The efficacy of complete blood count parameters in the diagnosis of tubal ectopic pregnancy

Skuteczność oznaczania parametrów morfologii w diagnostyce ciąży ektopowej jajowodowej

Fatma Eskicioğlu[1], Alper Tunga Özdemir[2], Gülüzar Arzu Turan[3], Esra Bahar Gür[3], Esin Kasap[3], Mine Genç[3]

[1] Celal Bayar University, School of Medicine, Department of Obstetrics and Gynecology, Manisa, Turkey
[2] Ege University, Institute of Health Sciences, Department of Stem Cell, Izmir, Turkey
[3] Şifa University, School of Medicine, Department of Obstetrics and Gynecology, Izmir, Turkey

Abstract

Objective: *Ectopic pregnancy (EP) is the major cause of maternal morbidity and is responsible for maternal mortality in the first trimester. In order to reduce undesirable results, it is necessary to find rapid and accurate, non-surgical diagnostic tests for EP. The goal of the study was to investigate the differences in complete blood count parameters between tubal EPs and healthy pregnancies in be used in the diagnosis of ectopic pregnancy.*

Study design: *White blood cell (WBC), neutrophil, monocyte, lymphocyte, platelet (PLT) counts, mean PLT volume (MPV) and PLT distribution width (PDW) levels in the complete blood count samples have been obtained from subjects with diagnosed tubal EP (n=78; study group) and women with healthy intrauterine gestations (n=79; control group). Statistical comparisons between groups were performed using the t test.*

Results: *PDW levels were found to be significantly higher in the control group than EP ($p<0.001$). However, no differences between the study and control groups with regard to PLT and MPV levels were observed. WBC levels were found to be significantly higher in the EP group as compared to controls ($p<0.001$). When leukocyte differentials were compared, monocyte counts in the EP group were significantly higher than in controls ($p=0.005$). No statistically significant differences in neutrophil and lymphocyte values were observed in either group.*

Conclusion: *PDW as an indicator of PLT activation is lower in tubal EP than intrauterine pregnancy so, possibly, endometrial invasion in the intrauterine pregnancy needs more PLT activation. Monocyte counts are higher in tubal EP, indicating that monocyte activation in the pathophysiology of EP could be effective in the formation of tubal motility and microenvironment regulation.*

Key words: **ectopic pregnancy** / **monocyte** / **platelet activation** / **leukocyte count** /

Address for correspondence:
Fatma Eskicioğlu
Dept. Of Ob&Gyn, Medical Faculty of Celal Bayar University, Manisa, Turkey 45050
Tel: +90(505)2595349 Fax:+90(236)2327462
e-mail: fatmaeskicioglu@gmail.com

Otrzymano: **26.02.2014**
Zaakceptowano do druku: **25.07.2014**

Streszczenie

Cel: *Ciąża ektopowa (EP) jest główną przyczyną śmiertelności matek w pierwszym trymestrze ciąży. Aby zmniejszyć niekorzystne wyniki tej choroby, konieczne jest znalezienie szybkiego i właściwego testu nieoperacyjnego służącego do rozpoznania EP. Celem badania była ocena różnic w wynikach morfologii pomiędzy pacjentkami z ciążą ektopową a zdrowymi ciężarnymi.*

Materiał i metoda: *W próbkach pełnej krwi zbadano następujące parametry: leukocyty (WBC), neutrofile, monocyty, limfocyty, płytki krwi (PLT), średnią objętość płytki krwi (MPV), szerokość rozdziału płytek (PDW) u pacjentek z rozpoznaną ciążą jajowodową (n=78, grupa badana) oraz u zdrowych ciężarnych (n=79, grupa kontrolna). Analiza statystyczna porównująca obie grupy została wykonana przy pomocy testu t.*

Wyniki: *PDW był istotnie wyższy w grupie kontrolnej niż w grupie EP (p<0,001). Jednak nie znaleziono różnic pomiędzy grupą badaną a kontrolną w odniesieniu do PLT i MPV. WBC było istotnie wyższe w grupie z EP niż w grupie kontrolnej (p<0,001). Odsetek monocytów był istotnie wyższy w grupie EP niż w grupie kontrolnej (p=0,005). Nie obserwowano istotnych różnic w odniesieniu do neutrofili i limfocytów w obu grupach.*

