

# DYNAMICS OF SERUM LEVELS OF C-REACTIVE PROTEIN AND ALBUMIN IN WOMEN OF LATE REPRODUCTIVE AGE DURING PREGNANCY INDUCED IN ASSISTED REPRODUCTIVE TECHNOLOGY PROGRAMS

## F.A. KHANCHA

PhD, assistant, Department of Obstetrics and Gynecology, Donetsk National Medical University, Kropivnytskyi  
ORCID:0000-0001-6383-7885

## O.M. NOSENKO

MD, professor, Obstetrics and Gynecology Department, Odesa National Medical University, Odesa  
ORCID: 0000-0002-7089-2476

## Contacts:

Nosenko Olena Nikolaevna  
ONMedU, Department of Obstetrics and gynecology, KNE «Puerperal building No. 7» OCC  
65080, Odesa,  
St. Cosmonauts, 11/13  
Phone: +38 (050) 638-38-28  
Email: nosenko.olena@gmail

## INTRODUCTION

Pregnancy causes a number of physiological changes and laboratory parameters of a pregnant woman differ from a healthy non-pregnant donor woman. The volume of circulating blood, as well as blood viscosity, undergo significant changes during pregnancy. In the middle of the III trimester, blood volume increases to 40%, and blood viscosity decreases [1]. Significant immunological changes occur during pregnancy, as the mother's immune system encounters foreign antigens derived from the semi-allogeneic fetus and placenta [2]. Immune reactivity changes in the dynamics of the gestational process. Biologically, the I trimester of pregnancy has been found to be proinflammatory to facilitate blastocyst implantation and placentation. The II trimester, the period of intensive intrauterine development, is characterized by an anti-inflammatory Th2 environment [2]. During the III trimester, there is a switch of immune reactivity to a pro-inflammatory Th1 response, which is necessary for birth [3–7].

In recent years, circulating levels of pro-inflammatory molecules have been found to be elevated in patients with recurrent miscarriage and recurrent implantation failure. These results demonstrate that an imbalance of pro- and anti-inflammatory factors may underlie reproductive failure [8].

One of the most important pro-inflammatory molecules is C-reactive protein (CRP). It belongs to the evolutionarily conserved family of pentraxins, produced by hepatocytes in response to pro-inflammatory cytokines, mainly interleukin (IL)-6 and to a lesser extent IL-1 $\beta$ . The concentration of CRP in the blood can increase from less than 1 to 600–1000 mg/l [9, 10]. This rapid and profound increase makes CRP useful as a marker for monitoring inflammatory activity in chronic diseases. Infections during pregnancy are common, and CRP is often measured as a surrogate marker of infection [3–7]. Studies examining longitudinal CRP levels during induced pregnancy, particularly in women of advanced reproductive age, are scarce.

Serum albumin can serve as a “negative” protein of the acute phase of inflammation [11, 12].

Pro-inflammatory cytokines (such as tumor necrosis factor  $\alpha$  and IL-6) inhibit its synthesis. Albumin can provide communication between intracellular, extracellular and tissue fluid and maintain the balance of colloid-osmotic pressure of blood. This is explained by the fact that albumin accounts for nearly 70–80% of the total osmotic pressure in plasma and is the main regulator of fluid distribution in body cavities [13]. Albumin is considered an extracellular molecule, mainly because it is released outside the cell as rapidly as cytokines after synthesis in hepatocytes. In turn, numerous cell types (including endothelial cells and hepatocytes) are able to take up albumin by receptor-mediated endocytosis and cleave it by lysosomes. Accordingly, albumin has multiple biological effects, and its non-colloidal function cannot be ignored in addition to its colloidal function. Non-oncotic functions of albumin mainly include antioxidant, anti-inflammatory, molecular transport, endothelial stabilization, antithrombotic and immune regulation [11, 12, 14], which indicates the important role of this protein for the physiological course of pregnancy. The characteristics of albumin discovered in recent years indicate that this main blood plasma protein, which until recently was assigned the “modest” role of an osmotically active component, is actually a molecular “core”, a connecting link between various tissues and organs, which indicates health. I of the whole body and in many respects determine this health [15]. Pregnancy is characterized by a decrease in plasma albumin levels with gestational age [16, 17].

