Ozonotherapy in complex treatment of autoimmune miscarriage

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Abstract

The problem of study of pathogenesis and improvement of prophylaxis and therapy of recurrent noncarrying of pregnancy remains one of the topical problems in modern obstetrics. In present report we present a result of comparative investigation the aim of which to increase the efficiency of treatment of patients with noncarrying of pregnancy of autoimmune genesis via including medical ozone into complex treatment. To elucidate the molecular mechanisms, the efficiency of ozonotherapy and the traditional methods of treatment, we studied the state of peroxidation of lipids (POL) and antioxidant system of blood serum protection (AOSP). The performed investigations showed that ozonotherapy makes it possible to limit the rise in cytokines in blood and cervical mucus, which is pathogenetically significant for the result of treatment of noncarrying of pregnancy of autoimmune genesis. This allowed us to reduce the glucocorticoid dosage. The traditional treatment did not stop the avalanche rise in cytokines.

1. Importance of the problem

The problem of study of pathogenesis and improvement of prophylaxis and therapy of recurrent noncarrying of pregnancy remains one of the topical problems in modern obstetrics. According to the data from foreign authors, the frequency of this pathology in a population amounts to 2-5% and does not tend to decrease. Recurrent losses of pregnancy are a weighty factor which has a negative effect not only on the reproductive and general somatic morbidity of the female population and the phychological and social welfare of a family, but also keeps the maternal and perinatal mortality at a high level. Progress in this field is of great medical, social, and economical importance.

The problem of recurrent miscarriage is the object of constant attention of the leading scientific schools of the world [1-3]. In the context of present-day practice, this problem seems polyaethiological and comprising causal factors and their combination.

Among the causes of miscarriage resulting in loss of pregnancy, autoimmune processes come to the forefront. Without adequate therapy, death of embryo (fetus) is observed in 90-95% of the women having this pathology. Patients also represent a risk group in which dangerous obstetric complications such as premature detachment of placenta, thrombosis during pregnancy and in the postnatal period, including thromboembolism of pulmonary arteria, can develop.

Certainly, finding new ways for correction of the immune status with the simultaneous improvement of microcirculation and rheological properties of blood is very topical.

2. Objective of investigation

Increase the efficiency of treatment of patients with noncarrying of pregnancy of autoimmune genesis by including medical ozone into complex treatment.

3. Materials and methods of investigation

To elucidate the molecular mechanisms, the efficiency of ozonotherapy and the traditional methods of treatment, we studied the state of peroxidation of lipids (POL) and antioxidant system of blood serum protection (AOSP)..For the preliminary estimation of the intensity of free-radical oxidation, we used the screening method of induced hemiluminescence (HL) of blood serum. As the activators, we used a 0.05M solution of ferrum sulfate and a 2% solution of hydrogen peroxide. The luminescence intensity was measured for 30 min using the BHL-06 biohemiluminometer by indices I_{max}, S, and tg2ά, where I_{max} is the maximum intensity of luminescence, which describes the potential capability of a biological object, including blood serum, for free-radical oxidation of lipids. Units of measure are mv/sm; S is the sum of light for 30 s, which shows the content of radicals corresponding to a break of the free-radical oxidation chain. This quantity is inversely proportional to the antioxidant activity of a sample. Units of measure are mv/s; the index tg2ά characterizes the rate of decay of free-radical oxidation processes, i.e., is the index which also describes the antioxidant system. Units of measure are relative.

For estimation of the POL intensity, we measured the levels of the molecular peroxidation products, both primary (diene and triene conjugates) and final (Schiff bases).

The content of diene conjugates (DC) was determined in the methanol-hexane lipid fraction (5:1) for absorption wavelength 233 nm, and the content of triene conjugates (TC), in the same fraction for wavelength 275 nm. The obtained results are presented in units of optical density per 1 mg of common lipids. The amount of final products of POL-polymer fluorescent Schiff bases was analyzed with the use of a fluorimeter for excitation wavelength 365 nm and emission wavelength 420 nm. The obtained results are represented in relative units of optical density per 1 mg of common lipids. The content of common lipids in blood serum was determined with the help of Lachema diagnostic devices.

