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The Efficacy of Immunoadjuvant-Containing Influenza Vaccines in Pregnancy

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Additional information is available at the end of the chapter

Abstract

The aim of the work was to determine the clinical safety and immunogenicity of immunoadjuvant vaccines against influenza (MonoGripol Plus and Grippol® Plus) in 182 pregnant women in the II and III trimesters of gestation , and further assessment of fetal conditions and infants of the first 6 months of life. Results: It was shown that immunoadjuvant vaccines do not have a negative effect on the physiological course of pregnancy and the functional state of the fetoplacental complex. In the early postpartum period, the rates of physical and neuro-psychological development and the nature of feeding of children did not differ from the control group. In pregnant women vaccinated with Grippol® plus, the levels of seroprotection to strains of A/H1N1/v are 82.0%, A/H3N2/—88.0%, B—88.3% that measure the CPMP criteria and last more than a year . After birth, transplacental antibodies in children in protective values were observed in 52.3–68.9% of cases, did not differ from the control group, and disappear after 6 months. Respiratory infections during the first 6 months of life of infants born from mothers vaccinated against influenza registered in 1.8 times less frequently.

Keywords: pregnant women, vaccine against influenza, post-vaccination immunity.

1. Introduction

The reports of Strategic Advisory Group of Experts (SAGE) on Immunization underscore that between 7 and 10% of all hospitalized patients with severe influenza are women in the second and third trimester of pregnancy. The requirements of pregnant women with influenza infection for providing medical care in an intensive care unit are 10 times that of other population groups diagnosed with influenza [1–4].

Vaccination of pregnant women using the subunit and split influenza vaccines is routinely performed in a number of countries of Europe and America for over 20 years, and the vaccine efficacy reaches 70–85% [5, 6]. Clinical studies have shown that vaccination of pregnant women using modern inactivated influenza vaccines neither affect the course of pregnancy and fetal growth nor cause undesirable post-vaccination effects. It was found that vaccination of pregnant women using inactivated influenza vaccines leads to 50–63% reduction of flu-related morbidity among infants up to 6 months of age [7, 8].

The WHO Global Advisory Committee on Vaccine Safety indicates that influenza vaccination is a non-alternative approach to safe and effective prevention of influenza in pregnancy [1, 9, 10]. In Russia, the indications for vaccination of pregnant women using modern vaccines are defined within the National Immunization Program Schedule of Russian Federation (RF) (order of Ministry of Healthcare of Russian Federation №125n of 21 March 2014). Federal clinical guidelines "Influenza vaccination of pregnant women" and manuals for physicians have been published which establish the main vaccination requirements for the primary health care in Russia [11–13].

The unfavorable epidemiological situation with influenza that occurred in 2009 has accelerated the development and implementation in healthcare practices of adjuvant-containing pandemic influenza vaccines such as Fluad (containing squalene) and Arepanrix™H1N1 (containing AS03—squalene + α-Tocopherol acetate) which confer enhanced immunogenicity [1, 14]. Adjuvants accelerate, change the dynamics of development of the immunity, and increase its level and the duration of persistence of post-vaccination antibodies. With the help of an adjuvant, durable and solid immunity is achieved by administering small doses of antigen and a less number of injections.

In Russia, two adjuvant-containing subunit influenza vaccines have been developed (monovalent (pandemic) and trivalent preparations). These drugs, in contrast to non-adjuvant subunit vaccines against influenza (e.g., Agrippal S1 containing 15 µg strains of influenza viruses type A and B), have 5 µg of both strains of the influenza virus and an adjuvant-immunomodulator polyoxidonium. In clinical trials, immunoadjuvant vaccines demonstrated high efficacy and safety in children aged 6 months and older and in adults. The trivalent adjuvant-based influenza vaccine is used in clinical practice for more than 20 years [15–24]. In experimental studies, these vaccines showed no teratogenic effect on the developing fetus. Despite extensive use of these vaccines for specific prevention of influenza in Russia, studies on their safety in pregnancy have not been conducted until recently. The information on the effects of adjuvant-containing vaccines on the fetus and post-natal development was missing. The information on vaccine immunogenicity for pregnant women at

different gestational age, as well as vaccine ability to confer an adequate passive immunity to a fetus, was insufficient.

The study aimed at determining clinical safety and immunogenicity of "MonoGrippol Plus" and "Grippol® Plus" vaccines in pregnant women in the second and third trimesters of pregnancy with assessment of fetal condition and condition of infants during the first 6 months of life.

2. Materials and methods

2.1. Legal basis of the research

The study was carried out according to the protocol which met the National standard of Russian Federation—GOST P 52379-2005 "Good Clinical Practice" and the international GCP (good clinical practice) standards. Vaccination of pregnant women was carried out with adherence to the ethical norms and guidelines of the WHO and Ministry of Health care of RF.

Women to be vaccinated and followed up were selected strictly in accordance with a case report form that was examined and approved by the Ethics Committee of the Ulyanovsk State University (protocol №35 of 14.01.2010).

The observation of pregnant women before and after vaccination was carried out jointly with an obstetrician-gynecologist in accordance with requirements of the Order of Ministry of Health care and Social Development of RF of 02.10.2009 N 808n "On the approval of the Order of providing obstetric and gynecologic care." Before vaccination, women underwent laboratory testing after they have given the informed consent to participate in the study (**Figure 1**).

During observation and examination of infants, we also adhered to the ethical requirements applicable to biomedical studies. Development of the order and scope of studied parameters was based on provisions listed in the Order № 370 of Ministry of Healthcare of RF of 28.04.2007.

2.2. Randomization

The study was a randomized, placebo-controlled, single-blind, comparative, parallel-group study conducted on pregnant women and infants.

All candidates for study program underwent a preliminary assessment of whether they met the protocol inclusion and exclusion criteria (in accordance with the GMP standards).

Eligibility criteria:

1. Healthy pregnant women aged 20–40.

2. Volunteers capable of fulfilling the protocol requirements (i.e., able to fill in the self-observation diary and turn up for the scheduled visits).

3. Written informed consent of the volunteers to participate in the clinical study.
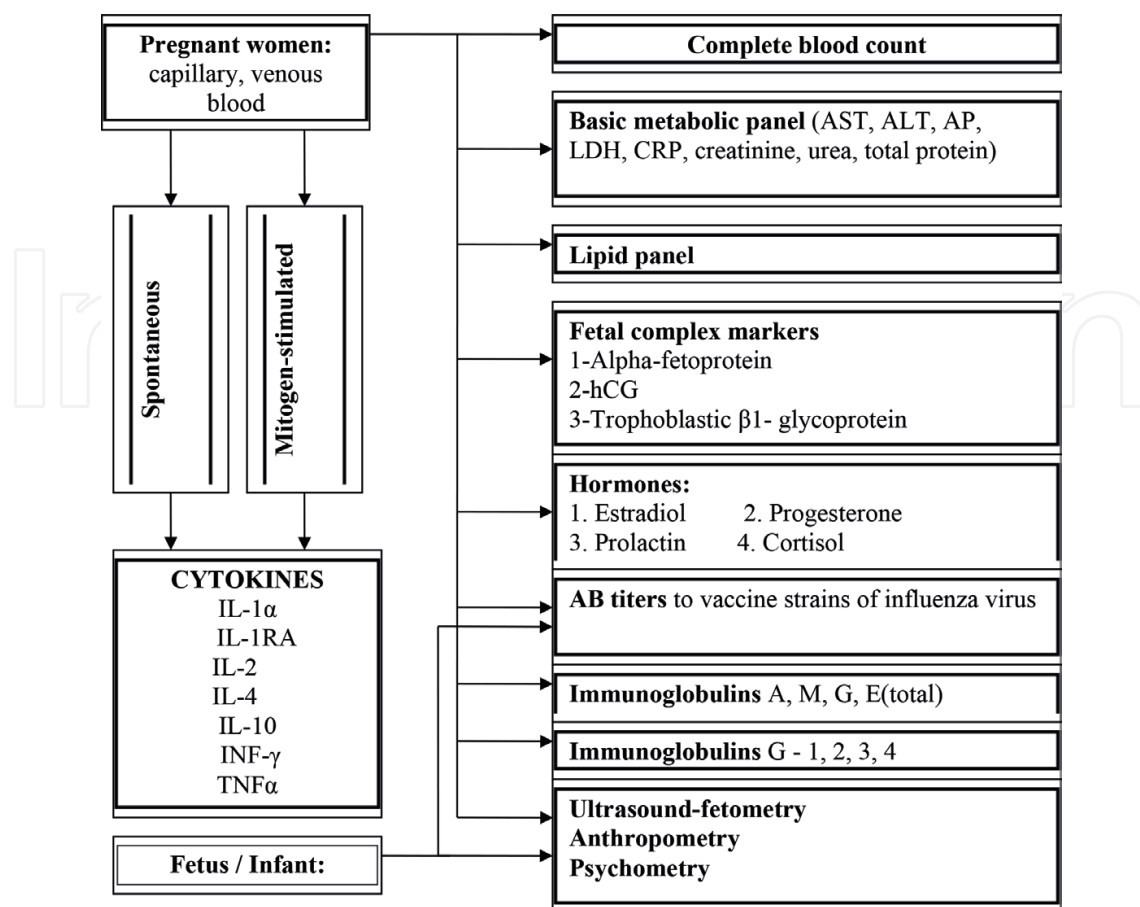
Figure 1. An algorithm of laboratory, physical and instrumental investigation.

