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## CHANGES OF SOME INDEXES OF HOMEOSTASIS AND OF HISTOLOGY OF PLACENTAS IN WOMEN WITH THE THREAT OF MISCARRIAGE IN EARLY TERMS OF PREGNANCY

**Key words:** *threat of abortion, trophoblast, placenta, pregnancy-specific beta-glycoprotein-1, progesterone-associated endometrial protein, hormones, hemostasis, histology, extract of ginkgo, erynithum.*

**Purpose.** *This study investigates the role of pregnancy-related proteins in pathogenesis of the threat of abortion in the 1<sup>st</sup> trimester of pregnancy in relation to the changes of hemostasis and histological changes of postpartum placenta. **Design/methodology/approach.** 30 women with uncomplicated course of pregnancy and 96 patients with the signs of the threat of abortion in the 1<sup>st</sup> trimester of pregnancy have been examined. The serum levels of pregnancy-specific beta-glycoprotein-1 (PSG1) and progesterone-associated endometrial protein (PAEP, placental protein 14) have been studied, as well as serum levels of the hormones (estradiol, progesterone, cortisol) and indexes of hemostasis. The extract of ginkgo and erynithum have been used in the therapeutic complex for treatment of the threat of abortion. The histological research of the postpartum placentas has been performed. The study uses the primary data.*

**Findings.** *The conclusion has been made that these medications improve functioning of trophoblast in the 1<sup>st</sup> trimester, and PSG1 and PAEP are the main factors which prevent development of the disorders of hemostasis in case of the threat of abortion. According to histological research, the proposed therapeutic complex is thought to increase compensatory reaction of the placental tissue in response to hypoxia. **Originality/value.** This study researches the changes in the placental proteins, hormones and the parameters of blood coagulation in pregnant women in the context of threat of abortion in the 1<sup>st</sup> pregnancy trimester in combination with histological examination of postpartum placentas.*

### Introduction

The problem of miscarriage and spontaneous abortion is one of the most important in modern obstetrics. The frequency of cases of miscarriage in Ukraine is 15-23% of all fixated pregnancy cases [3], and it has no tendency to decreasing і не має тенденції до зниження. The immune and vascular adaptation is considered to be basic in pregnancy process. The transformation of spiral arteries takes place during pregnancy: the cells of endothelium are replaced by trophoblastic cells, and the non-striated muscular cells are replaced by matrix, cells of trophoblast and fibrin [2, 5]. Insufficient invasion of the trophoblast in area of placental site results in occlusion of spiral and uterine-placental arteries what can lead to spontaneous abortion in early term of gestation or placental dysfunction in later pregnancy terms. If the pregnancy has been complicated by the threat of abortion, the average frequency of neonatal asphyxia rises up to 17,5% [7].

### Aim of study

To investigate the role of pregnancy-related proteins in pathogenesis of the threat of abortion in the 1<sup>st</sup> trimester

of pregnancy in relation to the changes of hemostasis and histological changes of postpartum placenta.

### Materials and methods

96 pregnant females with the clinical signs of the threat of abortion in 1<sup>st</sup> pregnancy trimester have been selected in the main *study group*. The clinical signs included: pain in the lower abdomen, spot-like bloody discharge from vagina, ultrasound signs of the threatened abortion (segmental uterine contractions). Patients with known severe extragenital pathology, verified cervical incompetence, ABO and Rh-immunization and TORCH-infections were not included in the group. 30 healthy pregnant women formed *control group*.

The main study group has been divided into two equal subgroups: 48 patients formed *subgroup with traditional treatment of miscarriage*, so they received pregnancy-saving complex therapy [1,4], including hormonal treatment (Duphaston 20-40 mg/day), spasmolytics, sedative and hemostatic medications. Other 48 women, who formed *subgroup of*

*correction* additionally were treated by our therapeutic complex which included extract of ginkgo (in 40 mg capsules 3 times daily) and erynithum (10 mg pills 3 times daily). Extract of ginkgo has the disaggregant properties meanwhile erynithum is the donor of nitric oxide (NO). The course of treatment (excluding Duphaston) was 10-12 days and had been prescribed since 6<sup>th</sup>-8<sup>th</sup> weeks of pregnancy. The course of treatment had been repeated 3 times with the intervals of 7-10 days. The proposed therapy was aimed to improve blood supply for trophoblast.

