ANTIPHOSPHOLIPID ANTIBODIES AND OUTCOMES OF ASSISTED REPRODUCTIVE TECHNOLOGY PROGRAMS IN PATIENTS WITH A HISTORY OF COVID-19

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Investigation of the effect COVID-19 mediated with autoantibodies has on reproductive outcomes is important. This study aimed to evaluate the profile of antiphospholipid antibodies (aPL) and their association with the outcomes of assisted reproductive technology (ART) programs in patients with a history of COVID-19. The study included 240 patients: 105 of them did not have a history of COVID-19 (group 1) and 135 of them had a history of COVID-19 (group 2) with a mild course (subgroup 2a, *n* = 85) or moderate course (subgroup 2b, *n* = 50). With the help of ELISA, serum antibodies (M, G) to cardiolipin, β_2-glycoprotein-I, annexin V (AnV), phosphatidylethanolamine (PE), phosphatidylserine, and phosphatidylserine/prothrombin complex were determined. The evaluated parameters were the indices of oogenesis, embryogenesis, ART intervention outcomes. In group 2, growing levels of anti-AnV and anti-PE IgG were observed more often (in 28 (20.7%) and 8 (5.9%) patients) than in group 1 (in 10 (9.5%) and 1 (0.95%); $p = 0.02$ and $p = 0.045$, respectively). In subgroup 2b we registered a higher level of anti-PE IgG and a higher incidence of early miscarriages (in 6 (12%) patients) than in group 1 (in 3 (2.9%)) ($p = 0.024$). Weak inverse correlations were found between the level of anti-PE IgG and the number of oocytes and zygotes. The results of this study suggest a negative impact of aPL-mediated COVID-19 on the outcomes of ART programs and the course of early pregnancy.

Keywords: COVID-19, SARS-CoV-2, assisted reproductive technologies, ART intervention outcomes, antiphospholipid antibodies

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Compliance with ethical standards: the study was approved by the Ethics Committee of Kulakov National Medical Research Center for Obstetrics, Gynecology and Perinatology (Minutes of meeting № 12 of November 26, 2020); all patients signed an informed voluntary consent to participation in the study.

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АНТИФОСФОЛИПИДНЫЕ АНТИТЕЛА И ИСХОДЫ ПРОГРАММ ВСПОМОГАТЕЛЬНЫХ РЕПРОДУКТИВНЫХ ТЕХНОЛОГИЙ У ПАЦИЕНТОК С COVID-19 В АНАМНЕЗЕ

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Исследование влияния COVID-19, опосредованного аутоантителами, на репродуктивные исходы имеет важное значение. Целью исследования было оценить профиль антифосфолипидных антител (аФЛ) и их связь с исходами программ вспомогательных репродуктивных технологий (ВРТ) у пациенток с COVID-19 в анамнезе. В исследование включили 240 пациенток: 105 из них не болели COVID-19 (группа 1), 135 перенесли COVID-19 (группа 2) в легкой (подгруппа 2а; *n* = 85) или среднетяжелой форме (подгруппа 2б; *n* = 50). С использованием ИФА определяли сывороточные антитела (M, G) к кардиолипину, β_2-гликопротеину-I, аннексину V (АнV), фосфатидилэтаноламину (ФЭ), фосфатидилсерину, комплексу фосфатидилсерин/протромбин. Оценивали показатели оогенеза, эмбриогенеза, исходы ВРТ. В группе 2 повышение уровня анти-АнV и анти-ФЭ IgG наблюдалось чаще (у 28 (20,7%) и 8 (5,9%) пациенток), чем в группе 1 (у 10 (9,5%) и 1 (0,95%); $p = 0,02$ и $p = 0,045$ соответственно). В подгруппе 2б был отмечен более высокий уровень анти-ФЭ IgG и более высокая частота ранних выкидышей (у 6 (12%) пациенток), чем в группе 1 (у 3 (2,9%)) ($p = 0,024$). Выявлены слабые обратные корреляционные связи между уровнем анти-ФЭ IgG и числом полученных ооцитов и зигот. Результаты исследования предполагают негативное влияние COVID-19, опосредованное аФЛ, на исходы программ ВРТ и течение беременности на ранних сроках.

Ключевые слова: COVID-19, SARS-CoV-2, вспомогательные репродуктивные технологии, исходы ВРТ, антифосфолипидные антитела

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The new coronavirus infection (COVID-19) pandemic, caused by the SARS-CoV-2 virus, affected over half a billion people throughout the world. In this connection, the task of studying the effect COVID-19 has on the female reproductive system is a very urgent one. Individual studies have reported discrepant data that suggest that COVID-19 increases the risk of pregnancy complications such as spontaneous miscarriages and preterm birth [1, 2]. The incidence of preterm birth in pregnant women with COVID-19 reaches 11.5–17% [3, 4], and the frequency of early spontaneous

miscarriages is 1.7 times higher compared to women not infected with SARS-CoV-2 [5].

The number of studies evaluating impact of COVID-19 and post-COVID syndrome on fertility and outcomes of assisted reproductive technology (ART) programs is insufficient. There is a paper [6] describing separate cases of development of premature ovarian failure (POF) in women after COVID-19, but the origin of the disorder is unclear. A 2022 meta-analysis did not register the impact of COVID-19 on the outcomes of ART programs [7]. However, the results of another study suggest the disease does have a negative effect and the number of oocytes received through an ART intervention depends on the time elapsed after COVID-19 [8].

It is known that infection caused by SARS-CoV-2 increases production of cytokines, such as IL6, TNFα, which can translate into a cytokine storm and adversely affect reproductive function through cytokine-driven suppression of the hypothalamic-pituitary-gonadal axis, development of systemic vasculitis and autoimmune lesions of the gonads [9, 10].