Wnioski: *PDW jako wskaźnik aktywacji płytek krwi jest niższy w ciąży ektopowej niż w ciąży wewnątrzmacicznej, gdyż prawdopodobnie inwazja ciąży w endometrium wymaga większej aktywacji PLT. Odsetek monocytów jest wyższy w ciąży jajowodowej co wskazuje na udział monocytów w procesie patofizjologicznym ciąży ektopowej, w perystaltyce jajowodu i regulacji jego mikrośrodowiska.*

Słowa kluczowe: **ciąża ektopowa** / **monocyty** / **aktywacja płytek krwi** / / **odsetek leukocytów** /

Introduction

Ectopic pregnancy (EP) is defined as the implantation of the fertilized ovum outside the uterine cavity. Approximately 98% of pregnancies implant in the fallopian tube [1]. Despite advances in contemporary medicine, EP remains an important cause of maternal morbidity and mortality worldwide due to misdiagnosis, incorrect or delayed treatment, resulting in failure to preserve fertility and minimize the associated morbidity. Therefore, proper diagnostic methods to reduce the undesirable consequences of EPs are needed [2].

Currently, the only biomarker used routinely in clinical practice is human chorionic gonadotropin (hCG). A single hCG measurement has limited usefulness. Therefore, hCG levels usually require a follow-up. Several serum biomarkers such as creatine kinase, progesterone, inhibin A, estradiol, relaxin, CA125 are currently being investigated as diagnostic parameters for faster and more accurate diagnosis of EP [3]. Leukocytosis is observed physiologically during a normal intrauterine pregnancy. The increase in the neutrophil number is especially remarkable [4]. In addition, dilutional thrombocytopenia, secondary to increased intravascular volume and compensatory increase in mean platelet volume (MPV), is also noted [5]. MPV is a simple platelet (PLT) index and the combined use of MPV and PLT distribution width (PDW), a more specific marker of PLT activation, could predict activation of PLT more efficiently [6]. An increase in the MPV, PDW levels and leukocyte counts, especially neutrophil numbers, is more noticeable in preeclampsia characterized by abnormal placental invasion and exaggerated inflammatory response compared to a normal healthy pregnancy [5, 7, 8]. EP increases inflammatory response, with angiogenesis, vascular permeability and immune response participating in the process [3]. Leukocytes, PLTs, and cytokines are involved in these mechanisms [3, 9, 7].

Early diagnosis of EP, obtained with the use of rapid and accurate tests, is necessary to eliminate the risk of mortality and preserve fallopian tube function and fertility [10].

The search for novel biomarkers which are capable of rapid diagnosis of EP, or support the existing tests to obtain accurate results, continues [3]. We found only one study investigating the leukocyte count and MPV in EP [9].

To the best of our knowledge, there are no reports on alteration in leukocyte subtypes and PDW in EP. The purpose of our study was to find the differences in the white blood count (WBC), subgroups, PLT, MPV and PDW levels between EPs and healthy pregnancies in order to accelerate the diagnosis of ectopic pregnancy.

Materials and methods

The study was approved by the Ethics Committee of Celal Bayar University. Seventy-eight women, operated on due to tubal EP and with the diagnosis confirmed by conclusive pathological results, constituted the study group. The control group consisted of 79 women at 6-8 weeks of healthy, intrauterine gestations confirmed by an ultrasound examination (positive fetal heart rates). The exclusion criteria were: unstable hemodynamic status, chronic inflammatory diseases, renal, cardiac or liver disease, oral contraceptives which might affect the parameters of coagulation, permanent use of anti-coagulants, smoking, hemoglobinopathy, anemia and coagulopathies.

Demographic data of 157 participants (age, gravidity including the current pregnancy, parity, abortions, the number of living children and gestational age) were collected. After admission, the time of diagnosis, WBC, neutrophil, monocyte, lymphocyte, PLT counts, MPV and PDW levels were evaluated in the complete blood count samples, drawn into vacutainer 2mL volume tubes containing 3.6 mg K2 EDTA. Blood samples were analyzed within one hour since sampling with a commercially available analyzer (Hematology analyzer Cell Dyn 3700, Abbott Laboratories, Abbott Park, Illinois, USA).