3.1. Clinical characteristic

To reach the objective, we inspected 105 pregnant women with autoimmune miscarriage. All the patients were given the traditional treatment which included glucocorticoids with dosage 5-10 mg (calculated for prednisolone), antiaggregants and/or anticoagulants as functions of

hemostasis indices. Among antiaggregants, in the first trimester we used curantyl with dosage 25 mg three times a day. Based on hemostasis indices, five thousand units of heparin taken three times a day or 0.3 ml of fraxiporin taken one time a day subcutaneously were added in the treatment. In the same way, we used polyvitamins. 72 patients (main group) had an additional treatment of ozonotherapy which included an everyday single-time injection of 400 ml of physiological solution obtained by processing with an ozone-oxygen mixture of ozone concentration 400 mcg/l. The course was 5 days. Ozonotherapy was applied in the 8^{th} to 12^{th} weeks of pregnancy and was combined with the traditional treatment.

The main group of patients can be divided into two subgroups.

The first group (I) without the infection-inflammation processes in anamnesis included 34 pregnant women with autoimmune miscarriage and having the normal level of cytokines in blood (TNF and IL1) and cervical mucus (IL6). The genital-tract infection (GTI) inspection (for chlamydiosis, ureaplasmosis, and mycoplasmosis) yielded the negative results in 100% of the cases (the methods of polymerization chain reaction (PCR) and direct immune fluorescence (DIF) were used). Data for chronical endometritis outside pregnancy in anamnesis were absent. 81% of the women of this subgroup in anamnesis had one abortion and 19% of the women had two abortions. Autoimmune genesis of miscarriage was established when antiphospholipid (APL) antibodies, lupous anticoagulant (LAC), and antibodies for antichorionic human gonadotropin (Anti-CHG) were found two times in blood with an interval of four to six weeks. The age of the women of the 1^{st} main group was 28±1.

The second main subgroup (II) with infection-inflammation factor included 38 women. The level of cytokines in blood (TNF and IL1) and cervical mucus (IL6) exceeded normal considerably. Before pregnancy, the patients had a treatment of chronical endometritis with the purpose of fertility recovery. In anamnesis, the patients of this subgroup had two abortions in 75% and three or more abortions in 25% of the women. The age of the women of the 2^{nd} main group was 30±1.

3.2. Methods of investigation

Cytokines (TNF, IL1, and IL6) were determined by the IFA method using the test systems produced by the “Protein Contour” open joint-stock company (St. Petersburg).

4. Clinical results and their discussion

4.2. Influence of ozonotherapy on the percent of POL in blood of pregnant women with autoimmune miscarriage and comparison with the traditional-therapy effect

Before treatment. The performed investigations showed (Table 1) that the initial level of DC in the first main subgroup on the average amounted to 0.45±0.09 opt.den.units/g of common lipids, which is a factor of 1.5 greater than normal. In the second main subgroup, the initial level of DC was 0.60±0.08 opt.den.units/g of common lipids, which exceeds twofold the normal values ($p < 0.05$). In the control group, this quantity was 0.51±0.04 opt.den.units/g of common lipids, which is a factor of 1.7 greater than normal ($p<0.05$). Increased content of DC was observed, correspondingly, in 69% of the patients in the first subgroup (Fig. 1), in 75% of the patients in the second subgroup (Fig. 2), and in 72% of the patients in the control group (Fig. 3).

Most patients in both main and control groups also had increased TC content (Table 2). For example, the initial level of TC was a factor of 1.8 greater than normal in 65% of the patients (Fig. 4) and amounted to 0.63±0.08 opt.den.units/g of common lipids ($p<0.05$) in the first main subgroup, the normal value was exceeded twofold in 69% of the patients (Fig. 5) and amounted to 0.70±0.09 opt.den.units/g of common lipids ($p<0.05$) in the second main subgroup, and the normal value was exceeded by a factor of 1.3, i.e., this quantity was 0.45±0.08 opt.den.units/g of common lipids in 65% of the patients (Fig. 6), in the control group.

The Schiff base (SB) concentration (Table 3) increased on the average by a factor of 1.5 in 70% of the patients and amounted to 5.3±0.5 con.units/g of common lipids in the first main subgroup (Fig. 7), increased by a factor of 1.4 and amounted to 4.9±0.1 con.units/g of common lipids in 75% of the patients in the second main subgroup (Fig. 8), and increased by a factor of 1.1 and amounted to 3.9±0.1 con.units/g of common lipids in 72% of the patients in the control group (Fig. 9).

The initial values of the antioxidant activity (AOA) of blood serum (Table 4) decreased in 53% of the women in the first main subgroup (Fig. 10) and in 63% of the women in the second main subgroup (Fig. 11) and were on the average 0.160±0.01 and 0.175±0.01 ($p<0.05$), which exceeded the normal values by factors 1.1 and 1.2, respectively. The performed investigations showed that in patients of the control group the AOA values on the average reached 0.158±0.01 (Table 4) in 40% of the patients (Fig. 12).

After treatment. The repeated study of the POL indices (Table 1) after the ozonotherapeutic course showed that in 70% of the patients (Fig. 1) the DC level decreased by 35% ($p < 0.05$) and amounted to 0.2±0.01 opt.den.units/g of common lipids in the first main subgroup, while in 80% of the patients in the second main subgroup (Fig. 2) the DC level decreased by 50% and the POL values amounted to 0.3±0.01 opt.den.units/g of common lipids for $p<0.05$.

Table 1. Influence of ozonotherapy and the traditional treatment on the DC level in blood of pregnant women with autoimmune miscarriage.

Index being studied	Main subgroup		Control group
	I	II	
DC before treatment (opt.den.units/g)	0.45±0.09	0.60±0.08	0.51±0.04
DC after treatment (opt.den.units/g)	0.29±0.01 p<0.05	0.30±0.01 p<0.05	0.59±0.03 p>0.05
DC normal (opt.den.units/g)	0.23±0.07		

Note: p is the reliability of the difference between the index after treatment and its initial value.

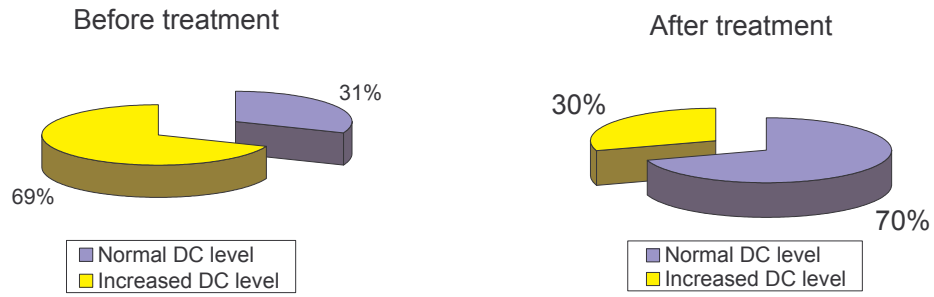

Figure 1. Influence of ozonotherapy on the level of diene conjugates in blood of pregnant women with autoimmune miscarriage in the first main subgroup.

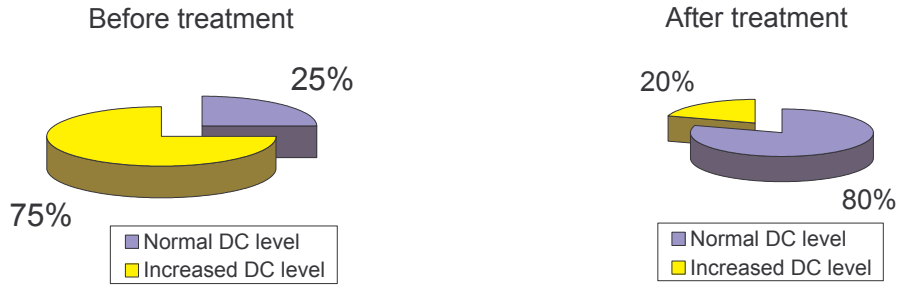

Figure 2. Influence of ozonotherapy on the level of diene conjugates in blood of pregnant women with autoimmune miscarriage in the second main subgroup.