25 pregnant randomly selected women from the subgroup of correction were examined using methods described below [1]. The levels of the hormones (estradiol, progesterone, cortisol) were investigated in the venous blood by radio-immune analysis using automatic diagnostic complex "Gamma-1" and its specific chemical reagents (manufactured in Minsk, Republik of Belarus). Concentrations of pregnancy-specific beta-glycoprotein-1 (PSG1) and progestagen-associated endometrial protein (PAEP, placental protein 14) were studied using diagnostic sets specific for those proteins (manufactured by "ДИА-М" company, Russia). The changes in hemostasis were established basing on the next indexes: recalcification time (RT), prothrombin time (PT), thrombin time (TT), quantity of platelets in 1 ml, percent of adhesive thrombocytes (PAT) and index of spontaneous aggregation of thrombocytes (ISAT).

Such extent of examinations was conducted twice: in 6-8 and 12-13 weeks of pregnancy. The blood samples were taken at the morning on fasting conditions of patients. The comparison was performed with the results of 24 randomly selected patients from subgroup with traditional treatment and with all 30 patients from control group.

### Results of study and their discussion

The serum levels of the hormones in examined women are represented in Table 1.

As seen from the table, the serum level of cortisol is significantly higher in both subgroups with threat of abortion, in comparison with healthy pregnant women ( $p < 0,05$ ). The mean concentration of estradiol in subgroup of correction was higher than in control group in 6-8 weeks of pregnancy with the background of treatment, and in 12-13 weeks of gestation ( $p < 0,05$ ). It might be caused by compensatory increasing of synthetic function of the trophoblast/placenta. The changes of concentration of the progesterone in the blood of pregnant with threat of miscarriage attract the most attention. The mean concentration of this hormone in subgroup of correction was the lowest by the start of treatment,

but at 12<sup>th</sup>-13<sup>th</sup> week of gestation it had no significant difference with similar index of control group but was higher in comparison with such index of group with traditional treatment ( $p < 0,05$ ).

The results of studying of the concentrations of the placental proteins are represented in Table 2.

It has been established that the mean concentration of PSG1 in both subgroups with complicated pregnancy is significantly lower than in control group ( $p < 0,05$ ). It is true for 6-8 and 12-13 weeks of gestation. The level of PSG1 was higher in subgroup of correction than in subgroup with traditional treatment by the end of the 1<sup>st</sup> trimester ( $p < 0,05$ ). Concerning PAEP it was unveiled that the mean level of this protein at 12<sup>th</sup>-13<sup>th</sup> week of pregnancy was higher both in subgroup of correction and control group, comparing with subgroup with traditional treatment ( $p < 0,05$ ).

The parameters of coagulation in women with the clinical signs of threatened abortion are represented in Table 3.

As seen from Table 3, time of re-calcification, prothrombin time and thrombin time are shortened at the end of the 1<sup>st</sup> pregnancy trimester in subgroup with traditional treatment in comparison with control group ( $p < 0,05$ ). In the subgroup of correction the coagulation indexes at 12<sup>th</sup> – 13<sup>th</sup> weeks of pregnancy get closer to the results of the control group. The mean concentration of fibrinogen had no significant differences between groups. Obviously, the received data is an evidence of appearance of the tendency to hypercoagulation which accompanies the clinic of threatened abortion during the 1<sup>st</sup> trimester of gestation.

The results of examination of the thrombocyte-related hemostasis are represented in the Table 4.

We found the signs of the tension of the thrombocyte-related hemostasis in women with complicated course of pregnancy. It manifests in increased count of the thrombocytes per 1 ml and in activation of membrane potential of the thrombocytes (seen as higher PAT and ISAT) in comparison with control group ( $p < 0,05$ ). At the same time, this component of hemostasis is normalized on the background of proposed treatment. For instance, the mean PAT and ISAT and count of thrombocytes are lower in the subgroup of correction than in subgroup with traditional treatment ( $p < 0,05$ ); moreover, PAT is even lower than in the control ( $p < 0,05$ ). Such changes, according to our opinion, lead to improvement of the blood supply of the trophoblast.