Statistical analysis

The statistical package SPSS for Windows 15.0 (Statistical Package for Social Sciences; SPSS Inc., Chicago, IL) was used to analyze the data. Statistical comparisons between groups were performed using the t test. Mean and standard deviations were used to describe data. P values <0.05 were accepted as significant.

Results

The median of the gestational age was 6.5 weeks (mean 6.5±0.8) in the control group. Demographic data for both groups were shown in Table I. Age, gravidity, parity, the number of abortions and of living children were found to be higher in the EP group as compared to controls.

MPV, PDW, PLT, WBC, neutrophil, monocyte, lymphocyte counts were shown in Table II. PDW levels were significantly higher in controls than the study group (p<0.001). There were no differences between EP and the control group in terms of PLT and MPV levels (p=0.26 and p=0.79). WBC levels were significantly higher in the EP group than controls (p<0.001). When leukocyte differentials were compared, monocyte counts in the EP group were significantly higher than in the control group (p=0.005). No statistically significant difference was observed between the groups in terms of neutrophil and lymphocyte values (p=0.06 and p=0.14).

Discussion

EP is a major cause of maternal morbidity and is responsible for high maternal mortality in the first trimester [10]. In the United States, the incidence of EP is estimated at 1-2%.

Since the 1980's, a significant decrease in mortality has been noted, from 1.15/100.000 deaths between 1980–1984 to 0.50/100.000 deaths between 2003 and 2007 [11]. Further reduction of the mortality rates due to ectopic pregnancy is possible with rapid diagnosis [3]. Alas, non-surgical tests for rapid and precise diagnosis of EP are unavailable. Contemporary ultrasonography and blood tests are used but they are able to establish the final diagnosis after a long-term follow-up and numerous visits. Delayed EP diagnosis results in morbidity, by damaging the oviduct entirety and negatively affecting the fertility, and mortality, by causing rupture and hemorrhage [10].

Currently, hCG is the only biomarker used in clinical practice. A single hCG measurement has limited usefulness. Therefore, hCG test should be accompanied by a pelvic ultrasound. The doubling time of hCG should be followed in the period when the pregnancy cannot be seen on ultrasound. However, even observing serial levels has its limitations as the expected minimum rate of increase during 48 hours in a viable pregnancy varies in reports from 35% to 66% [12]. Despite the limitations, serial hCG levels in combination with transvaginal ultrasound is the most commonly used clinical method for identifying patients at the highest risk for EP [3]. In recent years, numerous biochemical markers such as maternal serum creatine kinase, pregnancy-associated plasma protein C, relaxin, Ca 125, maternal serum α-fetoprotein, C-reactive protein, and vascular endothelial growth factor (VEGF), have been studied in the hope of establishing the final EP diagnosis more quickly. However, a test which (if combined with hCG and ultrasonography) might increases the velocity and precision of the diagnostic process, has not been identified yet [3].

Table I. Demographic data in ectopic pregnancy (EP) and control groups (mean±SD, Standard Deviation)

	EP (n=78)	Control (n=79)	*P* value
Age	30.65±6.07	26.39±6.21	<0.001
Gravidity	3.47±1.96	1.88±1.42	<0.001
Parity	1.55±1.35	0.65±0.97	<0.001
Abortions	0.69±1.04	0.22±0.65	0.001
Living children	2.12±5.96	0.54±0.93	0.022

Table II. Platelet (PLT), mean platelet volume (MPV), platelet distribution width (PDW) white blood cell (WBC), neutrophil, monocyte and lymphocyte levels in ectopic pregnancy (EP) and control groups (mean±SD, Standard Deviation)

	EP (n=78)	Control (n=79)	*P* value
PLT (K/uL)	256.64±92.11	270.58±62.92	0.269
MPV (fL)	8.38±0.97	8.69±1.14	0.79
PDW	11.55±1.78	16.36±3.00	<0.001
WBC (K/uL)	11.53±5.18	8.39±1.66	<0.001
Neutrophil (uL)	7.73±4.74	5.88±7.36	0.064
Monocyte (uL)	1.38±0.95	0.90±1.15	0.005
Lymphocyte (uL)	2.42±0.69	2.25±0.62	0.14

