EFFECTS OF COVID-19 VECTOR VACCINE ON AUTOANTIBODY PROFILE IN REPRODUCTIVE AGE WOMEN

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Autoimmune mechanisms have been implicated in the negative effects of vaccines on female reproductive health. This study evaluates the endogenous levels of self-reactive antibodies and ovarian reserve-associated hormones before and after immunization with the domestically developed Gam-COVID-Vac combined vector vaccine to check for possible reproductive sequelae. The prospective study enrolled 120 women aged 18–49, subject to vaccination with Gam-COVID-Vac. Ovarian reserve was assessed prior to vaccination and 90 days after the first component injection. Profiles of specific antibodies to self-antigens, including phospholipids, nuclear antigens, FSH, progesterone, and also thyroid, ovarian, trophoblast, and zona pellucida antigens, were assessed at the same time points by enzyme immunoassay. Overall, the vaccination had no effect on the levels of ovarian reserve-associated hormones and autoantibodies, apart from a transient increase in positivity for anti-phosphatidylethanolamine IgM and anti-dsDNA IgG. Seroprevalence of elevated serum autoantibodies constituted 70.8% before and 75% after vaccination. According to the results, immunization with Gam-COVID-Vac does not affect ovarian reserve or autoimmune status, thus being safe for the female reproductive potential.

Keywords: COVID-19, SARS-CoV-2, Gam-COVID-Vac (Sputnik V) vaccine, reproduction, autoantibody profile

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ВЛИЯНИЕ ВЕКТОРНОЙ ВАКЦИНЫ ОТ COVID-19 НА ПРОФИЛЬ АУТОАНТИТЕЛ У ЖЕНЩИН РЕПРОДУКТИВНОГО ВОЗРАСТА

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Один из механизмов негативного влияния вакцин на репродуктивное здоровье женщин носит аутоиммунный характер. Целью работы было оценить влияние иммунизации отечественной комбинированной векторной вакциной для профилактики коронавирусной инфекции, вызываемой вирусом SARS-CoV-2, на уровень аутоантител и гормонов, отражающих овариальный резерв, и связь между ними у женщин репродуктивного возраста. В проспективное исследование было включено 120 женщин в возрасте 18–49 лет, вакцинированных Гам-КОВИД-Вак. Овариальный резерв определяли перед вакцинацией и через 90 дней после введения первого компонента. С помощью ИФА изучали профиль антифосфолипидных антител, антител к ядерным антигенам, антигенам щитовидной железы, антиовариальных, антитрофобластических антител, к зоне пеллюцида, ФСГ, прогестерону. После вакцинации не обнаружено повышения уровня аутоантител, за исключением анти-ФЭ IgM и анти-дсДНК IgG-антител, которое носило транзиторный характер. Доля позитивных женщин до вакцинации составляла 70,8%, после вакцинации — 75%. Не выявлено корреляционной связи между уровнем аутоантител и гормонов, отражающих овариальный резерв. Согласно результатам, вакцинация Гам-КОВИД-Вак не оказывает негативного влияния на овариальный резерв, не вызывает развития аутоиммунных реакций и ассоциированного с ними снижения репродуктивного потенциала у женщин.

Ключевые слова: COVID-19, SARS-CoV-2, вакцина Гам-КОВИД-Вак (Спутник V), репродукция, профиль аутоантител

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Registered COVID-19 vaccines, mostly based on spike glycoprotein (S-protein) of SARS-CoV-2 [1], include whole inactivated vaccines, combined vector vaccines containing recombinant adenoviral particles with SARS-CoV-2 S protein gene, mRNA vaccines, and recombinant protein vaccines. The combined vector Gam-COVID-Vac (Sputnik V™) was the world’s first registered COVID-19 vaccine, approved by the Ministry of Health of Russia. The vaccine contains adenoviral vector cargoed with a coding sequence of S-protein intended for delivery to human cells in order to educate the immune system in a disease-specific manner [2]. The advent and ubiquitous propagation of COVID-19 vaccines has raised concerns on long-range safety of these medications, particularly on their possible influence on human reproductive health [3–7].