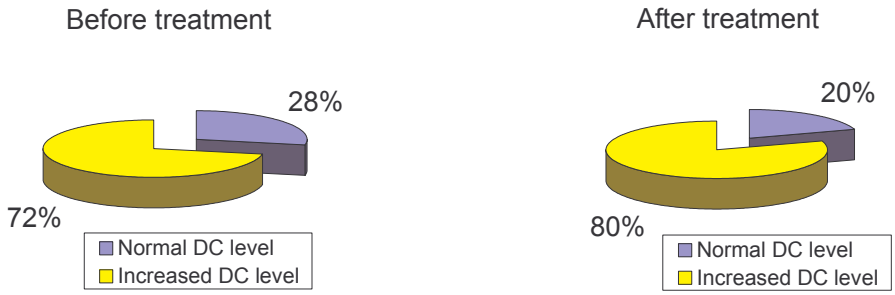

Figure 3. Influence of the traditional treatment on the level of diene conjugates in blood of pregnant women with autoimmune miscarriage in the control group.

The TC level decreased in the 1st and 2nd main subgroups after ozonotherapy. In the 1st main subgroup, we observed a reliable ($p<0.05$) decrease by 48% and the obtained results amounted to 0.035 ±0.01 opt.den.units/g of common lipids (Fig. 4). In the 2nd main subgroup, the results also reliably ($p<0.05$) decreased by 50% to values 0.029 ±0.01 opt.den.units/g of common lipids (Fig. 5).

Table 2. Influence of ozonotherapy and the traditional treatment on the TC level in blood of pregnant women with autoimmune miscarriage.

Index being studied	Main subgroup		Control group
	I	II	
TC before treatment (opt.den.units/g)	0.063±0.008	0.070±0.009	0.045±0.008
TC after treatment (opt.den.units/g)	0.035±0.001 p<0.05	0.029±0.01 p<0.05	0.052±0.007 p>0.05
TC normal (opt.den.units/g)	0.025±0.001		

Note: p is the reliability of the difference between the index after treatment and its initial value.

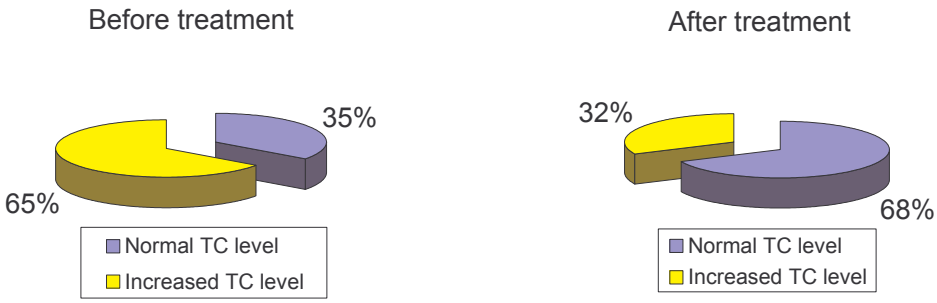

Figure 4. Influence of ozonotherapy on the TC level in blood of pregnant women with autoimmune miscarriage in the 1st main subgroup.

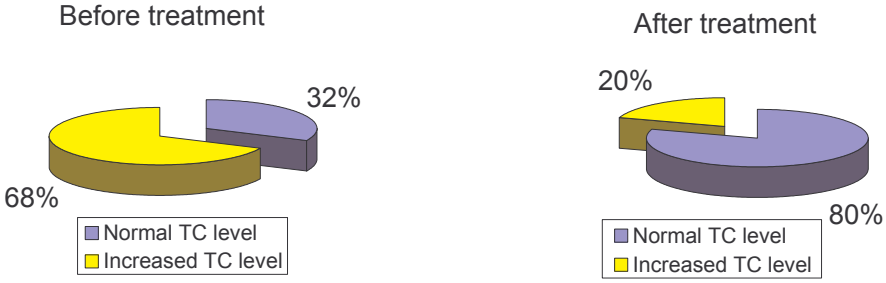

Figure 5. Influence of ozonotherapy on the TC level in blood of pregnant women with autoimmune miscarriage in the 2nd main subgroup.

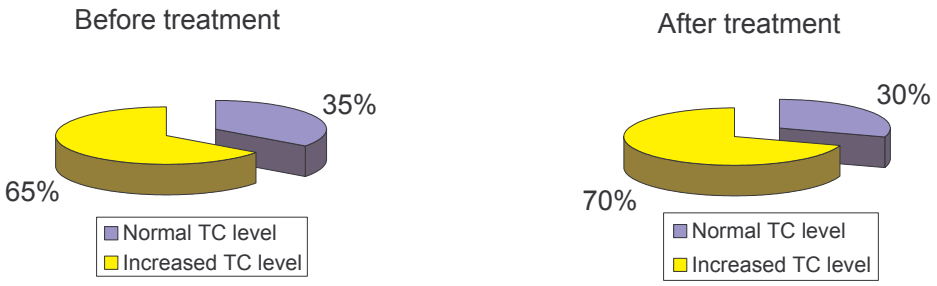

Figure 6. Influence of the traditional treatment on the level of triene conjugates in blood of pregnant women with autoimmune miscarriage in the control group.

The SB concentration (Table 3) in blood of women who had a treatment with the use of ozone reached the normal values in 70% of the patients (Fig. 7) and reliably ($p<0.05$) decreased to 3.3±0.1 con.units/g of common lipids, i.e, decreased by 37%, in the first main group of pregnant women. In the second main group, the SB values decreased by 30.5% ($p<0.05$) and amounted to 3.4±0.1 con.units/g of common lipids. A decrease was observed in 85% of the women (Fig. 8).

Table 3. Influence of ozonotherapy and the traditional treatment on the SB level in blood of pregnant women with autoimmune miscarriage.

Index being studied	Main group		Control group
	I	II	
SB before treatment (con.units/g)	5.3±0.5	4.9±0.1	3.9±0.1
SB after treatment (con.units/g)	3.3±0.5 p<0.05	3.4±0.1 p<0.05	5.2±0.1 p>0.05
SB normal (con.units/g)	2.5±1.0		

Note: p is the reliability of the difference between the index after treatment and its initial value

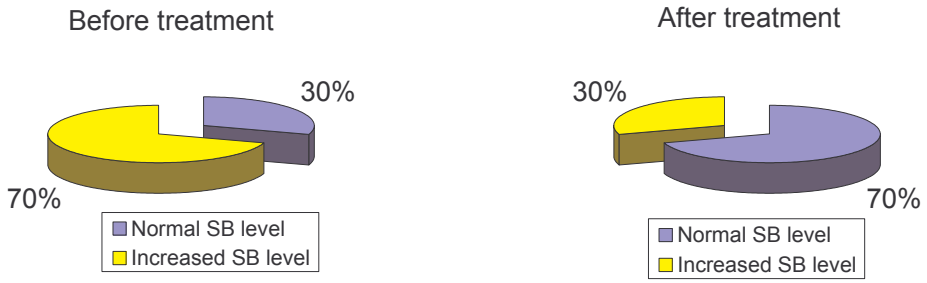

Figure 7. Influence of ozonotherapy and the traditional treatment on the SB level in blood of pregnant women with autoimmune miscarriage in the 1st main subgroup.

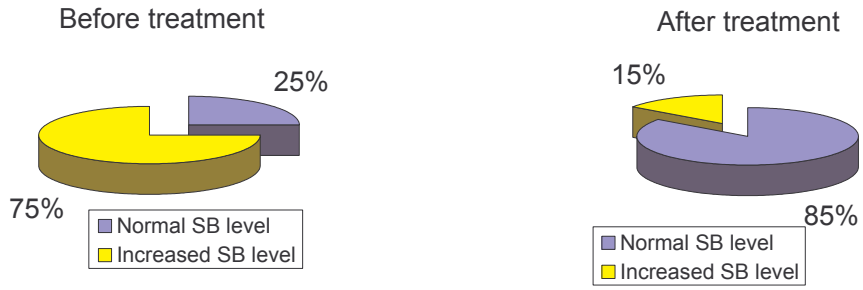

Figure 8. Influence of ozonotherapy and the traditional treatment on the SB level in blood of pregnant women with autoimmune miscarriage in the 2nd main subgroup.