Recently, research has focused on the relationship between albumin and CRP in inflammatory conditions. The obtained results are contradictory. Some researchers observed a decrease in albumin, which was accompanied by a significant increase in CRP [14, 18]. However, another study showed that albumin increased in inflammatory conditions [19]. A statistically significant inverse correlation was established between serum levels of CRP and albumin, illustrating the divergent relationship between “positive” and “negative” acute phase protein [20].

Elevated serum CRP levels have been associated with reduced fertility and poor outcomes of assisted reproductive technologies (ART) [21, 22]. In the work of H. Zhang et al. (2023) [8] it was shown that the frequency of clinical pregnancy in ART programs was significantly lower in the group of individuals with a high basal level of CRP compared to the group of women with low CRP (50.0 vs. 63.4%;  $p < 0.0167$ ), which contributed to a significant decrease in the birth rate (39.8 vs. 53.8%;  $p < 0.0167$ ) [8]. R.G. Radin et al. (2015) [23] showed that high pre- or post-implantation CRP levels reduce the survival of pre- or post-implantation male embryos among women trying to conceive naturally, and that daily treatment with low-dose acetylsalicylic acid before conception can reduce serum CRP concentrations blood, restoring the normal ratio of the sexes of the offspring. This deleterious effect of higher maternal inflammation on male embryos is consistent with *in vivo* animal studies [23].

Some studies show that reproductive aging is associated with a state of low-grade chronic inflammation and increased levels of serum inflammatory markers, such as CRP [24]. Despite numerous clinical trials, the mechanism behind this increase is not yet fully understood. However, higher levels of inflammatory markers are hypothesized to be associated with increased adipose tissue (especially visceral), decreased sex hormone levels, and increased oxidative damage, all of which are common in advanced reproductive and elderly people [24].

Thus, pregnancy is an inflammatory condition, the latter intensifies in advanced reproductive age. Decreased fertility in women, especially when ART is necessary, is also associated with increased inflammation, especially with such infertility factors as tubal-peritoneal factor, endometritis, endometriosis, polycystic ovary syndrome, obesity. Serum levels of CRP and albumin are important markers of inflammation in a woman's body, available for laboratory diagnosis in broad clinical practice.

**Objective of the study:** to determine the serum levels of CRP and albumin in women of advanced reproductive age in the dynamics of pregnancy induced in ART programs.

## MATERIAL AND METHODS

The study was conducted on the basis of the Department of Obstetrics and Gynecology of the Odesa National Medical University from 2020 to 2023. It is a fragment of the research topic "Improving methods of prevention, diagnosis and treatment of diseases of the female reproductive system using the latest medical and molecular genetic technologies" (No. of the state registration 0117U007494). The clinical bases of the study were LLC "Reproductive Medicine Clinic "Nadia Odesa" (Odesa), LLC "Profile Hospital AIRMED" (Odesa), Maternity House No. 7 of the Odesa City Council (Odesa). This study was approved by the Bioethics commission of the Odessa National Medical University (protocol No. 31 dated May 31, 2021) and performed according to the ethical principles of the World Medical Association Declaration of Helsinki. Written informed consent to the processing of personal data and their further use was obtained from all participants.

123 women with infertility cured in ART cycles were under observation:

- 65 pregnant women of late reproductive age were included in group I;
- 58 women of active reproductive age were included in group II.

Control group C included 57 pregnant women of late reproductive age after natural conception. All women's pregnancies ended by childbirth.

Fasting peripheral blood samples were collected from pregnant women at 7–8, 11–12, 18–20 and 29–31 weeks using EDTA vacutainer tubes. Serum was separated after centrifugation (3.500 rpm) for 10 min during the first 24 h after collection, stored in aliquots in a freezer at  $-80^{\circ}\text{C}$  until determination. The level of CRP was determined by the immunoturbidimetric method with latex amplification on a Cobas 6000 analyzer (c 501 module) using original Roche Diagnostics test systems (Switzerland). CRP agglutinate of human origin with latex particles coated with anti-CRP monoclonal antibodies was used. Aggregates were analyzed turbidimetrically. The albumin level was determined by colorimetric analysis on a Cobas 6000 analyzer (c 501 module) using original test systems from Roche Diagnostics (Switzerland).