Early (pre-pandemic) studies emphasized the potentially negative impact of adjuvant vaccines developed for other infectious diseases. The vaccines allegedly affected reproductive function by triggering autoimmune responses in

animals and humans [8]. For many immunological adjuvants, mechanisms of action remain understudied. The adjuvants are believed to amplify the innate immunity reactions by mimicking certain evolutionary conserved epitopes (e.g. those of bacterial lipopolysaccharides). These molecules act through binding Toll-like receptors (TLR) on dendritic cells and macrophages; the binding triggers cytoplasmic inflammasome signaling, production of pro-inflammatory cytokines, and expression of antigen-presenting proteins [9].

The normal immune response is sometimes accompanied by ancillary autoimmune reactions. Their diverse mechanisms involve (1) participation of cryptic antigens with subsequent activation of autoreactive Th1 cells; (2) exposure of intracellular epitopes upon tissue damage; (3) amassing of inflammation-specific autoantigens leading to systemic propagation of immune response with the incrementing role of autoantigens; (4) molecular mimicry based on structural similarities between foreign and host epitopes, resulting in activation of cross-reactive T cells and non-specific activation of self-reactive Th1 cells mediated by various TCR dependent and independent mechanisms [9]. Such reactions are collectively known as "autoimmune/inflammatory syndrome induced by adjuvants (ASIA)"; the term was introduced in 2011 [10]. Meta-analysis reveals about 500 cases of ASIA recorded in 2016–2019, most of them associated with vaccines against hepatitis B, influenza, and HPV [11].

Another meta-analysis assessed for correlations between vaccination and the risks of systemic lupus erythematosus (SLE) or rheumatoid arthritis to show significant correlation with the risk of SLE [12]. In women, SLE has been associated with autoimmune oophoritis leading to premature ovarian failure, which proves that rheumatoid conditions may result in infertility. Moreover, about one third of female patients with SLE develop the antiphospholipid syndrome accompanied by recurrent pregnancy losses and thromboembolic complications in connection with thrombocytopenia and antiphospholipid antibody (aPL) persistence. The antiphospholipid syndrome etiology clearly involves molecular mimicry based on the homology between certain exogenous agents and self-antigens, and the resulting cross-reactivity of corresponding antibodies. For instance, IgM antibodies produced in response to immunization with tetanus toxoid also react with cardiolipin and β2-glycoprotein I (β2GPI) self-antigens [13].

It should be noted that Gam-COVID-Vac is a combined vector vaccine and does not contain adjuvants. Preliminary data on the influence of Gam-COVID-Vac on the levels of aPL to cardiolipin (anti-CL), β2GPI (anti-β2GPI), annexin V (anti-AnV), and phosphatidylserine (anti-PS) have been published. According to the results of examination for 51 vaccinated female participants, immunization with Gam-COVID-Vac (Sputnik V) caused no elevation in circulating aPL levels [6]. The lack of correlation between circulating levels of aPL, follicle-stimulating hormone (FSH), and anti-müllerian hormone (AMH) indirectly confirmed the lack of negative impact on female reproductive potential.

A more dedicated assessment of the vaccination safety in terms of reproductive health requires studying a wide range of autoantibodies involved in the development of systemic autoimmune diseases before and after vaccination on more representative samples of reproductive age women.

This study evaluates endogenous levels of self-reactive antibodies and hormones, associated with ovarian reserve, before and after immunization with the domestically developed Gam-COVID-Vac combined vector vaccine against SARS-CoV-2, to check for possible reproductive sequelae of the vaccination.

METHODS

This prospective study was carried out at the Kulakov National Medical Research Center for Obstetrics, Gynecology and Perinatology, Moscow, Russia. The participants (n = 120) were enrolled within the period from December, 2020 to December, 2021; all participants received immunization with the Gam-COVID-Vac combined vector vaccine for the prophylaxis of SARS-CoV-2-induced new coronavirus infection. The inclusion criteria were as follows: age 18–49 years; preserved menstrual function; negative PCR test for SARS-CoV-2 RNA and negative tests for IgM and IgG antibodies to SARS-CoV-2 (before vaccination); no clinical history of COVID-19; and no contact with COVID-19 cases for at least 14 days prior to the start of the study. The non-inclusion criteria were as follows: listed contraindications for Gam-COVID-Vac use; pregnancy and lactation; acute inflammatory and infectious diseases; rheumatic diseases; oncological diseases of any localization; hormone therapy affecting the menstrual cycle; immunomodulatory therapy; or vaccination within three months preceding the enrollment. Patients with decreased ovarian reserve (FSH >12 mIU/mL and antral follicle count (AFC) below six in both ovaries) or morbid obesity (BMI ≥ 40.0 kg/m^2) were not included in the study. The exclusion criteria were COVID-19 during the vaccination period, pronounced side effects of immunization requiring the abolition of the second component, and vaccination refusal.