The proposed complex of treatment improves the integration between different systems of the organism which are responsible for normal

Table 1

**The indexes of the hormones in blood of women with the clinical signs of threatened abortion in the 1<sup>st</sup> trimester of gestation (M±m)**

	Cortisol, nmole/l		Estradiol, nmole/l		Progesterone, nmole/l	
	6-8 weeks	12-13 weeks	6-8 weeks	12-13 weeks	6-8 weeks	12-13 weeks
Subgroup of correction (n=25)	577,08± 36,27*	646,67± 30,84*	11,33± 0,60*	17,45± 1,08*	68,15± 2 32* **	119,79± 3,63**
Subgroup with traditional treatment (n=24)	489,65± 31,85*	513,23± 36,56*	12,09± 1,36	14,49± 1,92	98,43± 6,30	102,31± 6,87*
Control group(n=30)	336,54± 16,08	343,05± 25,16	9,78± 0,22	11,78± 0,46	108,90± 3,90	128,85± 3,74

Note. \* - the difference between indexes is significant in comparison with control group, \*\* - the difference between indexes is significant in comparison with subgroup with traditional treatment

Table 2

**The levels of the placental proteins in blood of women with the clinical signs of threatened abortion in the 1<sup>st</sup> trimester of gestation (M±m)**

	Pregnancy-specific beta-glycoprotein-1 (PSG1)		Progestagen-associated endometrial protein (PAEP)	
	6-8 weeks	12-13 weeks	6-8 weeks	12-13 weeks
Subgroup of correction (n=25)	87,75±5,91*	244,50±16,47*,**	17,58±1,30**	77,87±5,35**
Subgroup with traditional treatment (n=24)	83,30±9,56*	130,55±16,07*	29,05±4,60	23,14±5,02*
Control group(n=30)	186,50±13,60	569,33±12,51	19,87±2,14	96,30±4,04

Note. \* - the difference between indexes is significant in comparison with control group, \*\* - the difference between indexes is significant in comparison with subgroup with traditional treatment

Table 3

**The parameters of the general potential of blood coagulation and of fibrinogen in women with the clinical signs of threatened abortion in the 1<sup>st</sup> trimester of gestation (M±m)**

	Fibrinogen, g/l		Time of re-calcification, sec		Prothrombin time, sec		Thrombin time, sec	
	6-8 weeks	12-13 weeks	6-8 weeks	12-13 weeks	6-8 weeks	12-13 weeks	6-8 weeks	12-13 weeks
Subgroup of correction (n=25)	3,31± 0,17	2,82± 0,18	92,15± 2,15	99,94± 1,81**	18,50± 0,38	20,05± 0 45 * **	16,80± 0,31	18,90± 0,35**
Subgroup with traditional treatment (n=24)	2,90± 0,12	3,16± 0,23	94,80± 1,29	91,95± 1,71*	19,45± 0,61	17,95± 0,51*	17,45± 0,56	16,85± 0,66
Control group(n=30)	2,89± 0,10	2,80± 0,12	95,03± 1,44	99,50± 1,03	19,50± 0,45	21,47± 0,44	16,30± 0,38	17,37± 0,36

Note. \* - the difference between indexes is significant in comparison with control group, \*\* - the difference between indexes is significant in comparison with subgroup with traditional treatment

development of the fetal egg. It is confirmed by comparative analysis of the correlations between indexes which characterize different factors of pathogenesis of the threat of abortion in women of examined groups. For example, in patients with pregnancy course complicated by threat of abortion we unveiled the next correlations between protein-synthesizing and hormonal sections of adaptation: the index of PSG1 has the direct correlation with level of cortisol ( $r=0,63$ ,  $p<0,05$ ) at the 12<sup>th</sup> – 13<sup>th</sup>

weeks of pregnancy; the level of PAEP in 12-13 gestational weeks was negatively linked with concentration of progesterone measured in 6-8 weeks of pregnancy ( $r= -0,56$ ,  $p<0,05$ ). Studying the integration between protein-synthesizing function of the trophoblast/placenta and system of hemostasis we've established that concentration of PSG1 has the negative correlation with the count of the thrombocytes per 1 ml ( $r= -0,50$ ,  $p<0,05$ ), with PAT ( $r= -0,58$ ,  $p<0,05$ ) and with ISAT ( $r= -0,60$ ,  $p<0,05$ ).

Table 4

The results of examination of the thrombocyte-related hemostasis in women with the clinical signs of threatened abortion in the 1<sup>st</sup> trimester of gestation (M±m)

	Count of the thrombocytes, 10 <sup>3</sup> /ml		Percent of the adhesive thrombocytes (PAT), %		Index of spontaneous aggregation of the thrombocytes (ISAT), %	
	6-8 weeks	12-13 weeks	6-8 weeks	12-13 weeks	6-8 weeks	12-13 weeks
Subgroup of correction (n=25)	301,10± 4,36*	274,45± 1,48**	43,45± 0,58*	38,73± 0,60*,**	4,68± 0,27**	3,50± 0,14**
Subgroup with traditional treatment (n=24)	297,15± 4,65*	300,55± 3,63*	43,59± 0,65*	43,25± 0,43	5,22± 0,30*	4,67± 0,25*
Control group(n=30)	268,87± 3,00	277,40± 2,13	40,60± 0,47	42,32± 0,43	3,03± 0,17	3,19± 0,11

Note. \* - the difference between indexes is significant in comparison with control group, \*\* - the difference between indexes is significant in comparison with subgroup with traditional treatment

Table 5

Peculiarities of histology of the placentas in women who had the clinical signs of threatened abortion in the 1<sup>st</sup> trimester of gestation (%)

	Subgroup of correction (n=48)	Subgroup with traditional treatment (n=42)
Vascular hyperplasia	29 60,427,06%*	8 19,046,06%
Thickening of the syncytium-capillary membranes	4 8,333,99%*	17 40,487,57%
Accumulation of the syncytial proliferative "nodes"	27 56,257,16%*	9 21,436,33%
Fibrosis of the stroma of villi	4 8,333,99%	9 21,436,33%
Decrease of the quantity of the terminal villi	3 6,253,49%	6 14,295,40%

Note. \* - the difference between indexes is significant in comparison with control group, \*\* - the difference between indexes is significant in comparison with subgroup with traditional treatment

In addition, the level of PAEP measured in 12-13 weeks of pregnancy has the negative correlation with the count of the thrombocytes per 1 ml ( $r = -0,55$ ,  $p < 0,05$ ) and PAT ( $r = -0,50$ ,  $p < 0,05$ ). Cortisol is considered to have the strongest influence on the thrombocyte-related hemostasis among the studied hormones. Its level had the negative correlation with the count of the thrombocytes per 1 ml ( $r = -0,52$ ,  $p < 0,05$ ) and PAT ( $r = -0,57$ ,  $p < 0,05$ ) in 12-13 weeks of pregnancy.

After completion of pregnancy the pathohistologic examination of postpartum placenta has been conducted. It was performed in all 48 women from subgroup of correction and in 42 women from subgroup with traditional treatment. 6 women (12,504,77%) from subgroup of traditional treatment had spontaneous abortions in pregnancy term 14-21 weeks. Changes of placental histology (table 5) were studied according to criteria of placental insufficiency by A.Milovanov [5].

It has been established that in women who received our therapeutic complex, thickening of the syncytium-capillary membranes was seen significantly less commonly ( $p < 0,05$ ). At the same time,

vascular hyperplasia and accumulation of the syncytial proliferative "nodes" were found more frequently in placentas of women in subgroup of correction ( $p < 0,05$ ). The mentioned signs are thought to be histological markers of compensatory reaction of the placental tissue due to hypoxia [5]. To put in sum, the diagnosis of placental insufficiency based on histological changes had been established in 13 cases (27,08±6,41%) from the subgroup of correction and in 27 cases (64,297,39%) from the subgroup with traditional treatment ( $p < 0,05$ ).

### Conclusions

1. The proposed complex of treatment of the threat of abortion in the 1<sup>st</sup> trimester of pregnancy leads to improvement of the function of the trophoblast at the 12<sup>th</sup> – 13<sup>th</sup> weeks of pregnancy. It manifests in significant increasing of the serum levels of progesterone, PSG1 and PAEP, in comparison with patients who received traditional therapy, as well as in normalization of the parameters of hemostasis.

2. Pregnancy-specific beta-glycoprotein-1 (PSG1) and progesterone-associated endometrial protein (PAEP) are the main factors of suppression of the pro-coagulant mechanisms (especially, activation of the thrombocytes) in case of the threatened abortion at the 1<sup>st</sup> trimester of gestation. Progressive decreasing of the serum concentrations of the mentioned proteins is linked with the dysfunction of placenta which is forming. In our opinion, it can cause appearance of more severe disorders in the system of regulation of aggregation of the blood, what might lead to impairment of the blood supply of the fetal egg.

3. Histological signs of placental insufficiency are seen less commonly in placentas of women who had the threat of miscarriage at the 1<sup>st</sup> pregnancy trimester and received proposed therapeutic complex, in comparison with women with traditional treatment. The proposed therapeutic complex is thought to increase compensatory reaction of the placental tissue in response to hypoxia.