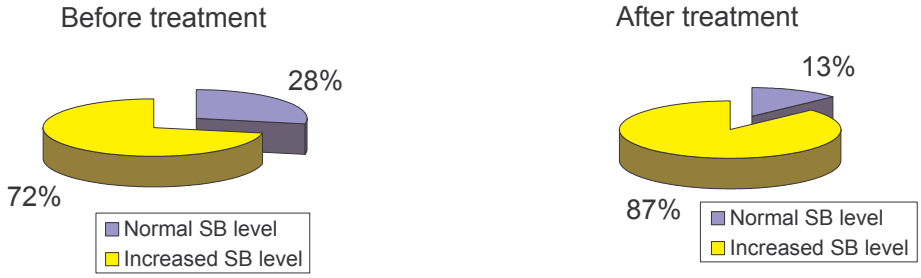

Figure 9. Influence of the traditional treatment on the SB level in blood of pregnant women with autoimmune miscarriage in the control group.

Table 4. Influence of ozonotherapy and the traditional treatment on the AOA level in blood of pregnant women with autoimmune miscarriage.

Index being studied	Main group		Control group
	I	II	
AOA before treatment	0.16±0.01	0.175±0.01	0.158±0.02
AOA after treatment	0.145±0.01 p<0.05	0.148±0.01 p<0.05	0.157±0.02 p>0.05
AOA normal	0.146±0.02		

Note: p is the reliability of the difference between the index after treatment and its initial value.

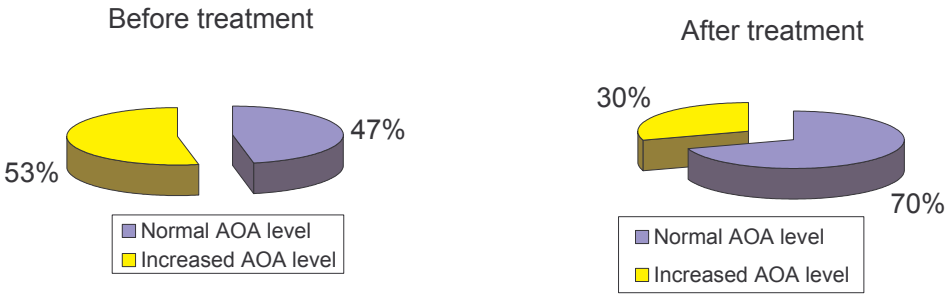

Figure 10. Influence of ozonotherapy and the traditional treatment on the AOA level in blood serum of pregnant women with autoimmune miscarriage in the 1st main subgroup.

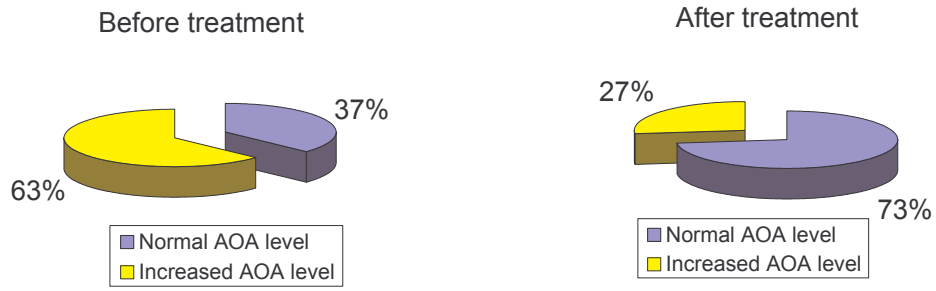

Figure 11. Influence of ozonotherapy and the traditional treatment on the AOA level in blood serum of pregnant women with autoimmune miscarriage in the 2nd main subgroup.

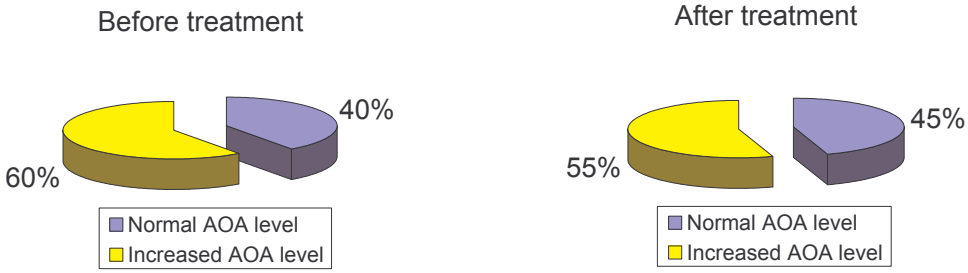

Figure 12. Influence of the traditional treatment on the AOA level in blood serum of pregnant women with autoimmune miscarriage in the control group.