The participants were examined twice: (1) before vaccination and (2) 90–100 days after injection of the first component. Patients with autoantibody levels above reference range (RR) were subject to additional round of tests performed at 6 months after the first component injection. Serum levels of FSH and AMH were determined by electrochemiluminescence assay on day 2–5 of the menstrual cycle.

Autoantibody levels were measured by enzyme immunoassay before and after vaccination. The scope included antiphospholipid IgM and IgG antibodies (Ab), both "criterial" (anti CL and anti-β2GPI) and "non-criterial" (anti-AnV, anti-PS) (reagents by ORGENTEC Diagnostika GmbH; Germany); phosphatidylethanolamine (anti-PE) and phosphatidylserine/prothrombin complexes (anti-PS/PT) (reagents by AESKU Diagnostics; Germany); antinuclear IgG Ab (ANA) and Ab to double stranded DNA (anti-dsDNA), ribonucleoprotein 70 (anti-RNP70), and cytoplasmic antigens SS-A(Ro) and SS-B(La) (respectively, anti-SS-A and anti-SS-B) (reagents by ORGENTEC Diagnostika; GmbH); IgG Ab to thyroid antigens: thyroglobulin (anti-TG) and thyroid peroxidase (anti-TPO) (reagents by ORGENTEC Diagnostika GmbH; Германия), and thyroid-stimulating hormone receptor (anti-TSH receptor) (reagents by Medipan GmbH; Germany). Immunoassay targets also included anti-trophoblast and anti-zona pellucida IgG (reagents by QAYEE-BIO for Life Science; China), anti-ovary Ab (reagents by DRG; Germany); and Ab to FSH and progesterone (respectively, anti-FSH and anti-PRG) (reagents by Xema-Medica; Russia).

The patients were vaccinated in accordance with the current recommendations issued by the Ministry of Health of Russia [14]. Handling of the vaccine strictly followed the official instructions for this medication.

Statistical processing of the data was performed using Microsoft Excel spreadsheet and Statistica V10 packages (TIBCO; USA). The categorical data were converted into percentages (%). McNemar's test was applied to analyze the paired binary data for the patients before and after vaccination. The distributions were assessed with Kolmogorov–Smirnov test

Table 1. Serum levels of antiphospholipid antibodies (aPL) in women before and after vaccination

Parameter	Reference range	Before vaccination	90 days after vaccination	*p*-value
anti-CL IgM, U/mL	< 7 U/mL	2.2 (1.4–3.5)	2.3 (1.7–3.1)	0.575
anti -CL IgG, U/mL	< 10 U/mL	2.9 (2.0–4.1)	2.4 (1.8–3.3)	< 0.001
anti-β2GPI IgM, U/mL	< 5 U/mL	2.6 (1.7–3.5)	2.3 (1.7–3.2)	0.022
anti- β2GPI IgG, U/mL	< 5 U/mL	1.8 (1.3–2.9)	1.7 (1.3–2.3)	0.003
anti-AnV IgM, U/mL	< 5 U/mL	2.9 (2.2–4.1)	3.4 (2.6–4.4)	< 0.001
anti-AnV IgG, U/mL	< 5 U/mL	2.3 (1.7–3.0)	4.0 (2.2–4.8)	< 0.001
anti-PS IgM, U/mL	< 10 U/mL	2.5 (1.8–3.4)	2.5 (1.9–3.2)	0.630
anti-PS IgG, U/mL	< 10 U/mL	2.6 (2.0–3.4)	3.5 (2.8–4.6)	< 0.001
anti-PE IgM, U/mL	< 12 U/mL	5.2 (2.6–10.4)	6.1 (3.4–13.4)	< 0.001
anti-PE IgG, U/mL	< 12 U/mL	1.0 (1.0–1.3)	1.5 (1.2–2.1)	< 0.001
anti-PS/PT IgM, U/mL	< 12 U/mL	1.9 (1.3–2.8)	1.9 (1.3–3.2)	0.949
anti-PS/PT IgG, U/mL	< 12 U/mL	2.3 (1.7–3.3)	2.8 (1.9–3.7)	0.002