In our study, we aimed to find a connection helping to discriminate between ectopic and intrauterine pregnancies in complete blood count parameters, which are frequently used as routine tests in clinical practice of care in pregnancy. To the best of our knowledge, the literature offers only one report on the topic in question [9]. Our control group consisted mainly of primiparas, so there were differences between the demographic data of the groups. The analysis of PLT and MPV values revealed no statistically significant differences between the groups. PDW values were significantly lower in the EP group. WBC count was significantly higher in the EP group. In leukocyte differential analysis, neutrophil, lymphocyte and monocyte counts were higher in the EP group as compared to controls, although statistical significance was determined only with regard to the monocyte number. While Turgut et al. [9], found similar correlations in WBC numbers in EPs, they detected higher MPV values in the EP group, what was different from our findings. In the literature, results of MPV values in pregnancy are contradictory. Numerous studies about preeclamptic patients with abnormal course of pregnancy, characterized by abnormal placental invasion, genetic disposition, immunologic or severe inflammatory responses, have been published [5, 8, 13]. Jaremo et al. [5], observed lower PLT counts and higher MPV values in preeclamptic patients as compared to healthy pregnancies, while Ceyhan et al. [13], did not report any differences between the two groups. The literature offers different results also for WBC numbers in the studies of preeclamptic pregnancies [7, 13]. Bernard et al. [7], determined an increase in leucocyte numbers in pregnancies with preeclampsia depending on elevated neutrophil level, while Ceyhan et al. [13], did not observe any differences. A physiological increase in the WBC count during pregnancy is a well-known fact [4]. Leukocyte levels during pregnancy vary between 5000-12000/μL [14].

In our study, the values were within that range. Leukocytosis is a result of an ongoing inflammatory process [7]. Leukocyte activation and various immune mediators such as adhesion molecules which are expressed from them, feature in the course of pregnancy, from implantation onward [15]. There is also an increase in the count of large granular lymphocytes which are thought to originate from the bone morrow in the mid-luteal phase, during which implantation occurs. These cells secreting granulocyte/macrophage stimulating factor (GM-CSF) are notably active and help trophoblastic invasion [16].

The etiology of EP remains unknown. It is claimed to be caused by the changes which allow retention in tubal transport of the embryo and implantation in tubal environment [17]. Mechanisms like angiogenesis activated with hypoxia, muscle cell damage and inflammatory process are believed to play a part in tubal environment changes. Elevated creatine kinase levels as the marker of smooth muscle damage have been observed in EP cases invasive of the tubal wall. Although fallopian tubes are not as rich as the uterus in terms of vascularization, the occurrence of implantation has been attempted to be explained via the activation of VEGF which is a marker of angiogenesis. There is a positive correlation between serum VEGF and beta-hCG levels [18]. It is justified by the fact that inflammatory cytokines like interleukin (IL)-6, IL-8 and tumor necrosis factor (TNF)-alpha also increase in EP and they are able to identify EP with 100% specificity and 52.9% sensitivity. An increase in the number of leukocytes supposed to take part in this inflammatory process is an expected result [9].

Since we were not able to find a study examining neutrophil, monocyte and lymphocyte numbers in EP in the literature, we were forced to rely only on the studies of intrauterine pregnancies and preeclamptic patients. Pitkin and Witte [4], demonstrated neutrophil numbers to be two times higher in pregnancy than the postpartum period and although monocyte numbers also showed a tendency to increase during pregnancy, there has been a decrease in the number of lymphocytes. Among others, the inflammatory process is an underlying mechanism of preeclampsia. The literature offers reports on elevated leukocyte numbers related to neutrophil count in preeclampsia as compared to normal healthy pregnancies. However, some studies demonstrated no differences in leukocyte counts [18, 13]. When we evaluated the leukocyte subunits, even though we observed elevated lymphocyte and neutrophil counts, we also noted a major statistically significant increase in the monocyte count, what could be linked to the increase of GM-CSF released by uterine large granular lymphocytes which take part in the implantation process. GM-CSF causes cell proliferation, maturation and monocyte activation and it also increases secretion of inflammatory mediators like IL-1 and TNF [19]. Release of mononuclear phagocyte from bone marrow rises during the inflammation process in EP. Proper recruitment and differentiation of macrophages play key roles in the development of EP. Monocyte recruitment, differentiation and function in the reproductive tract are regulated by numerous unique, and having substantial reproductive functions, cytokines like leukemia inhibitory factor, colony-stimulating factor 1 and transforming growth factor-b, GM-CSF. Cytokine types and levels are modulated by estrogen, progesterone and seminal plasma. Immunosuppressed trophic and scavenging macrophages regulate tubal motility by producing prostaglandin and increasing progesterone secretion [20]. Results of an investigation by Tonello and Poli [20], support the elevated monocyte level detected in our study by indicating that macrophages, which are the forms of monocytes in tissue, play a tissue remodeling and immune-regulatory role, leading to motility and receptivity in tuba.