Exclusion criteria:

1. History of leukemia, oncologic conditions or positive tests for HIV, hepatitis B and C.

2. Volunteers who had received the immunoglobulin preparations or blood transfusions within the last three months prior to the study.

3. Long-term (more than 14 days) administration of immunodepressants or other immunomodulating drugs within the last six months prior to the study.

4. Any confirmed or suspected immunosuppressive or immunodeficiency disorder.

5. History of chronic alcohol abuse and/or substance abuse.

6. Presence of respiratory or cardiovascular insufficiency, hepatic or renal impairment revealed during physical examination or by laboratory tests at visit 1.

7. Severe congenital defects or serious chronic diseases including any clinically significant diseases of lungs, kidneys, cardiovascular system, nervous system, psychiatric diseases or metabolic disorders confirmed by anamnestic data or objective clinical examination.

8. Presence of acute infectious and/or non-infectious diseases at the time of enrollment in the study.

9. Pregnancy via IVF procedure.

2.3. Duration of observation

A total number of pregnant women vaccinated against influenza during the epidemic seasons of 2009–2010, 2010–2011, and 2011–2012 were 345 subjects. Of those, the number of women and their children participated in an in-depth examination and their assignment to groups and subgroups is presented in **Table 1**.

The frequency of clinical examination and blood collection for laboratory testing in the post-vaccination period was based on the gestational age at the start of observation. Women vaccinated in the second and third trimesters underwent 7 and 6 examinations, respectively.

2.4. Assessment of fetal conditions

Fetometric measurements were carried out using the ultrasound (US) examination during pregnancy weeks 21–22 and 33–35 and included determination and calculation of biparietal diameter (BPD), fronto-occipital size (FOS), head circumference (HC), abdominal circumference (AC), estimated fetal weight (FW) and the femur length/abdominal circumference (FL/AC) ratio. The generally accepted guidelines were followed to evaluate the parameters obtained.

2.5. Assessment of infant conditions

Infant observation started from the first hours and days of life (day 2–3) and was conducted jointly with a neonatologist at maternity home. The basic signs of functional and morphological maturity of the newborn (Apgar score), blood work parameters/biochemical profile and antibody levels to influenza virus strains have been analyzed. All newborns at maternity home underwent neurosonography and cardiac sonography. Basic anthropometric measurements included body weight (BW), body length (BL), head circumference (HC), chest circumference (CC), and height-weight index (Ketle 1).

At the age of 3 and 6 months, the main parameters of physical and neuropsychological development and feeding pattern have been recorded.

2.6. Hormonal status in pregnant women

Hormone concentration in pregnant women was measured using the licensed immunoenzyme test-systems (IETS) such as "Estradiol-EIA" (LLC "Chema," Germany), "EIA-Progesterone,"

	Group I Pregnant women vaccinated with "MonoGrippol Plus"		Group II Pregnant women vaccinated with "Grippol® Plus"		Group III Pregnant women vaccinated with "Agrippal S1"		Group IV Pregnant women who had received "Placebo"		Group V Non-pregnant women, who had received	
	Trimesters of pregnancy								MonoGrippol Plus	Grippol Plus
	II	III	II	III	II	III	II	III		
Number of women participated in study program	28	15	27	23	27	21	22	19	30	19
Total:	43		50		48		41		49	
Average age (years)	25.1 ± 0.7		23.3 ± 0.4		27.8 ± 0.6		24.1 ± 0.3		23.1 ± 0.4	
Number of children born to women vaccinated during pregnancy	24	14	27	21	23	19	18	17		
Total:	38		48		42		35			

Table 1. Study participants' assignment to observation groups.

"EIA-Prolactin," "EIA-Cortisol" (LLC "Alcor Bio Company, Russia"). Fetoplacental complex markers, such as serum alpha-fetoprotein (AFP), human chorionic gonadotropin (hCG) and trophoblastic β1-glycoprotein (TBG), were tested using the IETS from CJSC "Vector-Best," Russia.

2.7. Cytokine profile

Serum cytokines were measured to determine levels of interleukin-1α (IL-1α), interleukin-2 (IL-2), interleukin-4 (IL-4), interferon-γ (IFN-γ), tumor necrosis factor α (TNFα), interleukin-1α receptor antagonist (IL-1RA), and interleukin-10 (IL-10). We used a dual cytokine assay to measure spontaneous and mitogen-induced cytokine production using a system of sample preparation from "Cytokine-Stimul-Best" (CJSC "Vector-Best", Russia). As the test systems, we used standard EIA kits (CJSC "Vector-Best" and LLC "Cytokine", Russia).

2.8. Humoral immune response to vaccination

Concentration of serum immunoglobulins A, M, G, E and IgG subclasses was determined using the appropriate IETS from CJSC "Vector-Best," Russia. Titers of antibodies to influenza virus strains A and B were measured in the hemagglutination inhibition test (HAI) as recommended by the WHO for this kind of studies. As viral antigens, we used the A/California/7/2009/H1N1/v-like, A/H3N2/(Victoria)-like and B (Brisbane)-like strains provided by the laboratory of artificial antigens (FSFI "State Research Center at the Institute of Immunology" of FMBA, Russia).

Vaccine immunogenicity was determined based on criteria established by the Committee for Proprietary Medicinal Products (CPMP) according to the protocol CPMP/BWP/214/96:

1. *Seroprotection level* (>70%).

2. *Seroconversion level* or *vaccine immunologic activity* (>40%).

3. *Seroconversion factor* or *geometric mean fold rise* (>2.5).

2.9. Vaccines

All vaccines used in the study were subunit inactivated preparations. Development of "MonoGrippol Plus" and "Grippol® Plus" vaccines (LLC "NPO Petrovax Pharm," Russia) is based on a special technology of coupling of highly purified protective influenza virus antigens with a polymeric, water-soluble, high-molecular weight adjuvant polyoxidonium. This technology enables a threefold reduction of hemagglutinin (HA) of each viral strain (down to 5 μg) in the vaccine compared to the analog subunit, adjuvant-free vaccine "Agrippal S1" ("Novartis Vaccines and Diagnostics," Italy).

"MonoGrippol Plus" contains antigens of only one influenza virus strain, namely A/California/7/2009/H1N1/v and belongs to the monovalent pandemic influenza vaccines, whereas

"Grippol Plus" and "Agrippal S1" additionally contain antigens of other strains, that is, A/H3N2/(Victoria)-like and B/Brisbane-like (trivalent vaccines).

2.10. Placebo

As a placebo, we used phosphate buffer saline ("GlaxoSmithKline Biologicals") which is used as a diluent for lyophilized vaccines.

2.11. Vaccination

Vaccination of pregnant women was performed in the vaccination room with adherence to sanitary and hygiene regulations, with emergency care available at once. Vaccine preparations were injected intramuscularly as a single-dose of 0.5 mL in the upper third of the arm (deltoid muscle).

2.12. Evaluation of vaccination safety

After an injection, the woman was observed for 40 min for the adverse reaction(s), which were scored to categorize reactions as described in **Table 2**.