Note: Me (X–Y), *p*-values by the sign test.

using graphical data representation. Given non-normality of the distributions, the data were analyzed non-parametrically, with the variables described by medians and interquartile ranges, Me (Q25–Q75), and the non-parametric sign test applied for paired samples. The differences were considered statistically significant at $p < 0.05$.

RESULTS

Median age of the participants was 33 years; median BMI constituted 22.4 kg/m2. All participants complied with the inclusion criteria; prevalence of gynecological diseases did not exceed 10%; non-gynecological diagnoses were dominated by allergic conditions (encountered in 30% of participants). Ovarian reserves were assessed before and after vaccination using FSH and AMH tests combined to AFC. No significant alterations in hormone levels and AFC were encountered. The medians/interquartile ranges fell within RR for all studied hormones, and similar numbers of patients presented with FSH levels above RR and AMH levels below RR before and after vaccination.

The study of serum antiphospholipid antibodies (aPL) revealed reciprocal dynamics for the levels of criterial and non-criterial aPL, respectively (Table 1).

In 30 pts (25%) aPL levels exceeded RR initially (Table 2). After vaccination, elevated aPL levels were observed in 28 pts (23.3%), most frequently for anti-PE IgM (20 pts, 16.7%). In 7 pts (5.8%) elevated aPL levels were encountered after vaccination only, whereas in 9 pts (7.5%) the initially elevated aPL levels decreased to normal after vaccination. Two pts (1.7%) with histories of autoimmune thyroiditis and allergic reactions revealed aPL persistence after vaccination. It should be noted that both of them had ovarian reserve parameters within the normal range, showing no decrease after vaccination.

Possible correlations in the dynamics of aPL and hormone levels we assessed by setting Δ values for the parameters (e. g. ΔAMH = AMH1–AMH2, ΔFSH = FSH2–FSH1, etc.); a positive Δ always indicated an adverse trend (Fig. 1). The only identified correlation (a weak negative one of negligible clinical relevance) reflected an increase in AMH levels accompanied by an increase in anti-PS/PT IgM.

All 7 pts with elevated aPL as measured 90 days after vaccination showed normal aPL in the repeated tests performed 6 months after vaccination.

Thus, all median values and interquartile ranges for serum aPL levels fell within RR. The analysis revealed no significant increase in the prevalence of elevated aPL levels after vaccination, except for anti-PE IgM. In patients revealing elevated serum aPL at the second reference time point of the study (90 days after the first component injection), the values returned to normal range within a 3 months follow-up. The analysis revealed no clinically relevant dynamic associations for the ovarian reserve-related hormones and aPL.

Table 2. Prevalence of elevated serum aPL in women before and after vaccination

Parameter	Before vaccination	90 days after vaccination	*p*-value
anti-CL IgM, U/mL	4 (3.3%)	4 (3.3%)	0.683
anti-CL IgG, U/mL	4 (3.3%)	1 (0.8%)	0.248
anti-β2GPI IgM, U/mL	0	1 (0.8%)	–
anti-β2GPI IgG, U/mL	4 (3.3%)	2 (1.7%)	0.479
anti-AnV IgM, U/mL	3 (2.5)	5 (4.2%)	0.723
anti-AnV IgG, U/mL	3 (2.5)	3 (2.5)	0.617
anti-PS IgM, U/mL	1 (0.8%)	1 (0.8%)	1.00
anti-PS IgG, U/mL	0	1 (0.8%)	–
anti-PE IgM, U/mL	15 (12.5%)	20 (16.7%)	0.043
anti-PE IgG, U/mL	0	2 (1.7%)	–
anti-PS/PT IgM, U/mL	1 (0.8%)	0	–
anti-PS/PT IgG, U/mL	0	1 (0.8%)	–
At least one type of aPL above RR	30 (25%)	28 (23.3%)	0.802

Note: *p*-values by McNemar's test, RR — reference range.