#### Prospective of the further studies

The further studies provide the investigations of the levels of the PSG1 and PAEP in later pregnancy terms, as well as studying of the link between the levels of the mentioned proteins and condition of the newborns.

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#### ЗМІНИ ДЕЯКИХ ПОКАЗНИКІВ ГОМЕОСТАЗУ ТА ГІСТОЛОГІЧНІ ЗМІНИ ПЛАЦЕНТ У ЖІНОК ІЗ ЗАГРОЗОЮ ВИКИДНЯ У РАННІ ТЕРМІНИ ГЕСТАЦІЇ

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**Мета дослідження.** Вивчити значення плацентарних білків в патогенезі загрози викидня в І триместрі вагітності та їх зв'язок з системою гемостазу та патогістологічними змінами в післяпологових плацентах.

**Дизайн дослідження.** Обстежено 30 жінок із неускладненим перебігом вагітності (контрольна група) та 96 жінок з ознаками загрози невиношування в І триместрі (основна група). Вивчалися сироваткові рівні білків вагітності (тро-

фобластичного в-глікопротеїну - ТБГ, б-2-мікроглобуліну фертильності - АМГФ), гормонів крові (естрадіолу, прогестерону, кортизолу) та показники гемостазу. Для лікування невиношування використовували в складі терапевтичного комплексу екстракт гінкго білоба та ериніт. Проведено патогістологічне дослідження плацент після пологів. В дослідженні використано первинні дані.

**Результати.** Екстракт гінкго білоба та ериніт покращують функцію трофобласта в І триместрі, а ТБГ та АМГФ є головними чинниками, що перешкоджають розвитку порушень у системі гемостазу при невиношуванні. Згідно результатів патогістології, запропонований терапевтичний комплекс збільшує компенсаторну реакцію плацентарної тканини у відповідь на гіпоксію.

**Новизна.** Вивчаються зміни рівнів плацентарних білків, гормонів та параметрів гемостазу у вагітних в контексті загрози викидня в І триместрі в поєднанні з патогістологічним дослідженням плацент після пологів.

**Ключові слова:** загроза викидня, трофобласт, плацента, трофобластичний бета-1-глікопротеїн, альфа-2-мікроглобулін фертильності, гормони, гемостаз, гістологія, екстракт гінкго білоба, ериніт.

#### ИЗМЕНЕНИЯ НЕКОТОРЫХ ПОКАЗАТЕЛЕЙ ГОМЕОСТАЗА И ГИСТОЛОГИЧЕСКИЕ ИЗМЕНЕНИЯ ПЛАЦЕНТ У ЖЕНЩИН С НЕВЫНАШИВАНИЕМ В РАННИЕ СРОКИ ГЕСТАЦИИ

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**Цель исследования.** Изучить значение белков беременности в патогенезе угрозы выкидыша в I триместре гестации и их связь с системой гемостаза и патогистологическими изменениями в послеродовых плацентах.

**Дизайн исследования.** Обследовано 30 женщин с неосложненным течением беременности (контрольная группа) и 96 женщин с признаками угрозы выкидыша в I триместре (основная группа). Изучались сывороточные уровни белков беременности (трофобластического в-глицопротейна - ТБГ, б-2-микрoглобулина фертильности - АМГФ), гормонов крови (эстрадиола, прогестерона, кортизола) и показатели гемостаза. Для лечения невынашивания использовали в составе терапевтического комплекса экстракт гинкго билоба и эринит. Проведено патогистологическое исследование плацент после родов. В исследовании использованы первичные данные.

**Результаты.** Экстракт гинкго билоба и эринит улучшают функцию трофобласта в I триместре, а ТБГ и АМГФ являются главными факторами, предупреждающими развитие нарушений в системе гемостаза при невынашивании. Согласно результатам патогистологии, предложенный терапевтический комплекс увеличивает компенсаторную реакцию плацентарной ткани в ответ на гипоксию.

**Новизна.** В исследовании изучается значение показателей плацентарных белков, гормонов и параметров гемостаза у беременных в контексте угрозы выкидыша в I триместре, в соединении с патогистологическим исследованием плацент после родов.

**Ключевые слова:** угроза выкидыша, трофобласт, плацента, трофобластический бета-1-глицопротейн, альфа-2-микрoглобулин фертильности, гормоны, гемостаз, гистология, экстракт гинкго билоба, эринит.

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