Local reactions	
0—absent	Absence of symptoms
1—mild	Hyperemia up to 50 mm in diameter or infiltrate up to 25 mm in diameter
2—moderate	Hyperemia over 50 mm in diameter or infiltrate 26–50 mm in diameter
3—severe	Infiltrate over 50 mm in diameter
Systemic reactions	
0—absent	Absence of symptoms
1—mild	Presence of mild symptoms
2—moderate	Symptoms which markedly impair normal daily activity
3—severe	Symptoms which interfere with normal daily activity
Fever	
0—absent	≤37°C
1—mild	>37°C to ≤37.5°C
2—moderate	>37.6°C to ≤38.5°C
3—severe	>38.6°C

Table 2. Assessment of undesirable post-vaccination reactions.

All possible changes in the well-being and health state were recorded in the case report form (CRF) and self-observation diary (SOD) which the women continued to fill in on a daily basis throughout the first month of the follow-up.

2.13. Statistical analysis

Statistical analysis of samples which did not follow a normally distributed pattern was carried out using the non-parametric tools or parametric methods when the samples followed a normal distribution. We used the applied software package "Microsoft Excel" with the "AtteStat" application (version 10.10.2.). The differences were considered significant at $p < 0.05$.

3. Results and discussion

3.1. Particulars of the course of pregnancy in women vaccinated with "Grippol® Plus"

Although many pregnant women had a history of somatic diseases before they entered the study, as a rule, no exacerbation of pre-existing disease has been observed. The most commonly encountered illness was a mild hypochromic anemia (60.5, 70.0, 61.3, and 60.9% of pregnant women in group I–IV, respectively). Markers of chronic urogenital infection were detected fairly often (44.2% in group I, 46.0% in group II, 59.1% in group III, and 41.5% in group IV). Also, the syndrome of vegetative dystonia (20.9% in group I, 38.0% in group II, 20.5% in group III, and 29.3% in group IV) and altered allergic response (16.3% in group I, 20.0% in group II, 18.2% in group III, and 19.5% in group IV) had been observed. All women had approximately the same frequency of cases of threatened miscarriage in the past (53.5% in group I, 48.0% in group II, 47.7% in group III, and 51.2% in group IV). Therefore, clinical condition of pregnant women was comparable among the groups [25].

3.1.1. Clinical course of the post-vaccination period

Evaluation of the clinical course of the post-vaccination period has shown that it was asymptomatic in 58.1% of women from group I, 60.0% from group II, 54.5% from group III, and 60.9% from group IV ($p > 0.05$). It came under notice that in groups I, II, and III, women vaccinated in the third trimester of pregnancy developed the post-vaccination local and systemic undesirable effects significantly less often than women vaccinated in the second trimester ($p < 0.05$ to $p < 0.01$). The local symptoms occurred in the first few days after vaccination included pain, hyperemia, and infiltration at the site of injection. Such reactions occurred more often in pregnant women immunized with trivalent vaccines (group II—8.0%, group III—10.4%) than in women from placebo group (4.9%), ($p < 0.05$). It was noted that pregnant women from group I developed no or minimal systemic adverse reactions (nausea, fatigability, dizziness or myalgia) where intensity was significantly lower compared to that in women vaccinated with trivalent vaccine, namely group I—6.9% ($p < 0.05$ versus group II), group II—12.0%, group III—10.4%, and group IV—10.2% [26, 27].

3.1.2. Clinical blood analysis

In the late (8–30 days) post-vaccination period, no local post-injection reactions have been reported in either group. Systemic reactions included frequent complaints on increased fatigability and headaches in women from group III ($p < 0.05$). With regard to other symptoms, the groups did not differ significantly between each other and the placebo group (group I—9.3%, group II—14.0%, group III—12.5%, and group IV—12.2%). All symptoms were of a transient nature and required no medication management [26, 27].

Analysis of complete blood count has shown that for the majority of formed elements, cell counts did not differ from normal values in both pre-vaccination and post-vaccination periods. Occasional differences were mostly related to the particulars of the pregnancy period. Analysis of basic metabolic panel performed in dynamics on day 7 and day 30 of the post-vaccination period in each group also did not reveal significant abnormalities which could reflect changes in the metabolic homeostasis ($p > 0.05$). Small changes in creatinine level (minimal value in group III at day 30 post-vaccination—58.04 ± 1.57 μmol/L) and alkaline phosphatase (AP) (maximum value in group III at day 30 post-vaccination—86.23 ± 7.84 IU/L) are not remarkable and fall within the average normal values, attesting to normal variability of this parameter [28, 29].

3.1.3. Lipid metabolism

Analysis of lipid panel obtained 30 days post-vaccination has shown that in all groups, the parameters of lipid/cholesterol metabolism are not significantly altered, and remain within physiological variations [12, 13].

3.1.4. Hormonal profile

Analysis of hormonal profile among vaccinated women has revealed only the intra-group changes in hormone levels which are not so much related to vaccination but rather are due to the gestational age.

Significant differences in prolactin, progesterone, estradiol, and cortisol serum levels were observed in women of different gestational age regardless of whether they received monovalent or trivalent influenza vaccine or placebo ($p < 0.05$). Therefore, it can be affirmed that, despite certain differences in the composition of influenza vaccines used in the study, there are no hormonal changes which could have influenced the state of the fetoplacental unit [12, 13].

3.1.5. Humoral immunity

Serum levels of immunoglobulins measured immediately after vaccination and on day 7 post-vaccination were comparable in pregnant women immunized with different influenza vaccines. At day 30, post-vaccination pregnant women who had received the monovalent influenza vaccine demonstrated higher IgA levels (2.56 ± 0.27 mg/mL) compared to women vaccinated with trivalent preparations (1.61 ± 0.09 mg/mL in group II, 1.34 ± 0.11 mg/mL in

group III, and 1.14 ± 0.14 mg/mL in group IV) ($p < 0.01$ to $p < 0.001$). Despite the above differences, the antibody levels reflect normal serum IgA variations. Despite the established difference, the IgA content in all comparison groups was recorded within the physiological norm. Levels of IgM and IgG antibodies did not differ significantly between the groups.

Some variations in the IgG subclasses (1, 2, 3, 4) in the early and late post-vaccination periods were found. However, these variations remained within the acceptable range. In pregnant women with a history of allergic diseases, vaccination against influenza had no subsequent effect on serum total IgE levels.

3.1.6. Cytokine profile

It was noted that all pregnant women vaccinated with different vaccine preparations had elevated levels of mitogen-stimulated IL-1α at day 7 post-vaccination. By day 30, concentration of IL-1α remained elevated as compared to placebo control ($p < 0.05$) although was significantly lower than in vaccinated non-pregnant women ($p < 0.01$). No changes in the IL-2 and TNFα levels have been observed in vaccinated pregnant women although were also significantly higher than in non-pregnant women ($p < 0.01$). The IL-1RA values in a spontaneous cytokine production assay were significantly elevated only after vaccination with trivalent preparations by day 7 (group II) and by day 30 (groups II and III) post-vaccination ($p < 0.01$). At the same time, following mitogen stimulation, no significant changes in the IL-2 concentration have been found in any group. All pregnant women demonstrated significant increase in the IL-IRA and IL-10 following mitogen stimulation regardless of the type of vaccine that reflected the mechanism of physiological control of immune activation.

The IL-4 levels were most stable, with no significant dynamic changes among the groups. The only exception was a subgroup of women immunized with a non-adjuvanted trivalent vaccine in different trimesters of pregnancy. It was noted that by day 7 post-vaccination, a higher level of stimulated IL-4 was found in vaccinated women in the third trimester of pregnancy (6.85 ± 0.11 pg/mL in group III) as compared to pregnant women who had received the adjuvant-containing vaccine during the same period (2.95 ± 0.09 pg/mL in group II) ($p < 0.05$). Subsequently (on day 30 post-vaccination), such differences between the groups could not be found.

Pregnant women had lower IFNγ levels in the mitogen-stimulated cytokine production assay (881.86 ± 92.93 pg/mL in group I, 784.17 ± 65.03 pg/mL in group II, 854.89 ± 68.71 pg/mL in group III, and 790.30 ± 45.55 pg/mL in group IV) than the non-pregnant women (1419.60 ± 69.45 pg/mL in group V) which reflected a natural background level of physiological immune response ($p < 0.05$). At the same time during the first 7 days, post-vaccination elevated IFNγ was detected only in pregnant women who had received the polymer-subunit vaccines (6.47 ± 1.68 pg/mL in group I and 5.89 ± 1.08 pg/mL in group II) as compared to group III (3.03 ± 0.39 pg/mL) ($p < 0.05$). These differences were short-lived, and by day 30, post-vaccination was undetectable [30].