Statistical analysis of the obtained data was carried out using the Excel electronic program with determination of the reliability of differences at  $p$  value  $< 0.05$ . The average value ( $M$ ), standard error of the mean ( $\pm$  SEM) was determined. The Mann-Whitney U-test was used to compare groups based on quantitative characteristics.

## RESULTS

The average age of examined women of group I was  $38.71 \pm 0.54$  years ( $p_{I-II} < 0.01$ ,  $p_{I-C} > 0.05$ ), group II –  $30.86 \pm 0.38$  years ( $p_{II-C} < 0.01$ ), group C –  $37.65 \pm 0.29$  years, body mass index, respectively –  $24.32 \pm 0.58$  kg/m<sup>2</sup> ( $p_{I-C} > 0.05$ ),  $23.47 \pm 0.66$  kg/m<sup>2</sup> ( $p_{II-C} > 0.05$ ),  $24.27 \pm 0.73$  kg/m<sup>2</sup>.

It was established that level of CRP in blood serum increased with the course of the gestational process, and was statistically significantly lower in the I trimester compared to the II and III trimesters in all studied groups. Pregnant women of advanced reproductive age with pregnancy induced in ART programs had higher levels of serum CRP compared to women of active reproductive age with pregnancy induced in ART programs and pregnant women of advanced reproductive age after natural conception. At 7–8 weeks of gestation, serum levels of CRP were increased in pregnant women of group I compared to the group II by 1.44 times and group C by 1.79 times ( $2.89 \pm 0.08$  vs.  $2.01 \pm 0.14$  pg/ml ( $p < 0.01$ ) and  $1.62 \pm 0.08$  pg/ml ( $p < 0.01$ )), in the period of 11–12 weeks – 1.42 and 1.83 times, respectively ( $6.02 \pm 0.19$  vs.  $4.24 \pm 0.29$  pg/ml ( $p < 0.01$ ) and  $3.29 \pm 0.18$  pg/ml ( $p < 0.01$ )), in the term 18–20 weeks – 1.26 and 1.97 times ( $7.32 \pm 0.24$  vs.  $5.79 \pm 0.40$  pg/ml ( $p < 0.01$ ) and  $3.72 \pm 0.22$  pg/ml ( $p < 0.01$ ) pg/ml), in 29–31 weeks – 1.30 and 1.91 times ( $9.29 \pm 0.30$  vs.  $7.16 \pm 0.50$  pg/ml ( $p < 0.01$ ) and  $4.87 \pm 0.27$  pg/ml ( $p < 0.01$ ) (Fig. 1).

As our study showed, the albumin content in blood serum in the first trimester of pregnancy was statistically significantly higher compared to the second and third trimesters in all studied groups of pregnant women. Lower levels of serum