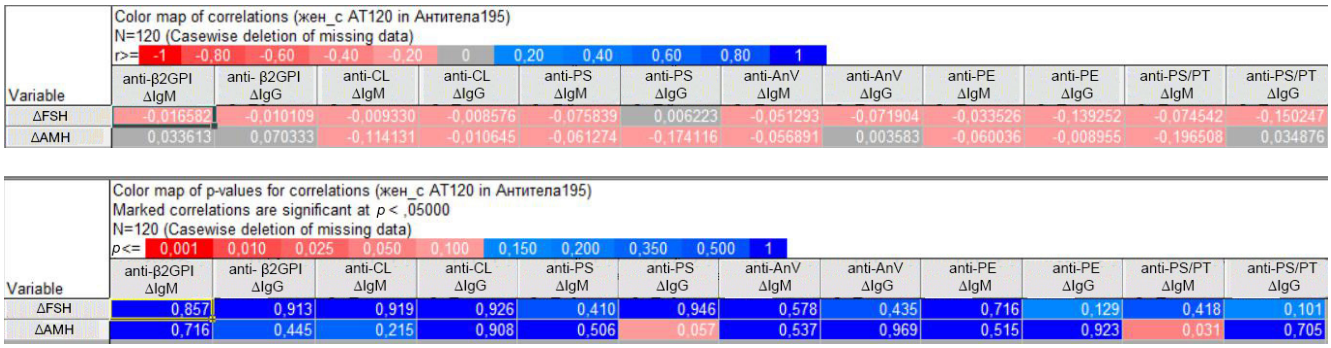

Fig. 1. Correlation analysis for possible dynamic associations between serum levels of hormones (ΔFSH, ΔAMH) and antiphospholipid antibodies (ΔaPL) in women before and after vaccination

Apart from aPL, we analyzed a scope of other self-reactive antibodies with diverse specificities (organ-specific, antinuclear, and anti-hormone) before and after vaccination (Table 3). Significantly decreased serum levels were observed for anti-FSH IgM, anti-TSH receptor IgG, ANA IgG, anti-SS-A IgG, and-RNP70 IgG, along with significantly increased serum levels of anti-trophoblast IgG, anti-ovary IgG, anti-PRG IgG, anti-SS-B IgG, and anti-dsDNA IgG.

However, these trends are hardly relevant: despite the statistically significant dynamics, Ab levels stayed within RR in the majority of patients. Comparing seroprevalence of elevated autoantibodies is more indicative (Table 4). Patients with at least one type of elevated serum autoantibodies constituted 70.8% before vaccination and 75% after vaccination. Patients with at least two autoAb above RR constituted 38.3% and 45.8%, respectively. It should be noted that different classes of anti-PRG and anti-FSH Ig showed reciprocal dynamics: the prevalence of elevated serum IgM decreased, whereas the prevalence of elevated serum IgG increased. Anti-dsDNA IgG were the only autoantibodies with the elevated serum levels more prevalent after vaccination than before, although in most of the cases their levels only slightly exceeded RR.

Correlation analysis revealed no dynamic associations between serum levels of ovarian reserve-related hormones (FSH, AMH) and self-reactive antibodies to nuclear antigens, thyroid-, ovarian-, and trophoblast-specific antigens, or hormones (Fig. 2).

The results indicate high incidence of serum autoantibody persistence in reproductive age women. The only antibodies showing increased seroprevalence after vaccination were anti-dsDNA IgG. In patients showing elevated serum Ab at the second reference time point of the study, these levels returned to normal within 6 months after the first component injection. Correlation analysis revealed no dynamic associations between serum levels of ovarian reserve-related hormones and autoantibodies.

DISCUSSION

According to *in vivo* studies and pilot clinical research, vaccination may cause reproductive failure by promoting ASIA and certain autoimmune disorders including anti-phospholipid syndrome and SLE [12, 13, 15]. It is known that aPL can destroy the annexin V protective layer formed over the negatively charged surface of syncytiotrophoblast and endothelium, thus promoting coagulation and development of a hypercoagulable state. In addition, aPL can initiate apoptosis in trophoblast cells, interfere with cell proliferation, expression of cell adhesion molecules, and chorionic gonadotropin secretion, while supporting pro-inflammatory reactions [16].

Autoantibody persistence is rather prevalent in women. This asymptomatic condition may favor development of autoimmune diseases in genetically predisposed individuals. In connection with this concern, the present study analyzed a wide range of

Table 3. Serum levels of autoantibodies in women before and after vaccination

Parameter	Reference range	Before vaccination	90 days after vaccination	*p*-value
anti-zona pellucida IgG, ng/mL	< 250 ng/mL	156.0 (126.5–183.5)	157.0 (133.7–218.2)	0.114
anti-trophoblast IgG, ng/mL	< 150 ng/mL	101.7 (84.0–117.0)	127.2 (106.0–137.2)	< 0.001
anti-ovary IgG, U/mL	< 10 U/mL	4.0 (3.3–5.1)	4.8 (4.0–5.7)	< 0.001
anti-PRG IgM, OD u	< 0.4 OD u	0.28 (0.22–0.40)	0.29 (0.23–0.35)	0.302
anti-PRG IgG, OD u	< 0.4 OD u	0.28 (0.21–0.37)	0.33 (0.25–0.44)	0.002
anti-FSH IgM, OD u	< 0.4 OD u	0.29 (0.22–0.38)	0.25 (0.19–0.30)	< 0.001
anti-FSH IgG, OD u	< 0.4 OD u	0.27 (0.20–0.34)	0.29 (0.23–0.35)	0.575
anti-TPO IgG, IU/mL	< 50 IU/mL	12.2 (8.7–18.7)	12.4 (9.5–18.9)	1.00
anti-TSH receptor IgG, IU/L	≤ 1 IU/L	0.5 (0.3–0.6)	0.3 (0.2–0.5)	< 0.001
anti-TG IgG, IU/mL	< 100 IU/mL	19.4 (15.0–28.5)	20.7 (14.8–31.5)	0.227
ANA IgG, PI	< 1 PI	0.5 (0.4–0.7)	0.45 (0.4–0.65)	< 0.001
анти-SS-A IgG, IU/mL	< 15 IU/mL	3.3 (2.6–5.3)	3.0 (2.6–4.3)	0.032
анти-SS-B IgG, IU/mL	< 15 IU/mL	3.2 (2.1–4.8)	3.3 (2.3–5.1)	0.038
anti-dsDNA IgG, IU/mL	< 20 IU/mL	11.8 (9.3–14.7)	15.3 (12.8–18.1)	< 0.001
anti-RNP70 IgG, U/mL	< 25 U/mL	4.1 (2.8–5.6)	2.3 (1.7–3.1)	< 0.001

Note: Me (X–Y), *p*-values by the sign test.

Table 4. Prevalence of elevated serum autoantibodies in women before and after vaccination

Parameter	Reference range	Before vaccination	90 days after vaccination	*p*-value
anti-zona pellucida IgG, ng/mL	< 250 ng/mL	17 (14.2%)	19 (15.8%)	0.844
anti-trophoblast IgG, ng/mL	< 150 ng/mL	7 (5.8%)	13 (10.8%)	0.211
anti-ovary IgG, U/mL	< 10 U/mL	1 (0.8%)	2 (1.6%)	1.00
anti-PRG IgM, OD u	< 0,4 OD u	32 (26.7%)	19 (15.8%)	0.012
anti-PRG IgG OD u	< 0,4 OD u	21 (17.5%)	41 (34.2%)	< 0.001
anti-FSH IgM, OD u	< 0,4 OD u	23 (19.2%)	11 (9.2%)	0.006
anti-FSH IgG, OD u	< 0,4 OD u	14 (11.7%)	21 (17.5%)	0.190
anti-TPO IgG, IU/mL	< 50 IU/mL	12 (10%)	13 (10.8%)	1.00
anti-TSH receptor IgG, IU/L	≤ 1 IU/L	2 (1.6%)	1 (0.8%)	1.00
anti-TG IgG, IU/mL	< 100 IU/mL	6 (5%)	7 (5.8%)	1.00
ANA IgG, PI	< 1 PI	11 (9.2%)	12 (10%)	1.00
anti-SS-A IgG, IU/mL	< 15 IU/mL	4 (3.3%)	7 (5.8%)	0.248
anti-SS-B IgG, IU/mL	< 15 IU/mL	1 (0.8%)	1 (0.8%)	–
anti-dsDNA IgG, IU/mL	< 20 IU/mL	4 (3.3%)	18 (15%)	0.003
anti-RNP70 IgG, U/mL	< 25 U/mL	4 (3.3%)	0	–
At least one type of autoAb above RR		85 (70.8%)	90 (75%)	0.423
Two or more types of autoAb above RR		46 (38.3%)	55 (45.8%)	0.176

Note: *p*-values by McNemar's test, RR — reference range.

autoantibodies to organ-specific and systemic self-antigens in reproductive age women before and after COVID-19 vaccination. The tests for criterial and non-criterial aPL were performed to assess the risks of antiphospholipid syndrome with possible complications including infertility, thrombosis, thrombocytopenia, and habitual miscarriage [17].

The study also included the antinuclear antibody (ANA) tests conventionally used in diagnostics of connective tissue autoimmune disorders (SLE, Sjögren syndrome, the mixed connective tissue disease (Sharp syndrome), polymyositis/dermatomyositis, and progressive systemic scleroderma) [18]. ANA are widely employed as biomarkers of particular connective tissue disorders. Apart from ANA, the women were tested for autoantibodies to double-stranded DNA (dsDNA) and extractable nuclear antigens SS-A/Ro, SS-B/La, and RNP70. Rheumatoid autoimmune diseases are often associated with the presence of autoantibodies to extractable nuclear and cytoplasmic antigens. The anti-SS-B and anti-SS-A autoantibodies are usually detectable in Sjögren syndrome [19]. The study also included serum and plasma tests for anti-RNP70 IgG conventionally used in diagnostics of the mixed connective tissue disease (Sharp syndrome) and related autoimmune disorders [20].

In addition, the participants were tested for autoantibodies to organ-specific antigens, including anti-ovary, anti-trophoblast, and anti-zona pellucida species, associated with the risks of reduced fertility, infertility, or premature ovarian failure. In particular, anti-oocyte autoantibodies have been associated with poor ovarian response upon ovarian stimulation in IVF cycles [21].

Other serological targets included autoantibodies to thyroid antigens including thyroglobulin (TG), thyroid peroxidase (TPO), and thyroid-stimulating hormone receptor (TSH receptor). These tests are used in differential diagnostics of autoimmune thyroid diseases including Hashimoto thyroiditis and Graves' disease [22].

We also used novel modifications of enzyme immunoassay enabling detection of antibodies to the most important female reproductive hormones, particularly FSH and progesterone. Such antibodies can interfere with the effects of exogenous and endogenous hormones and affect follicular growth, endometrial readiness, and the course of early pregnancy.

The results indicate significant decrease in blood levels of criterial aPL (anti-CL and anti-β2GPI) accompanied by significant increase in non-criterial aPL (anti-AnV, anti-PS, anti-PE, and anti-PS/PT) after vaccination. However, these findings have low clinical relevance: in the majority of patients, aPL levels stayed within the normal range despite the observed negative and positive dynamics.

The analysis of prevalence of elevated aPL levels with regard to vaccination is certainly more informative. We observed a significant increase in the prevalence of elevated anti-PE IgM in response to vaccination, which was transient.

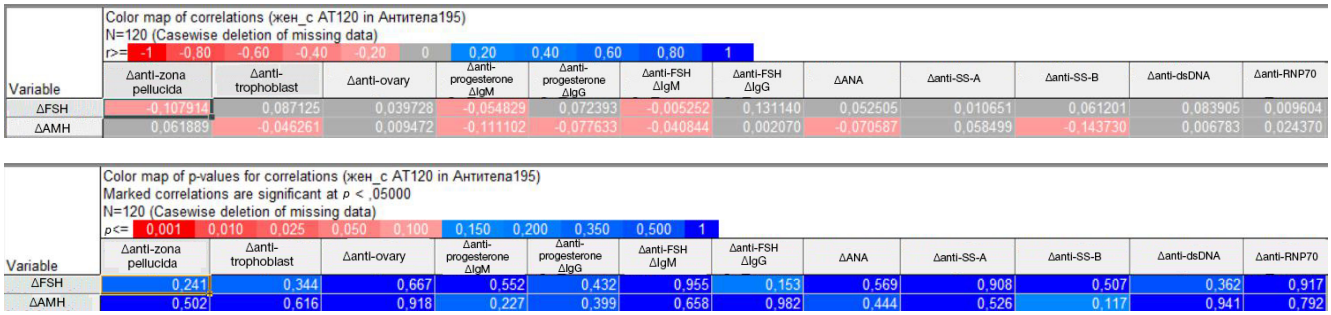

Fig. 2. Correlation analysis for possible dynamic associations between serum levels of hormones (ΔFSH, ΔAMH) and autoantibodies (ΔautoAB) in women before and after vaccination

It should be noted that anti-PE aPL typically arise during infectious processes induced by viral or bacterial pathogens and persist for a considerable period [23-24]. PE, a major lipid component of microbial membranes, is also found in human cell membranes. Immunologically compelling circumstances (immunization, activation of chronic infection foci, etc.) accompanied by release of pro-inflammatory mediators and tissue damage may facilitate exposure of PE in cell membranes, triggering the production of corresponding self-reactive autoantibodies capable of transient or long-term persistence. Infectious agents are capable of triggering autoimmune reactions, whereas anti-PE antibody persistence may indicate chronic infections possibly prone to the vaccination-mediated enhancement.

Overall, the observed trends were clinically irrelevant, except, perhaps, the transiently increased prevalence of elevated serum levels for anti-dsDNA IgG after vaccination. Elevated levels of anti-dsDNA antibodies may result from a variety of events (both physiological and pathological) where DNA fragments are released from cells along with other nuclear antigens. For instance, such release of nuclear antigens occurs in apoptosis or necrosis. Production of anti-dsDNA antibodies at systemic level can be induced by exogenous factors, e.g. bacterial lipopolysaccharide stimulation [25]. These findings explain the boosted synthesis of anti-dsDNA antibodies during Gram-negative bacterial infections [26].

One study focusing on the autoimmune effects of different types of vaccines demonstrates that production of autoantibodies occurs in healthy people in response to immunization against hepatitis B, influenza, or hepatitis A (including a transient increase observed for antinuclear antibodies). At the same time, none of the patients presented with autoimmune disease at follow-up. The authors suggest that a transient increase in serum titers observed for certain autoantibodies may reflect the non-specific activation of T and B cells in response to vaccination [27].

Importanly, in the current study, we found no dynamic associations between serum levels of ovarian reserve-related hormones and corresponding levels of aPL and other autoantibodies. This ultimately confirms the reproductive safety of vaccination with Gam-COVID-Vac by excluding adverse autoimmune reactions.

Moreover, anti-SARS-CoV-2 vaccination in women is increasingly justified by recent studies emphasizing the negative impact of COVID-19 on female reproductive function, reflected by reduced AMH levels [28]. The mechanism may involve direct infection of ovarian tissues, oocytes, and endometrial cells with SARS-CoV-2 leading to impaired ovulatory function, production of infected or aneuploid oocytes, reduced fertilization potential, and impaired embryo implantation [29].

CONCLUSIONS

The results indicate that immunization with Gam-COVID-Vac combined vector vaccine for the prophylaxis of SARS-CoV-2-induced new coronavirus infection has no adverse effects on serum levels of ovarian reserve-related hormones and self-reactive antibodies in reproductive age women. Immunization with Gam-COVID-Vac entails neither conspicuous autoimmune reactions, nor associated decrease in female reproductive potential. Further research will be required to elucidate the mechanisms of immune response to vaccination and dynamic immune status with an emphasis on the cellular wing of immunity.

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