In complete blood count repeated regularly in pregnancy care, the PLT counts decrease during the third trimester depending on the increase of plasma volume [21]. PLT volume, rather than PLT count, indicates PLT activation and raised production [5, 13]. Soluble factors released from active PLTs increase the invasion capacity of the trophoblast. In this way, PLTs enable maternal spiral arteries to transform into low-resistance large-caliber veins [22]. PLT activation factor (PAF) released from thrombocytes takes an important place in fertility physiology. PAF, whose secretion increases via progesterone and prostaglandin E2, behaves as paracrine by inducing the interaction of hypothalamus and stromal-epithelium cell in the uterus [23]. PDW and MPV are easily measured PLT indices, which increase during PLT activation. PLTs change in shape to obtain a larger surface during activation. An increase in both MPV and PDW due to PLT activation, resulting from PLT swelling and pseudopodia formation, has been suggested. PDW is a more specific marker of PLT activation since it does not increase during simple PLT swelling [6]. In the literature, there are many studies trying to relate elevated values of MPV and PDW to preeclampsia, which is a hypertensive disease of pregnancy [5, 8, 13]. The idea that increased levels of MPV values point to a probable risk of

preeclampsia was defended in some studies [5]. However, there are studies which do not support that connection [13].

Differences in measuring technology and substrates used as anticoagulants are among the reasons for these contradictory results. Generally, EDTA is used in clinical practice as the most common anticoagulant in blood count. When EDTA is used for anticoagulation instead of sodium citrate, MPV values increase depending on time. EDTA-induced PLT shape changes lead to progressive increase in MPV [5, 13]. Combined analysis of MPV and PDW is more effective in evaluating PLT activation [6]. No changes in MPV values were detected in our study. PDW, which is a stronger sign of PLT activation, was lower in EP, what allowed us to conclude that less PLT activation is needed in EP implantation than in the uterine cavity invasion. In intrauterine pregnancy, its trophoblastic growth and differentiation are realized by cytokines which are released from the trophoblasts, endometrium and decidual stromal cells [24]. However, lack of decidualization is a characteristic feature of EP, unlike in intrauterine pregnancies [25]. Thus, EP invasion may be rather limited when compared to intrauterine pregnancies and it needs less PLT activation.

As a consequence, significant differences in PDW values, which is an indication of PLT activation, lead us to think that EP necessitates less PLT activation when compared to intrauterine pregnancy, which needs endometrial invasion. Elevated monocyte levels that we detected in EP may show that the role of monocyte activation in the etiopathogenesis of EP consists in regulation of motility and ideal micro environment. Complete blood count parameters might accelerate the diagnosis of EP and prevent maternal deaths and adverse results. Further studies on the possible links and correlations between EP and each of PDW and monocyte numbers, which might be applied in the diagnosis of EP, are needed.

Authors' Contribution:

1. Fatma Eskicioğlu – concept, study design, acquisition of data, analysis and interpretation of data, article draft, writing article, corresponding author.
2. Alper Tunga Özdemir – concept, article draft, study design, acquisition of data, revised article critically.
3. Gülüzar Arzu Turan – analysis and interpretation of data, writing article, revised article critically.
4. Esra Bahar Gür – revised article critically, writing article.
5. Esin Kasap – revised article critically.
6. Mine Genç – revised article critically.

Authors' statement

- This is to certify, that the publication will not violate the copyrights of a third party, as understood according to the Act in the matter of copyright and related rights of 14 February 1994, Official Journal 2006, No. 90, Clause 63, with respect to the text, data, tables and illustrations (graphs, figures, photographs);
- there is no 'conflict of interests' which occurs when the author remains in a financial or personal relationship which unjustly affects his/her actions associated with the publication of the manuscript;
- any possible relationship(s) of the author(s) with the party/parties interested in the publication of the manuscript are revealed in the text of the article;
- the manuscript has not been published in or submitted to any other journal.

Source of financing: None.

