α level in patients of Group 2 can probably be associated with its effect on the initial stages of embryogenesis, by enhancing the adhesive properties of trophoblast. Moreover, an increase in the production of IL-4, which ensures the dominance of humoral immunity over cellular, explains the decrease in the IFN-γ level in this group of women. In addition, no increase in the level of anti-inflammatory IL-6, IL-10 may be due to the influence of superovulation, which can level the activity of humoral immunity.[12,13] In women of Group 3 with an ineffective IVF program, after stimulation of superovulation, the TNFα level remained high, along with high IFN-γ level and decreased IL-4 level. High values of IgM and IgG during preparation for pregnancy in patients with an IVF program, with a significant decrease after the procedure, can predict an unfavorable outcome of IVF.[14,15]

Thus, in healthy women, immunological changes occurring after conception are aimed at ensuring the physiological course of pregnancy. The revealed shifts in the functioning of the immune system in women with infertility, depending on the outcome of IVF, are multidirectional and may serve as a prognosis factor.

Competing Interests

The authors declare that they have no competing interests.

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