The case was different for patients in the control group, where, despite the complex treatment, enhancement of the POL processes was observed. The traditional treatment had almost no effect on the average levels of DC and TC in blood of pregnant women (Tables 1 and 2 and Fig. 3 and 6).

The SB content (Table 3) reliably ($p<0.05$) increased up to values 5.2±0.5 con.units/g of common lipids. Patients with increased SB concentration amounted to 87% in the control group (Fig. 9). A trend for decreased AOA in these pregnant women was also observed (Table 4 and Fig. 12).

Thus, the positive effect on the POL and AOA levels in blood serum of pregnant women with autoimmune miscarriage, which consists in a pronounced decrease in the initially high levels of POL molecular products (DC, TC, and SB) and enhancement of the previously weakened AOA, is obvious.

These changes become still more apparent if they are compared with the dynamics of POL and AOA in the control group (the accumulation of DC, TC, and especially SB, and the absence of AOA enhancement).

Therefore, unlike the traditional treatment, ozonotherapy is capable of correcting the POL, which is extremely important from the viewpoint of pathogenesis of autoimmune noncarrying of pregnancy [4].

4.2. Influence of ozonotherapy on the level of cytokines in blood and mucus of the cervical channel in pregnant women with autoimmune miscarriage and comparison with the traditional-treatment effect

To specify the influence of ozonotherapy and the traditional treatment on the cytokine level, we studied the TNF and IL1 indices in blood and IL6 in cervical mucus. For control, we used patients with noncarrying of autoimmune genesis who did not had the complex ozone treatment.

The performed investigations showed that the initial level of cytokines in blood and cervical mucus in pregnant women of the 1st main subgroup did not exceed the normal values ($p>0.05$) and on the average amounted to TNF - 45±0.8 pg/ml, IL1 - 42±0.7 pg/ml, and IL6 - 35±0.8 pg/ml (Tables 5a, 5b, and 5c). This was the case for 78% of the patients (Fig. 13). No reliable ($p>0.05$) changes in the cytokine level in blood and cervical mucus were observed after ozonotherapy in

pregnant women of the 1st main subgroup (Fig. 13): TNF = 47±0.8 pg/ml, IL1 = 50±0.2 pg/ml, and IL6 - 41±0.1 pg/ml (Tables 5a, 5b, and 5c).

A reliable ($p<0.05$) increase in the cytokine level in blood and cervical mucus was observed in pregnant women of the 2nd main subgroup: TNF increased up to 550±0.8 pg/ml, IL1 up to 250±0.7 pg/ml, and IL6 up to 150±0.1 pg/ml (Tables 5a, 5b, and 5c). The normal values were exceeded by factors 11, 5, and 3, respectively (Fig. 14).

A reliable ($p<0.05$) decrease in the cytokine values in blood and cervical mucus of pregnant women (Tables 5a, 5b, and 5c) was observed after ozonotherapy: TNF decreased to 48±0.2 pg/ml, IL1 to 50±0.1 pg/ml, and IL6 to 48±0.5 pg/ml. This was the case for 89% of the pregnant women with autoimmune miscarriage (Fig. 14).

A reliable ($p<0.05$) increase in the cytokine level also took place in the control group: TNF increased up to 370±0.1 pg/ml, IL1 up to 120±0.1 pg/ml, and IL6 up to 140±0.8 pg/ml, which exceeded the normal values by factors 7.4, 2.4, and 2.8, respectively (Tables 5a, 5b, and 5c). Such a situation was observed in 71% of the pregnant women with autoimmune miscarriage (Fig. 15). A reliable ($p<0.05$) increase in the cytokine indices occurred after the traditional treatment. The levels were TNF = 520±0.5 pg/ml, IL1 = 240±0.1 pg/ml, and IL6 = 230±0.2 pg/ml. Such a situation was observed in 85% of the patients (Fig. 15).

Table 5a. Influence of ozonotherapy and the traditional treatment on the TNF level in blood of pregnant women with autoimmune miscarriage.

Index under stady	Main subgroup		Control group
	I	II	
TNF before treatment (pg/ml)	45±0.8	550±0.9	370±0.1
TNF after treatment (pg/ml)	47±0.8 p>0.05	48±0.2 p<0.05	520±0.5 p<0.05
TNF normal (pg/ml)	No more than 50		

Note: p is the reliability of the difference between the index after treatment and its initial value.

Table 5b. Influence of ozonotherapy and the traditional treatment on the IL1 level in blood of pregnant women with autoimmune miscarriage.

Index being studied	Main subgroup		Control group
	I	II	
Il1 before treatment (pg/ml)	42±0.7	250±0.7	120±0.1
Il1 after treatment (pg/ml)	50±0.2 p>0.05	50±0.1 p<0.05	240±0.2 p<0.05
Il1 normal (pg/ml)	No more than 50		

Note: p is the reliability of the difference between the index after treatment and its initial value.

Table 5c. Influence of ozonotherapy and the traditional treatment on the IL6 level in blood of pregnant women with autoimmune miscarriage.

Index being studied	Main subgroup		Control group
	I	II	
Il6 before treatment (pg/ml)	35±0.8	150±0.1	140±0.8

Il6 after treatment (pg/ml)	41±0.1 p>0.05	48±0.5 p<0.05	230±0.2 p<0.05
Il6 normal (pg/ml)	No more than 50		

Note: p is the reliability of the difference between the index after treatment and its initial value.

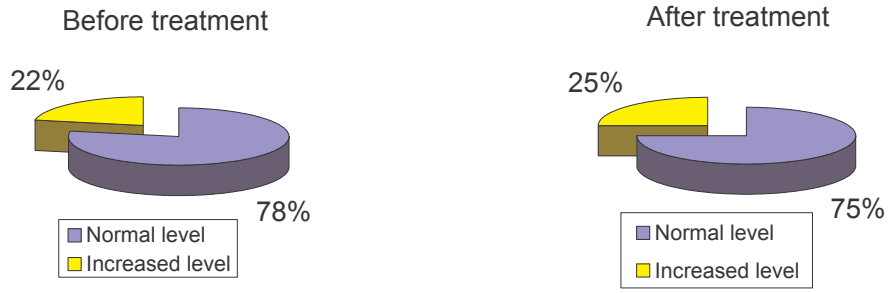

Figure 13. Influence of ozonotherapy and the traditional treatment on the cytokine level in blood and cervical mucus of pregnant women with autoimmune miscarriage in the 1st main subgroup..

Figure 14. Influence of ozonotherapy and the traditional treatment on the cytokine level in blood and cervical mucus of pregnant women with autoimmune miscarriage in the 2nd main subgroup.

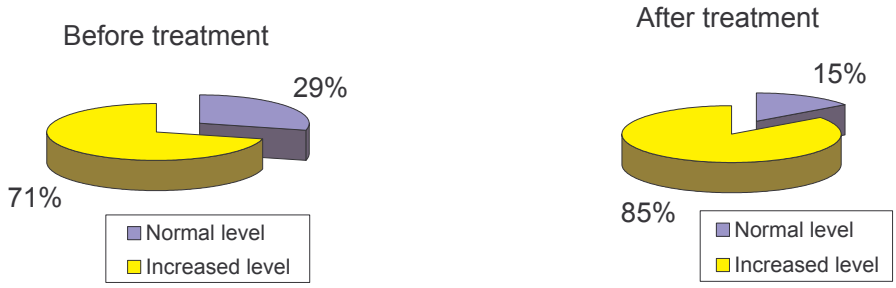

Figure 15. Influence of the traditional treatment on the cytokine level in blood and cervical mucus of pregnant women with autoimmune miscarriage in the control group.

Thus, the performed investigations showed that ozonotherapy makes it possible to limit the rise in cytokines in blood and cervical mucus, which is pathogenetically significant for the result of treatment of noncarrying of pregnancy of autoimmune genesis. This allowed us to reduce the glucocorticoid dosage. The traditional treatment did not stop the avalanche rise in cytokines. On the basis of the performed investigations it can be inferred that inclusion of ozonotherapy into treatment of autoimmune miscarriage is reasonable.

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