- There is no 'conflict of interests' which occurs when the author remains in a financial or personal relationship which unjustly affects his/her actions associated with the publication of the manuscript.
- Source of financing: The authors received no financial support for the research, authorship, and/or publication of this article.

References

1. Sivalingam VN, Duncan WC, Kirk E, [et al.]. Diagnosis and management of ectopic pregnancy. *J Fam Plann Reprod Health Care.* 2011, 37 (4), 231-240.
2. Orazulike NC, Konje JC. Diagnosis and management of ectopic pregnancy. Women's Health (Lond Engl). 2013, 9 (4), 373-385.
3. Rausch ME, Barnhart KT. Serum biomarkers for detecting ectopic pregnancy. *Clin Obstet Gynecol.* 2012, 55 (2), 418-423.
4. Pitkin RM, Witte DL. Platelet and leukocyte counts in pregnancy. *JAMA.* 1979, 242 (24), 2696-2698.
5. Järemo P, Lindahl TL, Lennmarken C, Forsgren H. The use of platelet density and volume measurements to estimate the severity of pre-eclampsia. *Eur J Clin Invest.* 2000, 30 (12), 1113-1118.
6. Vagdatli E, Gounari E, Lazaridou E, [et al.]. Platelet distribution width: a simple, practical and specific marker of activation of coagulation. *Hippokratia.* 2010, 14 (1), 28-32.
7. Canzoneri BJ, Lewis DF, Groome L, Wang Y. Increased neutrophil numbers account for leukocytosis in women with preeclampsia. *Am J Perinatol.* 2009, 26 (10), 729-732.
8. Rosevear SK, Liggins GC. Platelet dimensions in pregnancy-induced hypertension. *N Z Med J.* 1986, 99 (802), 356-357.
9. Turgut A, Sak ME, Ozler A, [et al.]. Alteration of peripheral blood cells in tubal ectopic pregnancy. *Ginekol Pol.* 2013, 84 (3), 193-196.
10. Barnhart KT. Clinical practice. Ectopic pregnancy. *N Engl J Med.* 2009, 361, 379–387.
11. Bachman EA, Barnhart K. Medical management of ectopic pregnancy: a comparison of regimens. *Clin Obstet Gynecol.* 2012, 55 (2), 440-447.
12. Cartwright J, Duncan WC, Critchley HO, [et al.]. Serum biomarkers of tubal ectopic pregnancy: current candidates and future possibilities. *Reproduction.* 2009, 138, 9–22.
13. Ceyhan T, Beyan C, Başer I, [et al.]. The effect of pre-eclampsia on complete blood count, platelet count and mean platelet volume. *Ann Hematol.* 2006, 85 (5), 320-322.
14. Taylor DJ, Phillips P, Lind T. Puerperal hematological indices. *Br J Obstet Gynaecol.* 1981, 88 (6), 601-606.
15. Gearing AJ, Hemingway I, Pigott R, [et al.]. Soluble forms of vascular adhesion molecules, E-selectin, ICAM-1, and VCAM-1: pathological significance. *Ann N Y Acad Sci.* 1992, 667, 324-331.
16. Johnson PM, Christmas SE, Vince GS. Immunological aspects of implantation and implantation failure. *Hum Reprod.* 1999,14 Suppl 2, 26-36.
17. Shaw JL, Dey SK, Critchley HO, Horne AW. Current knowledge of the aetiology of human tubal ectopic pregnancy. *Hum Reprod Update.* 2010, 16 (4), 432-444.
18. Rausch ME, Barnhart KT. Serum biomarkers for detecting ectopic pregnancy. *Clin Obstet Gynecol.* 2012, 55 (2), 418-423.
19. Khatami S, Brummer E, Stevens DA. Effects of granulocyte-macrophage colony stimulating factor (GM-CSF) in vivo on cytokine production and proliferation by spleen cells. *Clin Exp Immunol.* 2001,125 (2), 198-201.
20. Tonello A, Poli G. Tubal ectopic pregnancy: macrophages under the microscope. *Hum Reprod.* 2007, 22 (10), 2577-2584.
21. Edelstam G, Löwbeer C, Kral G, [et al.]. New reference values for routine blood samples and human neutrophilic lipocalin during third-trimester pregnancy. *Scand J Clin Lab Invest.* 2001, 61 (8), 583-592.
22. