

# Possible Effect of Extended Use of Hormonal Contraception on Increased Levels of Antiphospholipid Antibodies in Infertile Women

Mögliche Auswirkung der Langzeiteinnahme hormoneller Empfängnisverhütungsmittel auf den erhöhten Antiphospholipid-Antikörper-Spiegel bei unfruchtbaren Frauen

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## Key words

- infertility
- hormonal contraception
- antiphospholipid antibodies
- antiphospholipid syndrome

## Schlüsselwörter

- Infertilität
- hormonelle Empfängnisverhütung
- Antiphospholipid-Antikörper
- Antiphospholipid-Syndrom

## Abstract



**Purpose:** Increased levels of antiphospholipid antibodies (aPLs) are associated with the autoimmune disorder antiphospholipid syndrome (APS) and are known to play a role in infertility. We investigated the possible effect of prolonged use of hormonal contraception (HC) on autoimmunity after discontinuing HC in women with infertility problems.

**Material and Methods:** We analyzed hormonal status including ovulation and the humoral autoimmune response to eight phospholipids detected by ELISA in 1190 infertile women aged 21–46 (mean age: 29.3) years.

**Results:** Long-term HC did not affect ovulation, but in the absence of ovulation HC masked the hormonal disorders. The majority of patients taking HC for more than 10 years with fertility failure had significantly higher levels of aPLs of the IgG isotype against phosphatidylinositol and phosphatidylserine ( $p > 0.001$ ) than against  $\beta$ 2-glycoprotein I and annexin V.

**Discussion:** Thus, high levels of aPLs are significant for the anticoagulant treatment before and during pregnancy to prevent pregnancy loss.

## Zusammenfassung



**Zielsetzung:** Das erhöhte Auftreten von Antiphospholipid-Antikörpern im Blut ist mit der Autoimmunerkrankung Antiphospholipid-Syndrom (APS) assoziiert und spielt bekanntlich eine Rolle bei Infertilität. In Frauen mit Fertilitätsproblemen wurde die mögliche Auswirkung der Langzeiteinnahme hormoneller Empfängnisverhütungsmittel auf die Autoimmunität untersucht, die auch nach Absetzen des Mittels fortbesteht.

**Methoden:** In 1190 unfruchtbaren Frauen im Alter von 21–46 (Durchschnittsalter: 29,3) Jahren wurden Hormonstatus und Eisprung sowie die durch ELISA ermittelte humorale Immunreaktion auf 8 Phospholipide untersucht.

**Ergebnisse:** Die Langzeiteinnahme hormoneller Empfängnisverhütungsmittel hatte keine Auswirkung auf den Eisprung, aber bei Ausbleiben des Eisprungs hat die hormonelle Empfängnisverhütung die Hormonstörung überdeckt. Die Mehrheit der Patientinnen mit Fertilitätsproblemen, die Hormonpräparate zur Empfängnisverhütung über einen Zeitraum von mehr als 10 Jahren einnahmen, wiesen signifikant höhere Antiphospholipid-Antikörper-Spiegel des IgG-Isotyps gegen Phosphatidylinositol und Phosphatidylserin ( $p > 0,001$ ) auf als gegen  $\beta$ 2-Glykoprotein I und Annexin V.

**Diskussion:** Hohe Antiphospholipid-Antikörper-Spiegel erweisen sich also als signifikant für die antikoagulatorische Therapie vor und während der Schwangerschaft, um einem frühzeitigen Ende der Schwangerschaft vorzubeugen.

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## Bibliography

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## Introduction



Hormonal contraception (HC) is a birth control method that prevents ovulation by suppressing gonadotropins and changing the properties of cervical mucus. The most common combined con-

traceptives contain the both hormones, estrogen and progestin. HC has various well-known side effects such as bleeding, elevated liver tests, increased risk of cardiac disease, psychological and sexual changes, and deep vein thrombosis [1,2]. Although the history of HC commenced in 1921,

**Table 1** Fertility data of 1 190 patients after HC use with analysis of ovulation at 6 months after termination of HC.