Therefore, the overall picture of cytokine profile in pregnant women had a trend characteristic of physiologic immunosuppression in pregnancy, that is, moderately elevated IL-IRA and

IL-10 and the absence in the post-vaccination period of significantly elevated anti-inflammatory cytokines in the mitogen-stimulation cytokine production assay. Nonetheless, the adjuvanted subunit vaccines had certain differences in their ability to influence cytokine secretion and short-term elevation of IFNγ which is most prominent in women in the second trimester of pregnancy that may reflect active involvement of the Th_1-mediated mechanisms of post-vaccination immunity. Use of non-adjuvanted vaccines leads to immune processes in the early post-vaccination period which are accompanied by increased IL-4 synthesis by blood leukocytes (a sign of Th_2-mediated activation) especially in women vaccinated in late pregnancy. The indirect evidence in favor of this suggestion is the absence of significance changes in the IFNγ levels in the early and late post-vaccination periods. All found changes of parameters recorded in different groups of vaccinated women remained within an acceptable range of variation. Also, no changes pertaining to destabilization of regulation and functioning of immune system due to influenza vaccination of pregnant women have been found [31].

3.2. Effect of vaccination of pregnant women using "Grippol® Plus" influenza vaccine on the antenatal fetal development

3.2.1. Fetoplacental complex

Monitoring of fetal development was carried out using a complex of measures, which included analysis of markers of fetoplacental complex and ultrasound fetometry. In all groups of women in the early and late post-vaccination period, no changes in the basic parameters of embryo/fetogenesis (AFP, hCG, TBG) have been found (**Table 3**). Changes in the above parameters did not depend on the type of influenza vaccine used and corresponded to the gestational age (second and third trimesters of pregnancy). Thus, for example, the TBG level in all

Parameter		Group I "MonoGrippol Plus" (n = 43)	Group II "Grippol® Plus" (n = 50)	Group III "Agrippal S1" (n = 48)	Group IV "Placebo" (n = 41)
In 7 days	TBG Ng/mL	97.93 ± 20.97	72.69 ± 11.89	88.04 ± 15.46	92.69 ± 20.88
	AFP IU/mL	60.05 ± 13.39	69.59 ± 7.62	81.09 ± 17.88	75.41 ± 10.36
	hCG IU/mL	36.51 ± 4.62	39.74 ± 8.22	40.43 ± 3.10	36.15 ± 2.48
In 30 days	TBG Ng/mL	124.85 ± 14.43	109.17 ± 10.81	118.21 ± 13.99	110.35 ± 13.12
	AFP IU/mL	98.65 ± 8.33	100.43 ± 11.01	110.84 ± 11.19	115.29 ± 9.92
	hCG IU/mL	29.52 ± 3.62	33.84 ± 7.55	29.24 ± 5.20	28.95 ± 1.88

Note: $p > 0.05$ for differences between groups.

Table 3. Fetal complex markers in pregnant women vaccinated against influenza (M ± m).

groups (including placebo control) of women vaccinated in the second trimester of pregnancy was significantly lower than in the third trimester ($p < 0.05$ to $p < 0.01$). A direct relationship was found between the TBG and AFP concentrations ($r = 0.60$; $p < 0.05$) with TBG levels rising as pregnancy progresses ($p < 0.001$). The hCG levels were dropping during the follow-up and inversely correlated with the TBG levels ($r = -0.50$; $p < 0.01$). All serum markers had no deviations from the reference values and reflected physiological changes in pregnancy [32].

3.2.2. Fetometry

Fetometry performed in the second (21–22 weeks) and third (33–35 `) trimester of pregnancy failed to reveal differences among the groups of pregnant women (**Table 4**).

Therefore, study results indicate that vaccination of pregnant women using the adjuvant-containing influenza vaccines "MonoGrippol Plus" and "Grippol® Plus" has no effect on the intrauterine fetal development. Changes of the basic parameters of fetoplacental unit are comparable between the groups and reflect physiological changes during fetal growth.

3.3. Pregnancy outcomes in women vaccinated with "MonoGrippol Plus" and "Grippol® Plus" influenza vaccines

In the majority of cases (85.4–90.7%), pregnancy resulted in physiologic birth (**Table 5**). In a fraction of women, their pregnancy terminated prematurely with the birth of preterm babies (between 2.0 and 8.3%) which corresponds to the preterm birth rate in the Ulyanovsk region of Russia (3.7–5.8%) where the study was taking place. Such outcome was due to the obstetric pathology which was unrelated to prior vaccination. Also, cases of birth of babies with perinatally acquired neurological impairment were mostly associated with gestational immaturity (7.3–10.4%). A fraction of babies had the intrauterine infection-like syndrome (2.0–6.3%) and developmental abnormalities and defects in 2.0–4.9% of cases (3.8–5.9% across the Ulyanovsk region) [33]. Owing to the above abnormalities, such babies were excluded from further study.

3.4. Particulars of development of up to 6 month old infants born to mothers vaccinated during pregnancy with "MonoGrippol Plus" and "Grippol® Plus" influenza vaccines

3.4.1. Apgar scale

The early neonatal period of infants born to mothers vaccinated during pregnancy had a comparable dynamics between the groups. It was shown that, immediately after birth, the number of babies with Apgar score of 8–9 points was similar between the groups (group I—92.1%, group II—87.5%, group III—80.9%, and group IV—94.3%) which attests to the overall good functional maturity. The period of adaptation in newborns passed without complications [34, 35].

3.4.2. Feeding

The feeding of infants born to mothers vaccinated with different influenza vaccines did not differ significantly between the groups. The highest number of nursing mothers (100%) during the neonatal period was observed in groups I and IV and was somewhat lower in groups

Clinical groups	Gestational age	Parameter					
		BPD (mm)	FOS (mm)	HC (mm)	AC (mm)	Fetal weight (g)	$\frac{FL}{AC} \times 100$ (%)
Group I "MonoGrippol Plus" (n = 43)	21–22 weeks	53.18 ± 3.20	73.87 ± 8.11	188.12 ± 13.72	162.81 ± 17.08	527.10 ± 92.31	21.54 ± 0.57
	33–35 weeks	87.81 ± 5.52	111.67 ± 12.9	313.01 ± 24.18	311.60 ± 18.57	2539.40 ± 437.10	22.04 ± 0.44
Group II "Grippol® Plus" (n = 50)	21–22 weeks	54.04 ± 2.70	70.07 ± 7.42	183.90 ± 11.86	170.11 ± 15.98	504.70 ± 96.01	19.49 ± 0.86
	33–35 weeks	89.11 ± 5.31	109.80 ± 14.20	304.21 ± 38.73	295.90 ± 21.07	2489.70 ± 367.30	23.13 ± 0.78
Group III "Agrippal S1" (n = 48)	21–22 weeks	52.56 ± 2.62	68.55 ± 8.12	180.87 ± 18.66	175.44 ± 16.08	489.50 ± 110.10	20.26 ± 0.93
	33–35 weeks	81.49 ± 6.53	106.12 ± 11.40	301.01 ± 21.74	298.30 ± 33.15	2595.2 ± 455.1	22.96 ± 1.04
Group IV "Placebo" (n = 41)	21–22 weeks	52.01 ± 3.9	67.22 ± 9.02	181.01 ± 15.31	178.79 ± 13.9	497.72 ± 138.03	20.99 ± 1.02
	33–35 weeks	82.77 ± 7.71	103.07 ± 8.33	299.82 ± 28.53	301.02 ± 29.34	2607.7 ± 631.0	22.73 ± 1.38

Note: p > 0.05 for differences between groups.

Table 4. Ultrasound-fetometry data in pregnant women vaccinated against influenza (M ± m).

Parameter		Group I "MonoGrippol Plus" (n = 43)	Group II "Grippol® Plus" (n = 50)	Group III "Agrippal S1" (n = 48)	Group IV "Placebo" (n = 41)
Women	Physiological birth	39 (90.7%)	48 (96.0%)	43 (89.5%)	35 (85.4%)
	Miscarriage	0	1 (2.0%)	1 (2.1%)	1 (2.4%)
	Premature birth	4 (9.3%)	1 (2.0%)	4 (8.3%)	3 (7.3%)
Infants	Without pathology	38 (88.4%)	46 (92.0%)	42 (87.5%)	33 (85.4%)
	Birth of babies with abnormalities or developmental defects	1 (2.3%)	1 (2.0%)	2 (4.2%)	2 (4.9%)
	Perinatal CNS lesions	4 (9.3%)	3 (6.0%)	5 (10.4%)	3 (7.3%)
	Intrauterine infection-like syndrome	2 (4.6%)	1 (2.0%)	4 (6.3%)	2 (4.9%)

Note: p > 0.05 for differences between groups.

Table 5. Outcomes of pregnancy and birth in women vaccinated against influenza.

II and III (85.4 and 92.9%, respectively) (p > 0.05). Further onwards, the number of infants receiving only breast milk gradually diminished (92.1% at 3 months and 65.8% at 6 months in group I; 85.4% at 3 months and 72.9% at 6 months in group II; 83.3% at 3 months and 69.0% at 6 months in group III; and 88.6% at 3 months and 60.0% at 6 months in group IV) (p > 0.05). Therefore, vaccination of women with subunit adjuvanted influenza vaccines during pregnancy has no further impact on lactation and duration of breastfeeding.

3.4.3. Body weight and length

Parameters of physical development of infants of the first 6 months of life from different groups were generally comparable. Body weight and body length at different time points were within the percentile rank (25-50-75). The Ketle 1 index in group I newborns was 65.1 ± 0.67, in group II—63.8 ± 1.22, in group III—65.5 ± 1.72, and in group IV—67.1 ± 1.03 (p > 0.05).

In the majority of cases, the proportionality of physical development among infants in their first few months of life had the average values of harmonious development, namely 65–74% in group I, 70–76% in group II, 69–81% in group III, and 69–76% in placebo group (p > 0.05). The infants with the average values below harmonious development were found equally often (14–22% in group I, 12–16% in group II, 12–19% in group III, and 18–21% in group IV). The infants with the average values above harmonious development (6–22% in group I, 10–17% in group II, 7–12% in group III, and 6–15% in group IV) were considered as a variant of body constitutional norm (p > 0.05). Infants with a disproportional physical development have not been found.

Therefore, our results attest to the sufficiency of basic criteria for infant development and reflect the population maturity in terms of their physical development regardless of vaccination of their mothers during pregnancy with different influenza subunit vaccines.

3.4.4. Neuropsychological development

Parameters of neuropsychological development (NPD) of children born to vaccinated mothers did not differ significantly from those of the placebo group. Overall, no changes of NPD have been observed in 81.6% of group I infants in their first 6 months of life who had been born to mothers vaccinated during pregnancy with a monovalent influenza vaccine. In other clinical groups, this parameter was 83.3% (group II), 78.6% (group III), and 77.1% (group IV) (p > 0.05). Within the structure of occasional NPD disorders, there were conditions which number did not exceed the average statistical rate of neurological pathology in a given pediatric age group.

It was noted that infants born to women vaccinated during pregnancy with trivalent influenza vaccines were 1.8-times less likely to develop non-influenza respiratory infections within the first 6 months of life as compared to infants from placebo control group (**Figure 2**).

3.5. Immunogenicity of adjuvanted influenza vaccine "Grippol® Plus" in pregnant women vaccinated during different trimesters of pregnancy

In this study, the level of post-vaccination antibodies to influenza virus was evaluated only in a group of pregnant women and non-pregnant women vaccinated with a trivalent adjuvanted influenza vaccine with the aim of revealing the features of the effect of pregnancy on the synthesis of antibodies. Since it has been already proven that the introduction of subunit unadjuvanted vaccines in pregnant women is accompanied by the formation of antibodies to the influenza virus in values not differing from those in non-pregnant ones, it seemed to us interesting to investigate the interaction of the immunoadjuvant preparation with the transiently altered immune status of the pregnant woman [36].
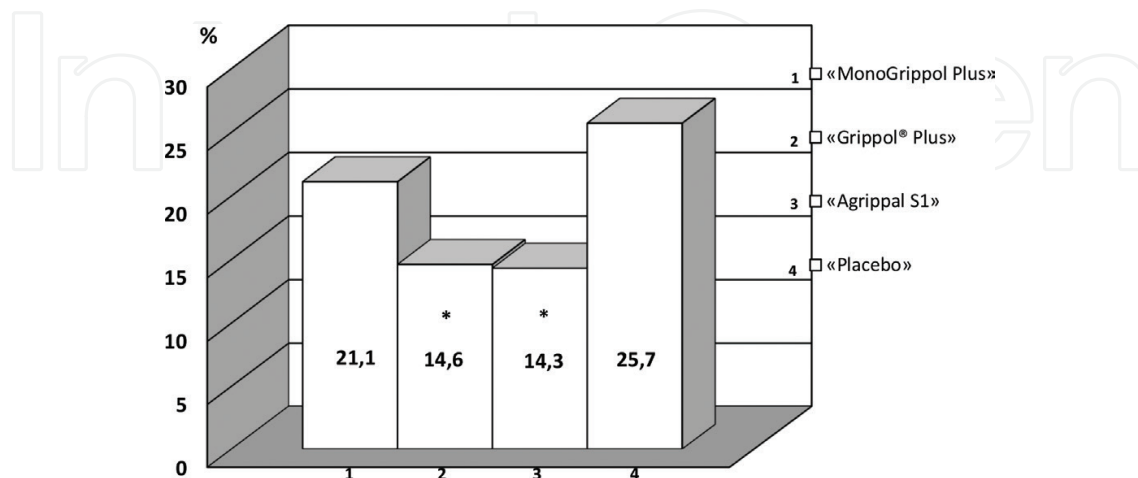

Figure 2. Incidence of morbidity due to non-influenza respiratory infections in infants during their first 6 months of life. *Note:* *- p < 0.05 for differences between groups I, II, III and group IV.

It was found that a part of women before immunization had a seroprotective (≥1:40) baseline antibody level to vaccine strains of influenza virus (**Table 6**). In all examined women, the antibody titer was higher to influenza virus B (22.2% of women in the second trimester, 26.1% in the third trimester and 25.7% in non-pregnant women). This is probably due to the duration of its circulation in the population and the formation of natural immunity. It should be noted that none of the participants in the study was vaccinated before and did not confirm an acute illness caused by the influenza virus. One month after vaccination, women of all groups demonstrated a significant rise in antibody titer that fully met one of the CPMP criteria. In the post-vaccination period, the antiviral antibody titer gradually declined reaching a significant difference against baseline by 3 months postpartum in women vaccinated in the second and third trimesters. The observed difference referred only to viral strain A/H1N1/v ($p < 0.05$). It is possible that the loss of antibodies to a pandemic strain is associated with the peculiarities of the formation of immunity after its first administration. Other authors have shown that specific antibodies to this strain in the post-vaccination period can be synthesized at lower values and therefore be accompanied by their faster loss. At 6 and 12 months post-vaccination, there was a marked regression of seroprotection level with regard to antibody titer against strains A/H1N1/v, A/H3N2/and B in women vaccinated during pregnancy in the second and third trimesters ($p < 0.01$). Such trend was also traced in a group of non-pregnant women; however, the changes were less remarkable, with a fairly significant fraction of subjects having a high level of protective antibodies. Similar dynamics of post-vaccination antibodies were noted in pregnant women vaccinated with subunit non-adjuvanted influenza vaccine [37].

The rate of development and intensity of protective immunity include the level and factor of seroconversion across all influenza virus strains. Those were compared between the groups, and it was found that their values met the CPMP criteria (**Table 7**). The majority of data obtained did not differ between the groups. One exception was the seroconversion factor for strain B in pregnant women vaccinated in the third trimester of pregnancy (5.1) when it was compared with the matching parameter in the non-pregnant women group (6.9) ($p < 0.05$).

The dynamics of influenza antibody (AB) titers based on the geometric mean titer (GMT) reflect the decline of antibody level with time in the post-vaccination period (**Table 8**). It was noted that at one month post-vaccination, the value of GMT AB to A/H1N1/v strain in women vaccinated in the second trimester of pregnancy was significantly lower (49.12 ± 0.29) compared to subjects vaccinated in the third trimester of pregnancy (60.99 ± 0.25) ($p < 0.05$). During all subsequent periods, this parameter showed no differences with regard to the trimester of pregnancy.

Pregnant women vaccinated in the third trimester of pregnancy showed at 3 months post-vaccination and throughout the follow-up period lower GMT AB titers to strain B compared to the non-pregnant women ($p < 0.05$ to $p < 0.01$). Similar trend was traced in women of the same group with regard to all influenza virus strains at 6 months postpartum compared to the non-pregnant women ($p < 0.05$ to $p < 0.01$).

Therefore, the post-vaccination immune response in women vaccinated with a trivalent adjuvanted influenza vaccine at different times of pregnancy, during the first month, did not differ

	Periods of observation	Second trimester (n = 27)			Third trimester (n = 23)			Non-pregnant women (n = 19)		
		A/H1N1/v	A/H3N2/	B	A/H1N1/v	A/H3N2/	B	A/H1N1/v	A/H3N2/	B
Seroprotection level (AB titer ≥ 1:40) (%)	Before vaccination (V)	3.7	11.1	22.2	8.7	13.0	26.1	2.6	14.1	25.7
	1 month post-V	77.0*	88.9*	85.2*	87.0*	87.0*	91.3*	83.1*	90.2*	94.4*
	3 months post-V	74.8	88.4	84.2	—	—	—	80.6	88.7	92.0
	6 (3) months post-V 2–3 days postpartum	74.1	81.5	77.8	62.0	76.2	71.4	78.5	83.1	88.2
	9 (6) months post-V 3 months postpartum	57.7 ×	69.2	65.4	57.1 ×	71.4	62.0	71.4	78.6	72.8
	12 (9) months post-V 6 months postpartum	48.2 ∇∇/×	65.4 ∇∇	57.7 ∇∇/×	50.0 ∇∇/×	61.1 ∇∇	55.6 ∇∇/×	67.6 ∇	72.5 ∇	69.1 ∇∇

Note: Time period elapsed since the moment of vaccination of women in the third trimester of pregnancy (group II) is given in brackets.

*: p < 0.01—the intra-group difference for the second/third trimesters of pregnancy; non-pregnant in-between pre-vaccination and 1 month post-vaccination.

∇: p < 0.05; ∇∇: p < 0.01—the intra-group difference for the second/third trimesters of pregnancy; non-pregnant in-between 1 month and 12 (9) months post-vaccination.

×: p < 0.05; ××: p < 0.01—difference between the second/third trimesters of pregnancy group and non-pregnant group.

Table 6. Seroprotection level in pregnant women vaccinated with "Grippol® Plus," allowing for trimester of pregnancy.

Parameter	Second trimester (n = 27)			Third trimester (n = 23)			Non-pregnant women (n = 19)		
	A/H1N1/	A/H3N2/	B	A/H1N1/	A/H3N2/	B	A/H1N1/	A/H3N2/	B
Seroconversion level (%)	70.4	77.8	74.1	69.6	78.3	65.2	71.5	81.1	70.3
Seroconversion factor	6.5	7.2	6.5	7.1	6.5	5.1*	6.8	7.6	6.9

Note: *p < 0.05 for difference between the third trimester group and non-pregnant group.

Table 7. Seroconversion level and seroconversion factor in pregnant women vaccinated with "Grippol® Plus," allowing for the trimester.

	Periods of observation	Second trimester (n = 27)			Third trimester (n = 23)			Non-pregnant (n = 19)		
		A/H1N1/v	A/H3N2/	B	A/H1N1/v	A/H3N2/	B	A/H1N1/v	A/H3N2/	B
Geometric mean antibody titer (log₂GMT AB)	Before vaccination (V)	7.54 ± 0.17	8.79 ± 0.23	13.96 ± 0.26	8.60 ± 0.23	10.62 ± 0.24	14.36 ± 0.25	8.02 ± 0.15	9.12 ± 0.21	14.19 ± 0.21
	1 month post-V	49.12 ± 0.29	63.49 ± 0.28	90.96 ± 0.36	60.99 ± 0.25 *	68.81 ± 0.26	73.08 ± 0.24	52.12 ± 0.22	59.47 ± 0.24	81.11 ± 0.37
	3 months post-V	47.87 ± 0.27	55.85 ± 0.26	65.15 ± 0.35	—	—	—	51.31 ± 0.24	57.73 ± 0.31	73.88 ± 0.26
	6 (3) months post-V 2–3 days postpartum	41.04 ± 0.23	44.33 ± 0.22	47.87 ± 0.32	41.34 ± 0.30	45.64 ± 0.25	42.72 ± 0.30 ×	48.56 ± 0.22	55.53 ± 0.24	63.13 ± 0.36
	9 (6) months post-V 3 months postpartum	30.64 ± 0.23	29.83 ± 0.22 ××	32.32 ± 0.29	30.72 ± 0.31	31.75 ± 0.32 ×	26.92 ± 0.35 ××	44.82 ± 0.25	51.79 ± 0.23	57.71 ± 0.31
	12 (9) months post-V 6 months postpartum	21.67 ± 0.24 ∇∇/×	24.10 ± 0.26 ∇∇/××	25.42 ± 0.25 ∇∇/××	20.79 ± 0.34 ∇∇/×	25.19 ± 0.33 ∇∇/××	19.24 ± 0.35 ∇∇/××	38.17 ± 0.26 ∇	44.47 ± 0.21 ∇	50.03 ± 0.28 ∇∇

Note: Time period elapsed since the moment of vaccination of women in the third trimester of pregnancy is given in brackets.

*: $p < 0.05$—for difference between the second trimester/third trimester groups.

∇: $p < 0.05$; ∇∇: $p < 0.01$—the intra-group difference for the second/third trimesters of pregnancy; non-pregnant between 1 month and 12 (9) months post-vaccination.

×: $p < 0.05$; ××: $p < 0.01$—for difference between the second trimester group, third trimester group and non-pregnant group.

Table 8. Geometric mean antibody titer in pregnant women vaccinated with "Grippol® Plus," allowing for the trimester.

from that in vaccinated non-pregnant women and fully met the CPMP criteria. The level of antibodies to strain A/H1N1/v following administration of a trivalent vaccine was nearly the same as with vaccination of pregnant women with the monovalent, subunit, adjuvanted vaccine [38, 39]

With time the postpartum women demonstrated a more pronounced reduction of seroprotection level, especially against the A/H1N1/v strain. After 6 months postpartum, the rate of regression of seroprotection level in subgroups of vaccinated pregnant women (taking into account the gestational age) has increased 1.6–1.7-fold (A/H1N1/v), 1.4-fold (A/H3N2) and 1.5- to 1.6-fold (B), whereas in the non-pregnant women group same parameter was 1.2-fold (A/H1N1/v), 1.2-fold (A/H3N2) and 1.4-fold (B), respectively. This trend was in line with dynamic reduction of the MGT AB values during the last months of the follow-up [38]. Consequently, the existing physiological immunological changes in the immune system during pregnancy may affect the formation and preservation of post-vaccination antibodies to strains of influenza virus when using subunit immunoadjuvant vaccines. However, this assumption should be confirmed by new data research.

3.6. Immunologic effectiveness of vaccination of pregnant women using "Grippol® Plus" influenza vaccine in mother-infant pairs

Analysis of transplacental immunity in the first months of life of infants born to women vaccinated during pregnancy with "Grippol® Plus" vaccine has shown that the level of seroprotection against influenza virus strains significantly differed only in the mother-infant pairs from the group of subjects vaccinated in the second trimester of pregnancy ($p < 0.05$), while no differences in the number of seroprotected infants have been found ($p > 0.05$) (**Table 9**). At 3 months after birth all infants, regardless of the time of their mothers' vaccination, demonstrated a significant reduction of protective titers of transplacental antibodies to vaccine strains of influenza virus as compared to their antibody titers obtained at birth and antibody titers in their mothers ($p < 0.01$). Further onwards, protective antibodies to vaccine strains of influenza virus completely vanished, and among the 6-month infants, the titer dropped to zero in both groups [40]. It should be noted that by 3 months of life, the rate of regression of antibody titer was higher in the subgroup of infants born to mothers vaccinated in late pregnancy, namely 2.8-fold higher for A/H1N1/v, 2.6-fold higher for A/H3N2/, and 4.0-fold higher for B strain.

Therefore, 52.3–61.9% of babies born to women vaccinated during pregnancy with "Grippol® Plus" vaccine had protective antibody levels against vaccine influenza strains at the time of their birth. This level of protection significantly declined with time and by 3 months of life remained at a protective level in only 14.2–24.0% of infants. At the age of 6 months, protective titers of maternal antibodies completely vanished in all infants. Infants born to women vaccinated in the second trimester of pregnancy had higher activity of protective antibodies and lower rate of reduction of seroprotection level which attests to a better preservation of the post-vaccination transplacental immunity. Thus, the advantage of vaccination of pregnant women with the use of immunoadjuvant subunit vaccine in the II trimester of gestation was revealed.

	Observation periods	Second trimester						Third trimester					
		A/H1N1/v		A/H3N2/		B		A/H1N1/v		A/H3N2/		B	
		M	I	M	I	M	I	M	I	M	I	M	I
Seroprotection level (%) (AB titer > 1:40)	Day 2–3 postpartum/ newborns	74.1	55.5 ×	81.5	59.3 ×	77.8	60.2 ×	62.0	52.3	76.2	61.9	71.4	57.1 ×
	At 3 months postpartum/3 months old	57.7	20.0 ××/⊗⊗	69.2	24.0 ××/⊗⊗	65.4	20.0 ▲××/⊗⊗	57.1	19.0 ××/⊗⊗	71.4	23.8 ××/⊗⊗	62.0	14.2 ××/⊗⊗
	At 6 months postpartum/6 months old	46.2	0 ⊗⊗	65.4	0 ⊗⊗	57.7	0 ⊗⊗	50.0	0 ⊗⊗	61.1	0 ⊗⊗	55.6	0 ⊗⊗
Geometric mean antibody titer ($\log_2$GMT AB)	Day 2–3 postpartum/ newborns	41.04 ± 0.23	25.85 ± 0.29 ×	44.33 ± 0.22	27.22 ±0.26 ×	47.87 ±0.32	30.94 ±0.31	41.34 ±0.30	23.59 ±0.28 ×	45.64 ±0.25	26.91 ±0.25 ×	42.72 ±0.30	25.19 ±0.28 ×
	At 3 months postpartum/3 months old	30.64 ± 0.23	11.30 ± 0.26 ××/⊗⊗	29.83 ± 0.22	13.86 ±0.24 ××/⊗⊗	32.32 ±0.29	13.19 ±0.32 ××/⊗⊗	30.72 ±0.31	13.46 ±0.25 ××/⊗⊗	31.75 ±0.32	15.87 ±0.24 ××/⊗⊗	26.92 ±0.35	12.59 ±0.24 ××/⊗⊗
	At 6 months postpartum/6 months old	21.67 ± 0.24	7.52 ± 0.26 ××/⊗⊗	24.10 ± 0.26	8.49 ±0.20 ××/⊗⊗	25.42 ±0.25	7.23 ±0.22 ××/⊗⊗	20.79 ±0.34	8.91 ±0.20 ××/⊗⊗	25.19 ±0.33	11.22 ±0.19 ××/⊗⊗	19.24 ±0.35	7.94 ±0.18 ××/⊗⊗

Note: **M**—mother; **I**—infant.

▲: $p < 0.05$—for differences between infants from different observation groups.

×: $p < 0.05$; ××: $p < 0.01$—for differences between "mother-infant" groups.

⊗: $p < 0.05$; ⊗⊗: $p < 0.01$—for infants' intra-group differences versus at birth data.

Table 9. State of transplacental post-vaccination immunity in "mother-infant" pairs following vaccination against influenza using "Grippol® Plus" vaccine.

4. Conclusions

1. Different underlying diseases diagnosed in women of reproductive age are not an impediment to influenza vaccination during pregnancy.

2. Influenza vaccination during pregnancy using Russian-made polymer-subunit monovalent and trivalent vaccines ("MonoGrippol Plus" and "Grippol® Plus") in 58.1–60.0% of cases is accompanied by asymptomatic post-vaccination period. The frequency of systemic (generalized) post-vaccination reactions in immunized women (6.9–14.0%) does not differ significantly from that in placebo control group (10.2–12.2%).

3. Administration of adjuvanted vaccines to pregnant women does not cause disturbances of their metabolic homeostasis, hormonal profile, and cytokine profile.

4. Vaccination of pregnant women against influenza does not affect trophoblast function and fetal growth. Vaccination neither bears the risk of miscarriage nor influences the pattern and duration of breastfeeding.

5. Considering that safety of adjuvanted influenza vaccines has been proven by clinical and laboratory investigations, additional safety studies in pregnant women before and post-vaccination are redundant.

6. Babies born to mothers vaccinated against influenza with adjuvanted vaccines ("MonoGrippol Plus" and "Grippol® Plus") have a high level of physiological maturity. The basic parameters of physical and neuropsychological development in the early postnatal period in such infants do not differ from those of infants from control group.

7. Infants born to women vaccinated during pregnancy with influenza vaccines are 1.8 times less likely to develop non-influenza respiratory infections within the first 6 months of life as compared to infants born to unvaccinated mothers.

8. Administration of adjuvanted trivalent vaccine to pregnant women elicits a pronounced immune response to influenza vaccine strains A and B that fully meets the CPMP criteria for seroprotection levels: A/H1N1/v—82.0%, A/H3N2/—88.0% and B—88.3%.

9. Women vaccinated with the polymer-subunit vaccine in the second trimester of pregnancy benefit from higher seroprotection level and longer retention time of influenza-specific antibodies.

10. Protective titers of transplacental antibodies to different influenza virus strains are found in 52.3–68.9% of infants that is comparable to control figures. Higher levels of protective antibodies to different influenza virus strains are found in infants whose mothers have been vaccinated with adjuvanted vaccine "Grippol® Plus" in the second trimester of pregnancy.

11. Analysis of mother-infant pairs showed a direct correlation in levels of post-vaccination IgG influenza-specific antibodies between mother and infant. However, after 3 months, protective antibodies to influenza virus strains were detectable in 14.2–36.1% of infants followed by their complete disappearance at 6 months of life versus 57.1–71.4% (3 months)

and 48.1–65.4% (6 months) in their mothers. This observation provides substantiation that vaccination against influenza in high-risk infants shall start at the age of 6 months.

12. The results obtained allow us to recommend the "Grippol® Plus" vaccine for use in healthcare practice for specific prevention of seasonal influenza in pregnant women and their offspring up to age 6 months inclusive, using a single-dose vaccination schedule.

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References

[1] ECDC scientific advice on seasonal influenza vaccination of children and pregnant women: ECDC Technical report/European Centre for Disease Prevention and Control. Stockholm: ECDC. 2012. 68

[2] Jamieson DJ, et al. H1N1 2009 influenza virus infection during pregnancy in the USA. Lancet. 2009;**374**. P. 451-458

[3] Tarbaeva DA, Kostinov MP, Zagorodnya E D, Josefson SA, Cherdantsev AP. Obstetrical and perinatal outcomes of pregnancy complicated with acute respiratory infection in the second trimester of pregnancy. Obstetrics and Gynecology (Russian). 2012. (2). pp. 67-71

[4] Tarbaeva DA, Kostinov MP, Josefson SA, Zagorodnya E D. Clinical course and outcomes of influenza A (HINI) 2009 infection in pregnant women. Journal of Microbiology, Epidemiology and Immunobiology (Russian). 2011;(4):pp. 71-76

[5] Mullooly JP. Risk of acute respiratory disease among pregnant women during influenza A epidemics [Text]. Mullooly JP, Barker WH, Nolan TF. Public Health Reports. 1986. Vol. 101:pp. 205-211

[6] Fiore AE. Prevention and control of influenza with vaccines: Recommendations of the advisory committee on immunization practices (ACIP). 2010. Fiore AE. et al. MMWR Recommendations and Reports. 2010;Vol. 59: pp. 1-62

[7] Zaman K, et al. Effectiveness of maternal influenza immunisation in mothers and infants. The New England Journal of Medicine. 2008;**359**(15):pp. 1555-1564

[8] Englund JA. Maternal immunization with inactivated influenza vaccine: Rationale and experience. Vaccine. 2003;**21**:pp. 3460-3464

[9] Fiore AE, et al. Prevention and control of influenza with vaccines: Recommendations of the advisory committee on immunization practices (ACIP). MMWR Recommendations and Reports. 2010;(59):pp. 1-62

[10] CDC. Guidelines for Vaccinating Pregnant Women: From Recommendations of the Advisory Committee on Immunization Practices (ACIP) [Electronic resource]. Centers for Disease Control and Prevention. 2007. Mode of access: http://www.cdc.gOv/vaccines/pubs/preg-guide.htm#5/

[11] Zverev VV, Kostinov MP, Cherdantsev AP, Kuselman AI, Kiselev OI, Erofeeva MK, Krasnopolskiy VI, Novikova S V, Serova OF, Briko NI, Chuchalin AG. Vaccination of Pregnant Women Against Influenza: Federal Clinical Guidelines. Moscow (Russian). 2015. p. 42

[12] Cherdantsev AP, Kostinov MP, Kuselman AI. Vaccine Prophylaxis of Influenza in Pregnant Women. Manual for Physicians. M. 4Mpress (Russian). 2013. p. 96

[13] Cherdantsev AP, Kostinov MP, Kuselman AI. Vaccine prophylaxis of influenza in pregnant women/Manual for physicians. 2nd edition with supplement. M. MDV Art Studio «Constellation» (Russian). 2014. p. 112

[14] Tsai T, et al. Exposure to MF59-adjuvanted influenza vaccines during pregnancy–retrospective analysis. Vaccine. 2010;**7**(28):pp. 1877-1880

[15] Kostinov MP, Kostinova TA, Zolotareva TA. Potentials of vaccination against influenza in high-risk patient groups. «Hospital physician» Journal (Russian). 2001;(10). pp.52-54

[16] Clinico-immunologic effectiveness of immunobiological preparations/Reference book. Kostinov MP, Ozeretskovsky NA. Eds. Moscow. 2004–«Miklosh» (Russian). p. 256

[17] Kostinov MP, Lavrov VF. New generation vaccines for the prevention of infectious diseases. 2nd edition, with supplement, M.: MDV (Russian). 2010. p. 192

[18] Chebykina AV, Kostinov MP, Magarshak OO. Evaluation of safety and effectiveness of vaccination against influenza in patients with syndrome of bronchial obstruction. Epidemiology and Vaccinoprophylaxis (Russian). 2010;**6**(55): pp. 50-53

[19] Protasov AD, Zhestkov AV, Lavrentyeva NE, Kostinov MP, Ryzhov AA. Effect of complex vaccination against pneumococcal, haemophilus type B and influenza infections in patients with chronic obstructive pulmonary disease. Journal of Microbiology. Epidemiology and Immunobiology (Russian). 2011;(4):pp. 80-84

[20] Kostinov MP, Yerofeeva MK, Kharit SM. Effectiveness and safety of influenza vaccinoprophylaxis in different populations. All-Russia Interdisciplinary Journal TERRA MEDICA® (Russian). 2011;(2):pp. 7-11

[21] Kostinov MP, Chuchalin AG, Chebykina AV. Particulars of formation of post-vaccination immunity to influenza in patients with chronic broncho-pulmonary pathology. Infectious Diseases (Russian). 2011;**9**(3):pp. 35-40

[22] Vaccination of adults with broncho-pulmonary pathology. Manual for physicians (Kostinov MP., Ed.), M: 2013. Art studio «Constellation» (Russian). p. 109

[23] Vaccination of Children with Impaired Health State. Manual for Physicians (Kostinov MP, Ed.), 4th edition, M.: 4Mpress (Russian). 2013. p. 432

[24] Vaccines and Vaccination. National Manual (brief version, Zverev VV, Khaitov RM, Eds.) M.: Publishing group «GEOTAR-Media» (Russian). 2014: p. 640

[25] Cherdantsev AP. Effectiveness of immunoadjuvanted vaccines against influenza in pregnant women and evaluation of their safety for fetus and newborn/D. Med. Sci. Thesis (Russian), Moscow. 2013: p. 39

[26] Cherdantsev AP, Kuselman AI, Sinitsyna MN, Shalyagina ME, Kostinov MP, Tarbaeva DA. Study of clinical safety of vaccination against influenza in pregnant women. Medical Almanac (Russian). 2011;**4**(17):pp. 120-122

[27] Kostinov MP, Cherdantsev AP, Savisco AA, Tarbaeva DA, Solovieva IL. True and false reactions to influenza vaccine in pregnant women. Problems of Gynecology, Obstetrics and Perinatology (Russian). 2011;**10**(6):pp. 44-48

[28] Serova OF, Semenova SS, Cherdantsev AP, Tsaregorodseva MV. Evaluation of clinic-laboratory parameters in pregnant women vaccinated against influenza. Russian herald of obstetrics and gynecology (Russian). 2015;(2):pp. 75-79

[29] Cherdantsev AP, Kostinov MP, Kuselman AI, Voznesenskaya NV. Vaccination of pregnant women against influenza A(HINI). Journal of microbiology, epidemiology and immunobiology (Russian). 2011;(4):pp. 46-50

[30] Kostinov MP, Cherdantsev AP, Semenova SS, Tarbaeva DA, Savisco AA, Serova OF, Josefson S A, Akhmatova NK, Kostinova TA, Praulova DA. Obstetrical and perinatal

outcomes after vaccination against influenza or past respiratory infection. Gynecology (Russian), 2015;**14**(3):pp. 43-46

[31] Kostinov MP, Cherdantsev AP. Clinical and immunological safety of inactivated, immunoadjuvanted, subunit vaccine against influenza in pregnant women. Obstetrics and Gynecology (Russian). 2016;(2):pp. 64-69

[32] Cherdantsev AP, Kostinov MP, Kuselman AI, Liutaya ZA, Kutbutdinova MH. Analysis of the ante- and postnatal development of infants against a background of vaccination of pregnant women against influenza. Russian Herald of Perinatology and Pediatrics (Russian). 2013;**58**(2):pp. 30-34

[33] Cherdantsev AP, Kuselman AI, Kostinov MP, Liutaya ZA, Deryabina EV, Azizova R Sh, Melnik RH. Postnatal development of infants born to mothers vaccinated against influenza. Ulyanovsk Medico-Biological Journal (Russian). 2012;(3):pp.80-84

[34] Kostinov MP, Cherdantsev AP, Praulova DA, Kostinova AM. Vaccination against influenza—A safe way to protect pregnant women and newborns from consequences of infection. Russian herald of perinatology and pediatrics (Russian). 2015;(5):pp. 69-74

[35] Kostinov MP, Cherdantsev AP. State of health of infants born to mothers vaccinated against influenza during pregnancy. Pediatrics. Journal named after G.N. Speransky (Russian). 2016;(195):pp. 67-71

[36] Kostinov MP, Khromova EA, Savisko AA, Kostinova AM. Features of the immune system functioning during physiological pregnancy and their relationship with vaccination against influenza. CONSILIUM MEDICUM (Russian). 2016;#6.;**18**. pp. 59-62

[37] Mikhail P. Kostinov, Alexander P. Cherdantsev, Anna D Shmitko, Dmitry Pakhomov, Evgenia O Demina. Duration of Preservation of Antibodies to the Flu Virus in the Mother-Child Pairs during the Vaccination of Women Depending on the Trimester of Pregnancy. International Journal of Biomedicine. 2015;**5**(4): 179-183

[38] Kostinov MP, Cherdantsev AP, Briko NI. Mono and trivalent immunoadjuvant influenza vaccines immunologenicity and safety in pregnant women, The Fifth International Conference & Exhibitiom on: Influenza Vaccines for the World, 6-9 October, Albufeira Algarve Portugal, 2015. Posters № 109

[39] Cherdantsev AP, Kostinov MP, Kuselman AI, Dagil Yu A, Savisko AA. Post-vaccination immunity to influenza virus A/California/2009(H1N1)v in immunized pregnant women. Medical Immunology (Russian). 2012;**14**(6): pp. 527-532

[40] Kostinov MP, Cherdantsev AP and Pakhomov DV. Anti-influenza antibody level in mother-infant pairs depending on trimester of vaccination of pregnant women using immunoadjivant vaccine. Vaccines. 2015;**6**:5. http://dx.doi.org/2157-7560.1000297