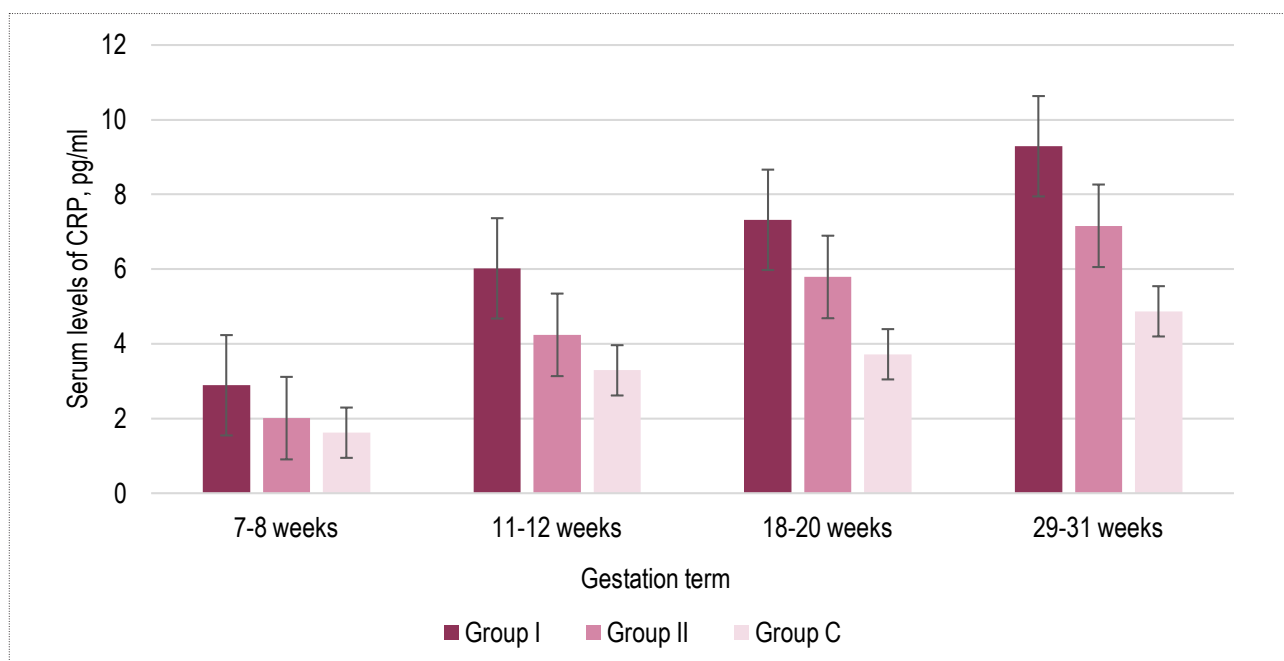


Figure 1. Levels of serum CRP in women of the studied groups in the dynamics of pregnancy

albumin were noted in subjects of advanced reproductive age with pregnancy induced in ART programs compared to pregnant women of advanced reproductive age after natural conception and pregnant women of active reproductive age after ART. Thus, serum albumin concentrations were reduced in pregnant women of group I compared to the group II by 1.24 times and in group C by 1.32 times ( $40.19 \pm 0.44$  vs.  $49.97 \pm 0.48$  g/l ( $p < 0.01$ ) and  $53.21 \pm 0.50$  g/l ( $p < 0.01$ )), in the period of 11–12 weeks – 1.20 and 1.31 times, respectively ( $37.29 \pm 0.41$  vs.  $44.62 \pm 0.75$  g/l ( $p < 0.01$ ) and  $49.01 \pm 0.42$  g/l ( $p < 0.01$ )), in the term 18–20 weeks – 1.15 and 1.38 times ( $33.82 \pm 0.44$

vs.  $38.82 \pm 1.08$  g/l ( $p < 0.01$ ) and  $46.76 \pm 0.55$  g/l ( $p < 0.01$ )), in 29–31 weeks – 1.14 and 1.40 times ( $31.90 \pm 0.44$  vs.  $36.22 \pm 1.14$  g/l ( $p < 0.01$ ) and  $44.55 \pm 0.59$  g/l ( $p < 0.01$ )) (Fig. 2).

The conducted study established an inverse correlation between albumin concentration and CRP level ( $r = -0.37$ ,  $p < 0.01$ ). Statistically significant correlations were also found between albumin and CRP in the first ( $r = -0.32$ ,  $p < 0.01$ ) and second ( $r = -0.23$ ,  $p = 0.03$ ) trimesters, but not in the third ( $r = -0.03$ ,  $p > 0.05$ ). Fetal gender did not affect CRP and albumin levels during any of the trimesters. No significant correlations were found between CRP levels during any of the trimesters

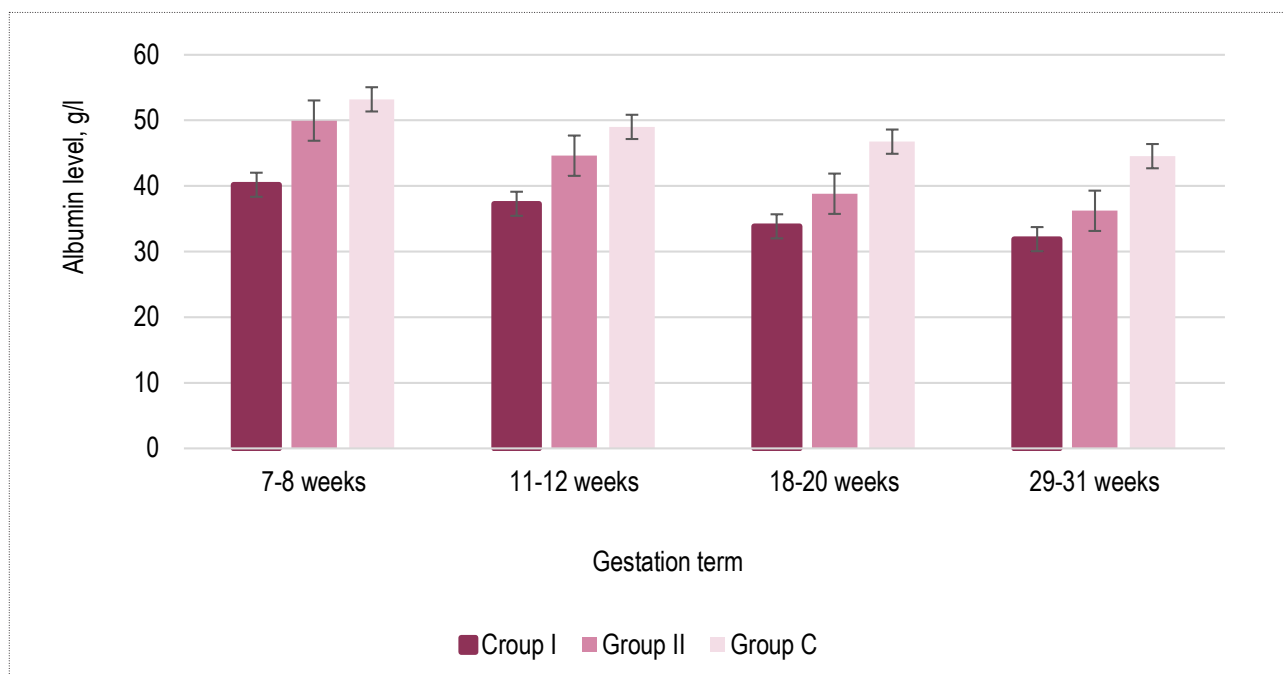


Figure 2. Levels of serum albumin in women of the studied groups in the dynamics of pregnancy

and fetal birth weight, and between CRP levels during any of the trimesters and fetal length of birth.

## DISCUSSION

The literature indicates that both endogenous and exogenous hormones of the reproductive system can affect protein synthesis by the liver. Endogenous progesterone has been shown to increase serum CRP levels during the normal menstrual cycle. A 10-fold increase in progesterone was reported to be associated with a 19.4% increase in serum CRP in the luteal phase. It was also observed that the concentration of CRP correlates with estradiol throughout the menstrual cycle. Oral estradiol, used in the maintenance of an induced pregnancy in the first trimester, can be rapidly and completely absorbed from the gastrointestinal tract and reach the liver in high concentrations via the portal blood. This so-called first-pass effect is responsible for enhancing hepatic protein synthesis [25].

Changes in the expression of CRP and albumin revealed in the work during pregnancy induced in ART programs compared to pregnancy after natural conception can be associated with inflammatory factors of infertility, intensive hormonal support

during pregnancy, and in women of advanced reproductive age – with reproductive aging.

## CONCLUSIONS

Pregnancy induced in ART programs in women of advanced reproductive age is characterized by increased levels of CRP and decreased levels of albumin in peripheral blood serum, which requires appropriate therapeutic and preventive correction during pregnancy.

Quantification of CRP may be a potential marker of ART outcome, but its prognostic value remains to be investigated in large prospective studies. In the future, quantification of circulating CRP before initiation of ART may help identify patients with a poor ART prognosis, leading to cancellation of the ART cycle or preconception treatment to minimize medical risks and costs. Studying the relationship between albumin and CRP may provide a screening tool for inflammation to guide therapeutic intervention and avoid overcorrection in patients with inflammation.

## Conflict of interest

The authors declare no conflict of interest.

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## ДИНАМІКА РІВНІВ С-РЕАКТИВНОГО БІЛКА ТА АЛЬБУМІНУ В ЖІНОК ПІЗЬНОГО РЕПРОДУКТИВНОГО ВІКУ ПІД ЧАС ВАГІТНОСТІ, ІНДУКОВАНОЇ У ПРОГРАМАХ ДОПОМІЖНИХ РЕПРОДУКТИВНИХ ТЕХНОЛОГІЙ

**Ф.О. Ханча**, к. мед. н., асистент кафедри акушерства та гінекології Донецького національного медичного університету, м. Кропивницький

**О.М. Носенко**, д. мед. н., професор кафедри акушерства та гінекології Одеського національного медичного університету, м. Одеса

**Обґрунтування.** Вагітність, з погляду біології, є запальним станом. Зниження плодючості, особливо за необхідності проведення допоміжних репродуктивних технологій, а також пізній репродуктивний вік жінок пов'язані з посиленням запалення.

**Метою** дослідження є визначення сироваткових рівнів С-реактивного білка (СРБ) та альбуміну в жінок пізнього репродуктивного віку в динаміці вагітності, індукованої в програмах допоміжних репродуктивних технологій.

**Матеріали та методи.** Спостереження охоплювало 123 жінки з вилікуванням у циклах допоміжних репродуктивних технологій безпліддям, із яких 65 вагітних – пізнього репродуктивного віку (група I), 58 – активного репродуктивного віку (група II). До контрольної групи К увійшли 57 вагітних пізнього репродуктивного віку після природної концепції. Рівень СРБ визначався імунотурбідиметричним методом, альбуміну – за допомогою колориметричного аналізу.

**Результати.** Встановлено, що протягом гестаційного процесу рівень СРБ в сироватці крові збільшувався і був значуще нижчим у першому триместрі порівняно з другим і третім триместрами у всіх досліджуваних групах вагітних. У жінок I групи спостерігалися більш високі показники сироваткового рівня СРБ порівняно з жінками групи II та вагітними групи К. Вміст альбуміну в сироватці крові у першому триместрі вагітності був статистично значуще вищим порівняно з другим і третім триместрами у всіх досліджуваних групах вагітних. У обстежених жінок групи I спостерігалися більш низькі показники сироваткового рівня альбуміну порівняно з вагітними групи II та групи К.

**Висновки.** Індукована у програмах допоміжних репродуктивних технологій вагітність у жінок пізнього репродуктивного віку характеризується підвищеною концентрацією СРБ та зниженим рівнем альбуміну в сироватці периферичної крові, що потребує розробки відповідної лікувально-профілактичної корекції під час ведення вагітності.

**Ключові слова:** безпліддя, допоміжні репродуктивні технології, індукована вагітність, пізній репродуктивний вік, С-реактивний білок, альбумін.

## DYNAMICS OF C-REACTIVE PROTEIN AND ALBUMIN LEVELS IN WOMEN OF LATE REPRODUCTIVE AGE DURING PREGNANCY INDUCED IN ASSISTED REPRODUCTIVE TECHNOLOGY PROGRAMS

**F.A. Khancha**, PhD, assistant, Department of Obstetrics and Gynecology, Donetsk National Medical University, Kropyvnytskyi

**O.M. Nosenko**, MD, professor, Obstetrics and Gynecology Department Odesa National Medical University, Odesa

**Background.** Pregnancy from a biological point of view is an inflammatory condition. Decreased fertility, especially when assisted reproductive technologies are required, as well as women's late reproductive age is associated with increased inflammation.

**Objective of the study:** to determine the serum levels of C-reactive protein (CRP) and albumin in women of late reproductive age in the dynamics of pregnancy induced in assisted reproductive technologies programs.

**Materials and methods.** Under observation were 123 women with infertility cured in assisted reproductive technologies cycles: 65 were pregnant women of late reproductive age (group I), 58 were of active reproductive age (group II). Control group C consisted of 57 pregnant women of advanced reproductive age after the natural concept. The level of CRP was determined using the immunoturbidimetric method, albumin level – with a colorimetric analysis.

**Results.** It was found that, the CRP level in the blood serum increased over the course of the gestational process and was significantly lower in the first trimester compared to the second and third trimester in all studied groups of pregnant women. Pregnant women of group I had higher levels of serum CRP compared to women of group II and group C. The albumin level in the blood serum in the first trimester of pregnancy was statistically significantly higher compared to the second and third trimesters in all studied groups. Women in group I had lower levels of serum albumin compared to pregnant women in group II and group C.

**Conclusions.** Pregnancy induced in assisted reproductive technologies programs in women of late reproductive age is characterized by increased levels of CRP and decreased levels of albumin in peripheral blood serum, which requires the development of appropriate therapeutic and preventive correction during pregnancy.

**Keywords:** infertility, assisted reproductive technologies, induced pregnancy, late reproductive age, C-reactive protein, albumin.