	Group 1	Group 2	Group 3	Subgroup 4 from group 3
N	220	221	749	213
Age range (mean)	20–39 (28.7)	21–44 (27.8)	22–46 (31.4)	25–46 (35.6)
Complications				
▶ RPL	2 (0.9%)	45 (20.4%)	65 (8.7%)	4 (1.9%)
▶ Fetal loss	1 (0.4%)	4 (1.8%)	6 (0.8%)	0
▶ IVF failure	64 (29.0%)	120 (54.3%)	540 (72.1%)	190 (89.2%)
▶ NP	153 (69.7%)	46 (20.8%)	134 (17.9%)	19 (8.9%)
▶ Delivery before pregnancy failure	0	6 (2.7%)	4 (0.5%)	0
Physiological ovulation	202 (92%)	102 (46%)	157 (21%)	23 (11%)

RPL – repeated pregnancy loss

NP – never pregnant

with studies done in a rabbit model [3], the first combined HC was used in Great Britain in 1957. Since then, a large number of women of reproductive age have used HC with or without side effects [4]. Heterogeneous autoantibodies specific for negatively charged membrane phospholipids were detected in 1906 by Wassermann [5] in patients with syphilis, and immunochemically isolated by Pangborn [6]. Antiphospholipid antibodies (aPLs) are detectable in almost 80% of patients with autoimmune disease. Furthermore, they are also present in patients with reproductive failure, with a wide range of potential complications before spontaneous and/or in vitro fertilization (IVF), during problems encountered in early or late pregnancy (including spontaneous miscarriage, missed abortion, fetal death, intrauterine growth retardation, prematurity, and preeclampsia), and especially in primary or secondary antiphospholipid syndrome (APS) [7–10]. Antiphospholipid antibodies can interfere with normal conception by binding to phospholipids in the ovary, thus preventing formation and release of a functional ovum, or in normal fertilization by binding to the surface of either the ova or the spermatozoa with cytoplasmic, mitochondrial, endothelial and trophoblast epithelial cell membranes and platelets [11, 12]. aPLs can negatively influence the remarkable tolerance mediated by complex immunoregulatory mechanisms which exists between mother and fetal trophoblast [13, 14]. Fertility problems caused by microthrombosis of the utero-placental vasculature are the result of endothelial cell activation, inhibition of protein complex formation and fibrinolysis, and annexin V displacement [15–17, 19, 20]; other mechanisms for non-thrombotic fetal damage have been also considered [16–18, 21]. During our routine practice in counseling infertile couples, we observed an increase in serum autoantibodies against phospholipids in oral contraceptive users who decided to discontinue HC and attempt to conceive. Problems with fertility were evident (no spontaneous pregnancy and unsuccessful IVF, often combined with spontaneous miscarriage or missed abortion). The aim of our present study was to establish a screening profile for all aPLs (aPLs of the IgG, IgM, IgA isotypes specific for cardiolipin, phosphatidyl (ph)-serine, ph-ethanolamine, ph-inositol, ph-glycerol, phosphatidic acid, annexin V and  $\beta$ 2-GPI) in selected infertile women with reproductive failure and to evaluate the possible side effects of prolonged HC on their autoimmunity.

## Material and Methods



### Patients

The study group consisted of 1190 patients, aged 21–46 years (mean age: 29.3) (Table 1) who were referred for pregnancy

planning to the Division for Infertility and Immunology of Reproduction at the Department of Obstetrics and Gynecology, Charles University and Faculty Hospital, Pilsen, Czech Republic between 2008 and 2013. All women of reproductive age were divided into three main groups according to the use and the length of HC intake (Table 1). The 1st group included 220 women who had never used HC and served as a control group. The 2nd group (221 women) were on HC for a maximum period of two years, and the 3rd group included 749 patients who had used HC for 3 to 20 years. We also studied a 4th subgroup, selected from the 3rd group of infertile women, who had taken HC for 10 and more years. The common goal of all patient groups was successful conception after repeated fertility failures. Their partners had normal spermograms evaluated according to WHO criteria [22]. Medical history indicated a good health status for both partners. Blood samples were obtained from each woman between 7:00–10:00 a.m. All obtained serum samples were frozen and stored at  $-18^{\circ}\text{C}$  in our serum bank. All sera were screened for the presence of eight aPLs. No female patient had a history of vascular thrombosis, thrombocytopenia, autoimmune or infectious disease. The study was approved by the local research ethics committee of the Charles University Medical Faculty Hospital, Pilsen, Czech Republic. Written informed consent was obtained from all female participants.

### Antiphospholipid antibodies (aPLs)

Enzyme-linked immunosorbent assay (ELISA) was used for the detection of aPLs of the IgG, IgA and IgM isotypes against ph-serine, ph-ethanolamine, ph-inositol, ph-glycerol, phosphatidic acid, annexin V,  $\beta$ 2-GPI and cardiolipin levels as previously described [19]. Briefly, serially diluted sera were incubated with plates coated with highly purified phospholipids. After washing the plates, anti-human immunoglobulins conjugated to horseradish peroxidase were incubated with the antigen-antibody complex. Unbound conjugate was then washed away. Addition of an enzyme substrate generated an enzymatic colorimetric reaction, which was stopped by diluted acid. The intensity of the color, which was directly proportional to the concentration of antibodies, was measured photometrically.

### Statistical analysis

Statistical analysis of all laboratory results was done using Mann-Whitney U-Test for Independent Samples in Statistics for Windows, Version 22.0 (IBM Corp., Armonk, NY, USA), with p-values  $\leq 0.05$  considered statistically significant.

**Table 2** Analysis of antiphospholipid antibodies in women without and with long-term HC use (2008–2013).

	Group 1	Group 2	Group 3	Subgroup 4 from Group 3	p-value
N	220	221	749	213	
Mean age (years)	28.7	27.8	31.4	35.6	NS
Presence of antiphospholipid antibodies against:	<b>56 (25%)</b>	<b>95 (43%)</b>	<b>588 (78.5%)</b>	<b>189 (89%)</b>	<b>0.001</b>
▶ Cardiolipin	IgG 3 (1.4%)	4 (1.8%)	18 (2.4%)	5 (2.3%)	NS
	IgM 2 (0.9%)	3 (1.3%)	0 (0%)	5 (2.3%)	NS
▶ Ph-inositol	IgG 54 (24.5%)	58 (26.2%)	273 (36.4%)	212 (99.5%)	<b>0.001</b>
	IgM 2 (0.9%)	1 (0.4%)	5 (0.6%)	5 (2.0%)	NS
▶ Ph-serine	IgG 19 (8.6%)	21 (9.5%)	321 (42.8%)	208 (98.0%)	<b>0.001</b>
	IgM 0 (0%)	4 (1.8%)	8 (1.0%)	6 (2.8%)	NS
▶ Ph-glycerol	IgG 11 (5.0%)	8 (3.6%)	57 (7.60%)	41 (19.0%)	NS
	IgM 0 (0%)	4 (1.8%)	12 (1.6%)	8 (4.0%)	NS
▶ Ph-ethanolamine	IgG 12 (5.4%)	8 (3.6%)	61 (8.1%)	51 (24.0%)	0.01
	IgM 1 (0.4%)	2 (0.9%)	16 (2.0%)	9 (4.2%)	NS
▶ β2-GPI	IgG 5 (2.3%)	20 (9.0%)	39 (5.2%)	29 (13.6%)	0.01
	IgA 0 (0%)	1 (0.4%)	10 (1.3%)	3 (1.4%)	NS
▶ Phosphatidic acid	IgG 16 (7.2%)	8 (3.6%)	60 (8.0%)	23 (10.8%)	NS
	IgM 0 (0%)	0 (0%)	0 (0%)	3 (1.4%)	NS
▶ Annexin V	IgG 5 (2.3%)	9 (4%)	37 (4.9%)	32 (15.0%)	0.01

NS – not significant

Group 1 – not on HC; Subgroup 4 (HC use of between 10 years and 20 years)

## Results

Table 1 shows the medical history of 1190 women before immunological examination. Long-term use of HC interferes with physiological ovulation but also depends on the age of patients. Normal ovulation was present in 92% of women who never used HC. In the 2nd and 3th groups with long-term HC use, ovulation was restored in 46% (2nd group), 21% (3rd group), and 11% in the 4th subgroup (Table 1).

Serum samples from 1190 infertile patients were evaluated for antibodies of the IgG, IgM and IgA isotypes specific for eight different phospholipids. We assessed individual aPLs with respect to their specificity, prevalence (given as a percentage) and significance. The results in Table 2 showed overall positivity for aPLs in 78% of women who used HC for more than 2 years ( $p \leq 0.001$ ) and also demonstrated significantly higher levels of antibodies against ph-inositol and ph-serine ( $p \leq 0.001$ ). The antibodies predominantly present were of the IgG isotype. Results also demonstrated significant differences between patients who never used HC (group 1) and those who had used HC for more than 10 years (subgroup 4). Increased aPLs positivity was found in 89% of patients, with higher levels of aPLs against ph-inositol ( $p \leq 0.001$ ), ph-serine ( $p \leq 0.001$ ), ph-ethanolamine ( $p \leq 0.01$ ), β2-GPI and annexin V ( $p \leq 0.01$ ). Our detailed analysis suggests that the prevalence of positive levels of aPLs against five different phospholipids was much higher in the group of HC users than in non-HC users, where we found only very low levels of such antibodies.

## Discussion

In a normal pregnancy, the mother is exposed to a great number of autoimmune and alloimmune antigens which modulate her immune activities. The immunologic mechanisms of pregnancy loss have been the subject of several reviews e.g. [7–9, 12, 13, 16–18, 20, 23]. This paper summarizes our experience in a long-termed study of women who used HC for more than ten years and displayed significantly increased levels of IgG aPLs, predom-

inantly against ph-inositol, ph-serine, ph-ethanolamine, β2-GPI, and annexin V. These autoantigens contribute to the pathogenesis of pregnancy failure through multiple mechanisms in which the central feature could be thrombosis and/or microthrombosis [23]. We did not find information on these findings in the literature. However, the effects of estrogen and progesterone in autoimmune diseases such as systemic lupus erythematosus (SLE) have been previously described [24–30]. Therefore, we aimed to determine whether HC played a role in the elevation of autoimmune factors such as aPLs which contribute to fertility failure. It is well known [29, 30] that estrogens have both pro-inflammatory and anti-inflammatory effects, with certain immunomodulatory and immunosuppressive activities enhancing the proliferation and activation of B-cells. Estrogen administration has the potential effect to exacerbate autoimmune processes and can also increase the risk of thrombosis, especially in women positive for aPLs. Our patients had taken oral combined hormonal contraceptives with different concentrations of the biologically active synthetic estrogen (ethinyl estradiol) and one of the 17-alpha ethinyl analogs of 19-nortestosterone as the progesterone component. However, it is not known which drug combination led to the induction of aPLs. Thromboembolism is a major complication of HC, the risk being further increased by the presence of positive autoimmune factors such as lupus coagulant and aPLs [15]. Could HC be the reason for the long-term effects of exogenous estrogen and progesterone through the impact on female autoimmunity? Our study focused on screening for aPLs positivity and determination of aPLs profiles in women with varying lengths of HC use. Although we also considered other causes of fertility problems (through the evaluation of gynecological and endocrinological information, reproductive immunology and genetics), the results of the aPLs panels were clear. A minority of women who did not use HC but had infertility problems also displayed certain immunological abnormalities such as the presence of aPLs antibodies. Although the current classification of APS is based only on positive identification using one or more of three standardized laboratory assays (anti-cardiolipin antibodies, lupus anti-coagulant, and anti-β2-GPI according to the report of

aPLs congresses [7,31]), it is clear that other antiphospholipid antibodies may also play an important role in pregnancy failure. To determine the immunological cause of infertility this definition is insufficient. This is the reason why our laboratory, which specializes in reproductive immunology, uses a wider range of aPLs. Results generated suggest that women with previous long-term use of HC have significantly increased levels of aPLs, especially of the IgG isotype, against ph-inositol and ph-serine, and that these levels are higher than those against  $\beta$ 2-GPI, and annexin V. Based on our results and extensive experience with human infertility and reproductive immunology, we stress that prolonged use of HC is likely to affect female autoimmunity through the induction of various autoantibodies such as aPLs. Immune response could be also associated with aging, particularly in older patients [32–34], but none of our patients were elderly; in all groups, only a minority of patients with proven anovulation and higher number of positives for aPLs were aged 40 or older. We speculate that the long-term intake of a combination of artificial hormones such as those present in HC and patient age of more than 35 years could influence hormonal and immunological responses.

## Conclusion

We suggest that prolonged HC use represents a new and significant risk factor for immunological reproductive failure [33,34]. The presence of high levels of aPLs could alert clinicians, prompting them to initiate timely anticoagulant treatment to prevent APS.

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## Conflict of Interest

None declared.

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