Autoimmune response is one of the possible mechanisms that can result in damage to the woman's reproductive system under the influence of the infection. It has been shown that people with a certain HLA haplotype are most susceptible to the development of autoimmune processes after COVID-19 [11]. Activation and maturation of autoreactive B lymphocytes from naïve B cells may follow an extrafollicular pathway lacking some tolerance checkpoints [12], which is proven by higher levels of extrafollicular B cells and plasma cells in patients with severe COVID-19.

A recent discovery of 28 human proteins containing domains homologous to SARS-CoV-2 peptides supports the hypothesis about the role of autoimmunity in a COVID-19 case. These peptides can act as autoantigens and trigger production of autoantibodies that is based on molecular mimicry [13]. COVID-19 patients have shown to have significant amounts of autoantibodies of various specificities, including antinuclear antibodies, antineutrophil cytoplasmic antibodies, antibodies to cardiolipin (CL), and β_2-glycoprotein-I (β_2-GP-I) [14]. Thyroid peroxidase antibodies have also been found in patients with post-COVID syndrome [15].

Vascular complications associated with COVID-19, such as deep vein thrombosis, stroke, disseminated intravascular coagulation, were initially linked to antiphospholipid antibodies (aPL) [16], primarily antibodies to CL and β_2-GP-I, classified as laboratory criteria for antiphospholipid syndrome (APS) [17]. However, it has been shown that aPL in COVID-19 patients are not detected frequently (8.9%), they may be transient and are not always associated with thrombosis [18]. At the same time, genetically predisposed patients may have a long-term persistence of pathogenic aPL, as well as an autoimmune disease developed [19].

In infertile women, autoimmune processes can affect fertilization, implantation, and placental development [20]. The pathogenetic mechanisms that link autoimmunity and infertility remain unclear. The association of aPL with infertility is an actively discussed matter currently. A 2016 scientific literature analysis has showed that, in most studies, infertility was associated with antibodies to β2-GP-I and "non-criteria" aPL, including phosphatidylethanolamine (PE) [21]. Five out of 18 studies reported detrimental effects aPL ("criteria" aPL, mainly) may have on the outcome of ART programs. Thus, the study of various autoantibodies in infertile patients that had COVID-19 and investigation of their impact on reproductive outcomes is of great scientific and practical importance.

This study aimed to evaluate the profile of antiphospholipid antibodies and their association with the outcomes of ART programs in patients with a history of COVID-19.

METHODS

The study was conducted at the V.I. Kulakov National Medical Research Center for Obstetrics, Gynecology and Perinatology of the Ministry of Health of Russia. The design of the study was prospective observational; it included a total of 240 infertile patients, who were stratified into two groups depending on the history of COVID-19: group 1 included patients who did not have COVID-19 (n = 105), group 2 included patients who had COVID-19 (n = 135) for 12 and less months prior to an ART intervention. Group 2 was further divided into two subgroups: subgroup 2a comprised of patients who had a mild form of COVID-19 (n = 85) and subgroup 2b where the form of the disease was moderate (n = 50).

The inclusion criteria were: age 18–40 years; normal ovarian reserve (anti-Müllerian hormone (AMH) ≥ 1.2 ng/mL, follicle-stimulating hormone (FSH) < 12 mIU/mL, antral follicle count (AF) ≥ 5 in both ovaries). The exclusion criteria were: vaccination against COVID-19; contraindications to ART, morbid obesity (BMI ≥ 40.0 kg/m^2); participation in donor programs, surrogacy programs; HIV infection. All patients were examined as prescribed in the 2021 Female Infertility clinical guidelines.

The patients' testimony of their COVID-19 cases were cross-checked with the Uniform State Health Information System and proven by determining antibodies to SARS-CoV-2 in the blood serum with the help of "Kit of reagents for detection of class G antibodies to SARS- CoV-2 spike protein by ELISA" (DS-ELISA-ANTI-SARS-CoV-2-G(S)) (Diagnostic Systems; Russia), which enables qualitative determination of antibodies to SARS-CoV-2 using enzyme-linked immunosorbent assay (ELISA). To evaluate the results of the analysis, we calculated positivity index (PI) using the formula: PI = sample OD / Cut-off, where sample OD is the optical density of the sample. With PI > 1.2 the result was considered positive, with PI < 0.8 — negative, with PI in the range from 0.8 to 1.2 — doubtful (uncertain).

The aPL study relied on ELISA and kits for quantitative determination of antibodies of classes M and G to CL, β_2-GP-I, annexin V (An V), phosphatidylserine (PS) (ORGENTEC Diagnostika GmbH; Germany). The reference values (RV) of blood antibody content were as follows: anti-CL IgM — < 7 MPL-U/ml, anti-CL IgG — < 10 GPL-U/ml; anti-β_2-GP-1 IgM and IgG — < 5 U/ml; anti-AnV IgM and IgG — < 5 U/ml; anti-PS IgM and IgG — < 10 U/ml. The quantity of antibodies (M, G) to PE and the phosphatidylserine/prothrombin complex (PS/PT) in blood serum was determined with the help of enzyme immunoassay kits (AESKU Diagnostics; Germany). The reference range for anti-PE antibodies and anti-PS/PT antibodies was < 12 U/mL.

Ovarian stimulation followed the protocol with gonadotropin-releasing hormone (ant-GnRH) antagonists, recombinant FSH (rFSH) preparations and/or preparations containing a luteinizing hormone (LH) component: human menopausal gonadotropin (hMG) or a combined preparation containing rFSH/rLH. On average, all patients who recovered from COVID-19 underwent ovarian stimulation six months (two to nine months) after the disease. The dose of gonadotropins was selected individually, taking into account age, medical history and ovarian reserve parameters. Ovulation was triggered with a single dose of chorionic gonadotropin (CG), 8,000–10,000 IU, or a combination of CG and a gonadotropin-releasing hormone (a-GnRH) agonist. Transvaginal follicle puncture (TFP) was

Table 1. Serum aPL level growth incidence in the study groups

Parameter	Group 1, *n* = 105	Group 2, *n* = 135		*p* value
		Subgroup 2a, *n* = 85	Subgroup 2б, *n* = 50	
anti-CL IgM	2 (1,9%)	4 (2,9%)		0,60*
		3 (5,2%)	1 (2,0%)	0,75**
anti-CL IgG	0 (0,0%)	0 (0,0%)		–
		0 (0,0%)	0 (0,0%)	–
anti-β_2-GP-1 IgM	3 (2,9%)	3 (2,2%)		0,75
		2 (2,4%)	1 (2,0%)	0,95
anti-β_2-GP-1 IgG	4 (3,8%)	7 (5,2%)		0,61
		3 (3,5%)	4 (8%)	0,43
anti-AnV IgM	9 (8,6%)	10 (7,4%)		0,74
		3 (3,5%)	7 (14%)	0,09
anti-AnV IgG	10 (9,5%)	28 (20,7%)		0,02
		20 (23,5%)	8 (16%)	0,03
anti-PS IgM	1 (0,9%)	0 (0,0%)		0,26
		0 (0,0%)	0 (0,0%)	0,52
anti-PS IgG	0 (0,0%)	0 (0,0%)		–
		0 (0,0%)	0 (0,0%)	–
anti-PE IgM	23 (21,9%)	23 (17,0%)		0,34
		11 (12,9%)	12 (24,0%)	0,28
anti-PE IgG	1 (0,95%)	8 (5,9%)		0,045
		5 (5,9%)	3 (6%)	0,13
anti-PS/PT IgM	3 (2,9%)	2 (1,5%)		0,46
		2 (2,3%)	0 (0,0%)	0,49
anti-PS/PT IgG	4 (3,8%)	4 (2,9%)		0,72
		4 (4,7%)	0 (0,0%)	0,32

Note: abs (%), χ^2 test; * — comparison of groups 1 and 2; ** — comparison of group 1 and subgroups 2a and 2b.

performed under ultrasound guidance 36 hours after triggering the ovulation.

In the aspirated follicular fluid we determined the resulting number of oocyte-cumulus complexes (OCC), and then, after oocyte denudation, assessed the degree of cell maturity. All mature oocytes were fertilized by in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI); after 16–18 h, if there were two symmetrical pronuclei in the cytoplasm, normal fertilization was recorded. The zygotes were then transferred to the COOK culture medium (COOK Medical; USA) for further cultivation. Morphological evaluation of the embryos was done after 120–122 hours (on the fifth day) of cultivation, relying on the Gardner blastocyst grading system (degree of blastocyst maturity, quality of trophectoderm and intracellular mass).

On the fifth day of cultivation one or two embryos were transferred (embryo transfer, ET) into the uterine cavity. Post-transfer support measures involved administration of micronized progesterone (600 mg daily) or dydrogesterone (30 mg daily). Pregnancy was identified by the level of β-CG in the blood serum 14 days after ET. A pregnancy test was considered to have returned positive when the level of β-CG exceeded 20 IU/L. Twenty-one days after ET, if ultrasound examination revealed a fetal egg in the uterine cavity, a clinical pregnancy was recorded.

Statistica 10 (StatSoft Inc.; USA) was used for statistical analysis. To evaluate qualitative data, we calculated proportions (%). The χ^2 test enabled comparison of categorical data and assessment of the differences between them. The analysis of quantitative data in comparison groups relied on the Kolmogorov–Smirnov test and graphical data evaluation, which allowed learning data distribution. For abnormal data distribution we applied nonparametric statistical methods: determination of median with an interquartile range ($Me(Q_{25}–Q_{75})$), Mann–Whitney test or Kruskal–Wallis test to compare data in unrelated populations. Spearman's rank correlation coefficient enabled evaluation of correlation dependence between variables. The differences between statistical values were considered significant at $p < 0.05$.

RESULTS

The mean age of the patients was 34 years (34 (30–36) years in group 1 and 34 (31–37) years in group 2 ($p = 0.39$). In group 1, 24 (22.8%) women were in the late reproductive age (35 years and older), and in group 2 there were 36 (26.7%) such patients ($p = 0.49$). Group 2 patients had a significantly higher serum level of specific antiviral antibodies compared to patients in group 1: the mean PI values in groups 1 and 2 were 0.16 ± 0.13 and 6.8 ± 4.1 ($p < 0.001$), respectively. Patients who had COVID-19 had greater body mass index than those who did not have the disease (22.9 (20.4–25.5) kg/m^2 and 21.9 (20.0–24.5) kg/m^2; $p = 0.009$); the former also exhibited higher incidence of ENT diseases (24 (17.8%) and 9 (8.6%); $p = 0.04$) and allergic conditions (23 (17%) and 9 (8.6%); $p = 0.055$).

As for the gynecological diseases, the most common diagnosis for women of groups 1 and 2 was endometriosis (in 25 (23.8%) and 38 (28.1%) patients, respectively; $p = 0.45$) and uterine myoma (in 21 (20%) and 33 (24.4%) patients; $p = 0.41$), followed by chronic salpingo-oophoritis (in 13 (12.4%) and 15 (11.1%) patients; $p = 0.76$), chronic endometritis (in 11

Table 2. The average level of blood serum aPL, both study groups

Parameter	Group 1, $n = 105$	Group 2, $n = 135$		p value
		Subgroup 2а, $n = 85$	Subgroup 2б, $n = 50$	
anti-CL IgM, MPL-U/ml	3,03 (1,94–4,05)	2,52 (1,59–3,91)		0,14*
		2,43 (1,59–1,04)	3,04 (1,50–3,83)	0,30**
anti-CL IgG, GPL-U/ml	1,87 (1,41–2,56)	2,10 (1,59–3,01)		0,06*
		2,01 (1,50–2,86)	2,14 (1,68–3,31)	0,08**
anti-β_2-GP-1 IgM, U/ml	1,51 (0,81– 2,43)	1,41 (0,98–2,17)		0,87
		1,41 (0,95–2,38)	1,42 (1,06–2,07)	0,96
anti-β_2-GP-1 IgG, U/ml	2,98 (2,12– 3,59)	2,37 (1,21–3,26)		0,001
		2,09 (0,94–2,30)	2,52 (1,94–3,54)	0,001
anti-AnV IgM, U/ml	2,52 (1,76–3,52)	2,22 (1,23–3,22)		0,03
		2,22 (1,26–3,18)	2,25 (1,45–3,35)	0,07
anti-AnV IgG, U/ml	2,88 (2,26–3,94)	3,37 (2,13–4,65)		0,19
		3,37 (2,20–4,95)	3,23 (2,00–4,58)	0,25
anti-PS IgM, U/ml	2,53 (1,56–3,76)	2,54 (1,25–4,01)		0,64
		2,31 (1,11–3,78)	2,95 (1,50–4,14)	0,33
anti-PS IgG, U/ml	1,76 (1,41–2,15)	1,69 (1,32–2,16)		0,76
		1,67 (1,27–2,10)	1,73 (1,51–2,23)	0,32
anti-PE IgM, U/ml	12,23 (8,70–16,98)	11,85 (8,67–15,58)		0,54
		11,93 (7,78–15,2)	11,61 (9,09–7,89)	0,51
anti-PE IgG, U/ml	3,63 (2,96–4,80)	4,78 (3,27–6,82)		0,001
		4,39 (3,20–5,89)	5,20 (3,74–7,93)	0,001
anti-PS/PT IgM, U/ml	1,72 (1,10–3,28)	2,39 (1,47–3,58)		0,01
		2,39 (1,53–3,73)	2,33 (1,28–3,55)	0,03
anti-PS/PT IgG, U/ml	4,24 (3,00–5,36)	3,38 (2,28–5,31)		0,03
		3,43 (2,32–5,48)	3,02 (2,24–5,11)	0,06

Note: Me (Q_{25}–Q_{75}), тест Манна–Уитни или Краскела–Уоллиса; * — при сравнении групп 1 и 2, ** — при сравнении группы 1 и подгрупп 2а и 2б.

(10, 5%) and 8 (5.6%) patients; $p = 0.19$) and PCOS (in 9 (8.6%) and 6 (4.4%) patients; $p = 0.19$). The share of patients with primary infertility was similar in both groups: 61 (58.1%) and 79 (58.5%) women; $p = 0.95$. There were no differences in terms of the average number of pregnancies, deliveries and miscarriages between the groups.

According to the results of the serum aPL content study, patients of both groups frequently had the level of antibodies growing above the RV. Cumulatively, 66 (62.9%) women in group 1 had at least one aPL of class M or G increased, and for group 2 this value was 81 (60%) ($p = 0.65$). A simultaneous increase in the level of several aPL was observed in 18 (17.1%) and 33 (24.4%) patients of groups 1 and 2, respectively ($p = 0.17$). Fifty-five (52.4%) patients of group 1 exhibited an increased level of antibodies to PE most frequently, whereas in group 2 there were 66 (48.9%) such patients; a less frequent observation — increased level of antibodies to An V, which was registered in 20 (19%) and 37 (27.4%) women, respectively ($p < 0.001$). The level of other antibodies (different specificity) has been registered growing up not as often as that to An V: antibodies to β2-GP-I in 6 (5.7%) and 8 (5.9%) cases, respectively, to the PS/PT complex — in 6 (5, 7%) and 6 (4.4%) cases, to CL — in 2 (1.9%) and 4 (3.0%) women, to PS — only a single case (0.95%) in group 1 ($p < 0.001$). A comparative analysis of incidence of increased level of either aPL class M or aPL class G revealed significant differences only in IgG antibodies to PE and AnV (Table 1); the incidence was significantly higher in group 2 than in group 1.

Assessment of serum aPL in patients of groups 1 and 2 has shown that, for all the studied antibodies, the mean levels with interquartile ranges were within the RV. Herewith, the average level of anti-β_2-GP-I IgG, anti-AnV IgM and anti-PS/PT IgG was higher in group 1, while the average level of anti-PE IgG and anti-PS/PT IgM, on the contrary, was higher in group 2 (table 2). A slightly higher level of IgG antibodies to AnV was noted in group 2 compared to group 1.

The indicators of spermatogenesis, oogenesis and early embryogenesis in groups 1 and 2 did not differ significantly. A factor that should be noted here is the high incidence of pathospermia in partners of the participants: it was registered in 72 (68.6%) partners of group 1 patients and 86 (63.7%) partners of group 2 patients ($p = 0.43$). The average number of

mature oocytes recorded in groups 1 and 2 was, respectively, 8 (5–11) and 7 (4–11) (p = 0.26), the level of fertilization was 0.90 (0.75– 1.0) and 0.92 (0.80–1.0) (p = 0.39), the number of zygotes — 6 (4–9) and 6 (4–10) (p = 0.37), the number blastocysts — 3 (1–5; similar in both groups), the number of excellent quality blastocysts — 1 (0–3) and 1 (0–2) (p = 0.19), the number of poor quality blastocysts — 1 (0–2) in both groups.

Investigation of the relationship between the level of aPL and the parameters of oogenesis and embryogenesis revealed a significant weak negative correlation between the level of anti-PE IgG antibodies and the number of mature oocytes (r = –0.129, p = 0.045) and zygotes (r = –0.132, p = 0.041). In other cases, correlations between other aPL and parameters of oogenesis and embryogenesis were not significant. It should be noted here that the mean level of anti-PE IgG antibodies was significantly higher in group 2 patients and especially in subgroup 2b (patients who had moderate COVID-19). In addition, the share of patients with elevated levels of anti-PE IgG antibodies in group 2 was significantly higher than in group 1.

Evaluation of the outcomes of ART programs has shown that the frequency of pregnancy occurrence (FPO) and childbirth did not differ significantly between the groups: biochemical pregnancy was observed in 32 (30.5%) and 39 (28.9%) women (p = 0, 79), clinical pregnancy in 30 (28.6%) and 39 (28.9%) (p = 0.96), childbirth in 27 (25.7%) and 30 (22.2%) (p = 0.53). It is important to note that in subgroup 2b (with a history of moderate COVID-19) the incidence of spontaneous abortions up to 12 weeks of pregnancy was higher than in group 1: 6 (12%) and 3 (2.9%) women, respectively (p = 0.024). Compared to group 1, the OR for spontaneous miscarriage in subgroup 2b was 2.1 (95% CI = 1.1; 19.4) (p = 0.036). A fact to be underscored here is that emphasized that 3 out of 6 miscarrying patients had elevated serum levels of IgM antibodies to PE and AnV.

DISCUSSION

Some scientific studies demonstrate the absence of a negative impact of COVID-19 on the outcomes of ART programs [2, 7, 22]. Similar statements can be found in papers comparing the results of ART programs in pre-covid and covid periods [23, 24]. However, there are publications that discuss the negative effect the infection may have on the reproductive function of women [6, 25, 26]. Post-COVID syndrome may manifest in reproductive dysfunction, including, inter alia, impaired fertility and miscarriage [6]. The scientific literature describes individual cases when young fertile patients became infertile or suffered from POF after recovery from COVID-19 [25, 26]. The severity of COVID-19 may have an impact on the incidence of complications of pregnancy [27].

It is assumed that COVID-19 can trigger autoimmune processes in genetically predisposed people [28]. Researchers describe development of such autoimmune pathologies as immune thrombocytopenic purpura, Guillain–Barré syndrome, and Miller–Fischer syndrome in patients that recovered from COVID-19 [16]. Those who contracted SARS-CoV-2 exhibit a significant prevalence of autoantibodies of different specificity: antinuclear autoantibodies — in 57.5%, antineutrophil cytoplasmic antibodies — in 25%, anti-CL antibodies — in 12.5%, anti-β_2-GP-I antibodies — in 5% [14].

This study shows a high overall rate of detection of aPL classes M and G in infertile patients that did or did not have COVID-19. The most common observation is an elevated level of "non-criteria" antibodies to PE and AnV, significantly less frequently registered — elevated level of classic aPL, namely, antibobies to CL and β_2-GP-I, which are currently considered to be laboratory criteria for APS. These results are consistent with our earlier findings, which demonstrate that in patients with COVID-19, antibodies to CL and β_2-GP-I are detected less frequently compared to antibodies to AnV [29].

An individual analysis of the frequency of identification of aPL classes M and G has shown that, compared to women who did not have a history of COVID-19, patients who recovered from the disease less than 12 months before ART intervention have higher levels of IgG antibodies to PE and AnV significantly more often. It is known that COVID-19 patients are predisposed to pro-inflammatory and hypercoagulable conditions and run a higher risk of thrombotic events and impaired coagulation. Activation of vascular endothelial cells, externalization of phospholipids and higher content of natural anticoagulants that bind phospholipids on the surface of the damaged endothelium, in particular AnV, can induce increased formation of autoantibodies to AnV [29].

In addition, patients with a history of COVID-19 had a higher average level of IgG antibodies to PE than those who did not have COVID-19 in anamnesis; moreover, the former had that level increasing more often than the latter. Antibodies to PE are known to form in infectious and inflammatory processes of a viral or bacterial nature and can persist for a long time in the human body. This is due to the fact that PE is the main lipid component of microbial membranes and is also abundant in human cell membranes with asymmetric distribution. Production of pro-inflammatory mediators and damage to cells and tissues in the context of infectious and inflammatory processes contribute to the exposure of PE in cell membranes and formation of autoantibodies to PE.

The revealed negative correlation between the level of IgG antibodies to PE and the number of mature oocytes and zygotes may indirectly indicate the possible negative effect some aPL persisting after COVID-19 have on the outcomes of ART programs.

The present study showed that the parameters of oogenesis, embryogenesis, FPO and frequency of childbirth did not differ significantly in the groups of patients who did and did not have a history of COVID-19, which is consistent with the data reported by other researchers and mentioned above. However, patients who had COVID-19 in its moderate form were more prone to spontaneous abortion up to 12th week of gestation; for them, the risk of miscarriage was 2.1 times higher compared with patients who did not have COVID-19. Since half of the patients that miscarried early were found to have antibodies to PE and AnV, it can be assumed that autoimmune mechanisms are involved in the development of these pregnancy complications. As is known, antibodies to PE and AnV are associated with recurrent miscarriage; they are significant risk factors for this complication of pregnancy.

CONCLUSIONS

Infertile patients often have antiphospholipid antibodies detected in them. Patients who had a moderate form of COVID-19 before entering the ART program have elevated serum levels of IgG antibodies to PE and AnV more often than patients who did not have COVID-19. It is assumed that COVID-19 may have a negative impact on reproductive outcomes, decrease the number of mature oocytes, zygotes and, as a result, embryos in the context of ART interventions, as well as increase the risk of spontaneous abortion in the early stages of pregnancy, which may be associated with the involvement of autoimmune mechanisms mediated by the formation of aPL, in particular antibodies to PE and AnV.

References

1. Bentov Y, Beharier O, Moav-Zafrir A, Kabessa M, Godin M, Greenfield CS, et al. Ovarian follicular function is not altered by SARS–CoV-2 infection or BNT162b2 mRNA COVID-19 vaccination. Hum Reprod. 2021; 36 (9): 2506–13.
2. Wang M, Yang Q, Ren X, Hu J, Li Z, Long R, et al. Investigating the impact of asymptomatic or mild SARS-CoV-2 infection on female fertility and in vitro fertilization outcomes: A retrospective cohort study. E Clinical Medicine. 2021; 38: 101013. DOI: 10.1016/j.eclinm.2021.101013.
3. Mullins E, Perry A, Banerjee J, Townson J, Grozeva D, Milton R, et al. Pregnancy and neonatal outcomes of COVID-19: The PAN-COVID study. Eur J Obste. Gynecol Reprod Biol. 2022; 276: 161–7.
4. Allotey J, Stallings E, Bonet M, Yap M, Chatterjee S, Kew T, et al. Clinical manifestations, risk factors, and maternal and perinatal outcomes of coronavirus disease 2019 in pregnancy: Living systematic review and meta-analysis. BMJ. 2020; 370: m3320. DOI: 10.1136/bmj.m3320.
5. Balachandren N, Davies MC, Hall JA, Stephenson JM, David AL, Barrett G, et al. SARS-CoV-2 infection in the first trimester and the risk of early miscarriage: a UK population-based prospective cohort study of 3041 pregnancies conceived during the pandemic. Hum. Reprod. 2022; 37 (6): 1126–33.
6. Bechmann N, Maccio U, Kotb R, Dweik RAl, Cherfane M, Moch H, et al. COVID-19 Infections in Gonads: Consequences on Fertility? Horm Metab Res. 2022; 54 (08): 549–55.
7. Hu W, Zhu Y, Wu Y, Wang F, Qu F. Impact of COVID-19 pandemic on the pregnancy outcomes of women undergoing assisted reproductive techniques (ARTs): a systematic review and meta-analysis. J Zhejiang Univ Sci B. 2022; 23 (8): 655–65.
8. Youngster M, Avraham S, Yaakov O, Landau Rabbi M, Gat I, Yerushalmi G, et al. IVF under COVID-19: treatment outcomes of fresh ART cycles. Hum Reprod. 2022; 37 (5): 947–53.
9. Maiorino MI, Bellastella G, Giugliano D, Esposito K. From inflammation to sexual dysfunctions: a journey through diabetes, obesity, and metabolic syndrome. J Endocrinol Invest. 2018; 41 (11): 1249–58.
10. Huang C, Ji X, Zhou W, Huang Z, Peng X, Fan L, et al. Coronavirus: A possible cause of reduced male fertility. Andrology. 2021; 9 (1): 80–7.
11. Matyushkina D, Shokina V, Tikhonova P, Manuvera V, Shirokov D, Kharlampieva D, et al. Autoimmune Effect of Antibodies against the SARS-CoV-2 Nucleoprotein. Viruses. 2022; 14 (6): 1141.
12. Knight JS, Caricchio R, Casanova J-L, Combes AJ, Diamond B, Fox SE, et al. The intersection of COVID-19 and autoimmunity. J Clin Invest. 2021; 131 (24): e154886. DOI: 10.1172/JCI154886.
13. Mohkhedkar M, Venigalla SSK, Janakiraman V. Untangling COVID-19 and autoimmunity: Identification of plausible targets suggests multi organ involvement. Mol Immunol. 2021; 137: 105–13.
14. Sacchi MC, Tamiazzo S, Stobbione P, Agatea L, De Gaspari P, Stecca A, et al. SARS-CoV-2 infection as a trigger of autoimmune response. Clin Transl Sci. 2021; 14 (3): 898–907.
15. Lui DTW, Lee CH, Chow WS, Lee ACH, Tam AR, Pang P, et al. Long COVID in Patients With Mild to Moderate Disease: Do Thyroid Function and Autoimmunity Play a Role? Endocr Pract. 2021; 27 (9): 894–902.
16. Ehrenfeld M, Tincani A, Andreoli L, Cattalini M, Greenbaum A, Kanduc D, et al. Covid-19 and autoimmunity. Autoimmun. Rev. 2020; 19 (8): 102597. DOI: 10.1016/j.autrev.2020.102597.
17. Zhang Y, Xiao M, Zhang S, Xia P, Cao W, Jiang W, et al. Coagulopathy and Antiphospholipid Antibodies in Patients with Covid-19. N Engl J Med. 2020; 382 (17): e38. DOI: 10.1056/NEJMc2007575.
18. Harzallah I, Debliquis A, Drénou B. Lupus anticoagulant is frequent in patients with Covid-19. J Thromb Haemost. 2020; 18 (8): 2064–5.
19. Soriano A, Blank M, Shoenfeld Y. Genetics and origin of antiphospholipid syndrome. In: Meroni PL, editor. Antiphospholipid antibody syndrome. From bench to bedside. Cham: Springer I, 2014; p. 1–12.
20. Khizroeva J, Nalli C, Bitsadze V, Lojacono A, Zatti S, Andreoli L, Tincani A, Shoenfeld Y, Makatsariya A. Infertility in women with systemic autoimmune diseases. Best Practice & Research Clinical Endocrinology & Metabolism. 2019; 33 (6): 101369. Available from: https://doi.org/10.1016/j.beem.2019.101369.
21. Chighizola CB, de Jesus GR, Branch DW. The hidden world of anti-phospholipid antibodies and female infertility: A literature appraisal. Autoimmunity Reviews. 2016; 15 (6): 493–500. Available from: https://doi.org/10.1016/j.autrev.2016.01.018.
22. Kolanska K, Hours A, Jonquière L, Mathieu d'Argent E, Dabi Y, et al. Mild COVID-19 infection does not alter the ovarian reserve in women treated with ART. Reprod Biomed Online. 2021; 43 (6): 1117–21.
23. Aharon D, Gounko D, Lee JA, Copperman AB, Flisser E. The Impact of the Coronavirus Disease 19 Pandemic on Early Pregnancy Outcomes Among Patients Undergoing In Vitro Fertilization Treatment. Women's Heal Reports. 2021; 2 (1): 473–8.
24. Setti PEL, Cirillo F, Immediata V, Morenghi E, Canevisio V, Ronchetti C, et al. First trimester pregnancy outcomes in a large IVF center from the Lombardy County (Italy) during the peak COVID-19 pandemic. Sci Rep. 2021; 11 (1): 16529. DOI: 10.1038/s41598-021-96134-9.
25. Madaan S, Jaiswal A, Kumar S, Talwar D. Premature ovarian failure-A long COVID sequelae. Med Sci. 2021; 25 (112): 1286–90.
26. Wilkins J, Al-Inizi S. Premature ovarian insufficiency secondary to COVID–19 infection: An original case report. Int J Gynecol Obstet. 2021; 154 (1): 179–80.
27. Shams T, Alhashemi H, Madkhali A, Noorelahi A, Allarakia S, Faden Y, et al. Comparing pregnancy outcomes between symptomatic and asymptomatic COVID-19 positive unvaccinated women: Multicenter study in Saudi Arabia. J Infect Public Health. 2022; 15 (8): 845–52.
28. Caso F, Costa L, Ruscitti P, Navarini L, Del Puente A, Giacomelli R, et al. Could Sars-coronavirus-2 trigger autoimmune and/or autoinflammatory mechanisms in genetically predisposed subjects? Autoimmun Rev. 2020; 19 (5): 102524. DOI: 10.1016/j.autrev.2020.102524.
29. Dolgushina NV, Menzhinskaya IV, Beznoshchenko OS, Mullabaeva SM, Gorodnova EA, Krechetova LV. Profil antifosfolipidnyx antitel i sostoyanie sistemy komplementa u bol'nyx COVID-19 raznoj stepeni tyazhesti. Medicinskaya immunologiya. 2022; 24 (2): 355–370. Medical immunology. 2022; 24 (2): 355-370. Russian.

Литература

1. Bentov Y, Beharier O, Moav-Zafrir A, Kabessa M, Godin M, Greenfield CS, et al. Ovarian follicular function is not altered by SARS–CoV-2 infection or BNT162b2 mRNA COVID-19 vaccination. Hum Reprod. 2021; 36 (9): 2506–13.
2. Wang M, Yang Q, Ren X, Hu J, Li Z, Long R, et al. Investigating the impact of asymptomatic or mild SARS-CoV-2 infection on female fertility and in vitro fertilization outcomes: A retrospective cohort study. E Clinical Medicine. 2021; 38: 101013. DOI: 10.1016/j.eclinm.2021.101013.
3. Mullins E, Perry A, Banerjee J, Townson J, Grozeva D, Milton R, et al. Pregnancy and neonatal outcomes of COVID-19: The PAN-COVID study. Eur J Obste. Gynecol Reprod Biol. 2022; 276: 161–7.
4. Allotey J, Stallings E, Bonet M, Yap M, Chatterjee S, Kew T, et al. Clinical manifestations, risk factors, and maternal and perinatal outcomes of coronavirus disease 2019 in pregnancy: Living systematic review and meta-analysis. BMJ. 2020; 370: m3320. DOI: 10.1136/bmj.m3320.
5. Balachandren N, Davies MC, Hall JA, Stephenson JM, David AL, Barrett G, et al. SARS-CoV-2 infection in the first trimester and the risk of early miscarriage: a UK population-based prospective cohort study of 3041 pregnancies conceived during the pandemic.

Hum. Reprod. 2022; 37 (6): 1126–33.
6. Bechmann N, Maccio U, Kotb R, Dweik RAl, Cherfane M, Moch H, et al. COVID-19 Infections in Gonads: Consequences on Fertility? Horm Metab Res. 2022; 54 (08): 549–55.
7. Hu W, Zhu Y, Wu Y, Wang F, Qu F. Impact of COVID-19 pandemic on the pregnancy outcomes of women undergoing assisted reproductive techniques (ARTs): a systematic review and meta-analysis. J Zhejiang Univ Sci B. 2022; 23 (8): 655–65.
8. Youngster M, Avraham S, Yaakov O, Landau Rabbi M, Gat I, Yerushalmi G, et al. IVF under COVID-19: treatment outcomes of fresh ART cycles. Hum Reprod. 2022; 37 (5): 947–53.
9. Maiorino MI, Bellastella G, Giugliano D, Esposito K. From inflammation to sexual dysfunctions: a journey through diabetes, obesity, and metabolic syndrome. J Endocrinol Invest. 2018; 41 (11): 1249–58.
10. Huang C, Ji X, Zhou W, Huang Z, Peng X, Fan L, et al. Coronavirus: A possible cause of reduced male fertility. Andrology. 2021; 9 (1): 80–7.
11. Matyushkina D, Shokina V, Tikhonova P, Manuvera V, Shirokov D, Kharlampieva D, et al. Autoimmune Effect of Antibodies against the SARS-CoV-2 Nucleoprotein. Viruses. 2022; 14 (6): 1141.
12. Knight JS, Caricchio R, Casanova J-L, Combes AJ, Diamond B, Fox SE, et al. The intersection of COVID-19 and autoimmunity. J Clin Invest. 2021; 131 (24): e154886. DOI: 10.1172/JCI154886.
13. Mohkhedkar M, Venigalla SSK, Janakiraman V. Untangling COVID-19 and autoimmunity: Identification of plausible targets suggests multi organ involvement. Mol Immunol. 2021; 137: 105–13.
14. Sacchi MC, Tamiazzo S, Stobbione P, Agatea L, De Gaspari P, Stecca A, et al. SARS-CoV-2 infection as a trigger of autoimmune response. Clin Transl Sci. 2021; 14 (3): 898–907.
15. Lui DTW, Lee CH, Chow WS, Lee ACH, Tam AR, Pang P, et al. Long COVID in Patients With Mild to Moderate Disease: Do Thyroid Function and Autoimmunity Play a Role? Endocr Pract. 2021; 27 (9): 894–902.
16. Ehrenfeld M, Tincani A, Andreoli L, Cattalini M, Greenbaum A, Kanduc D, et al. Covid-19 and autoimmunity. Autoimmun. Rev. 2020; 19 (8): 102597. DOI: 10.1016/j.autrev.2020.102597.
17. Zhang Y, Xiao M, Zhang S, Xia P, Cao W, Jiang W, et al. Coagulopathy and Antiphospholipid Antibodies in Patients with Covid-19. N Engl J Med. 2020; 382 (17): e38. DOI: 10.1056/NEJMc2007575.
18. Harzallah I, Debliquis A, Drénou B. Lupus anticoagulant is frequent in patients with Covid-19. J Thromb Haemost. 2020; 18 (8): 2064–5.
19. Soriano A, Blank M, Shoenfeld Y. Genetics and origin of antiphospholipid syndrome. In: Meroni PL, editor. Antiphospholipid antibody syndrome. From bench to bedside. Cham: Springer I, 2014; p. 1–12.
20. Khizroeva J, Nalli C, Bitsadze V, Lojacono A, Zatti S, Andreoli L, Tincani A, Shoenfeld Y, Makatsariya A. Infertility in women with systemic autoimmune diseases. Best Practice & Research Clinical Endocrinology & Metabolism. 2019; 33 (6): 101369. Available from: https://doi.org/10.1016/j.beem.2019.101369.
21. Chighizola CB, de Jesus GR, Branch DW. The hidden world of anti-phospholipid antibodies and female infertility: A literature appraisal. Autoimmunity Reviews. 2016; 15 (6): 493–500. Available from: https://doi.org/10.1016/j.autrev.2016.01.018.
22. Kolanska K, Hours A, Jonquière L, Mathieu d'Argent E, Dabi Y, et al. Mild COVID-19 infection does not alter the ovarian reserve in women treated with ART. Reprod Biomed Online. 2021; 43 (6): 1117–21.
23. Aharon D, Gounko D, Lee JA, Copperman AB, Flisser E. The Impact of the Coronavirus Disease 19 Pandemic on Early Pregnancy Outcomes Among Patients Undergoing In Vitro Fertilization Treatment. Women's Heal Reports. 2021; 2 (1): 473–8.
24. Setti PEL, Cirillo F, Immediata V, Morenghi E, Canevisio V, Ronchetti C, et al. First trimester pregnancy outcomes in a large IVF center from the Lombardy County (Italy) during the peak COVID-19 pandemic. Sci Rep. 2021; 11 (1): 16529. DOI: 10.1038/s41598-021-96134-9.
25. Madaan S, Jaiswal A, Kumar S, Talwar D. Premature ovarian failure-A long COVID sequelae. Med Sci. 2021; 25 (112): 1286–90.
26. Wilkins J, Al-Inizi S. Premature ovarian insufficiency secondary to COVID–19 infection: An original case report. Int J Gynecol Obstet. 2021; 154 (1): 179–80.
27. Shams T, Alhashemi H, Madkhali A, Noorelahi A, Allarakia S, Faden Y, et al. Comparing pregnancy outcomes between symptomatic and asymptomatic COVID-19 positive unvaccinated women: Multicenter study in Saudi Arabia. J Infect Public Health. 2022; 15 (8): 845–52.
28. Caso F, Costa L, Ruscitti P, Navarini L, Del Puente A, Giacomelli R, et al. Could Sars-coronavirus-2 trigger autoimmune and/or autoinflammatory mechanisms in genetically predisposed subjects? Autoimmun Rev. 2020; 19 (5): 102524. DOI: 10.1016/j.autrev.2020.102524.
29. Долгушина Н. В., Менжинская И. В., Безнощенко О. С., Муллабаева С. М., Городнова Е. А., Кречетова Л. В. Профиль антифосфолипидных антител и состояние системы комплемента у больных COVID-19 разной степени тяжести. Медицинская иммунология. 2022; 24 (2): 355-370.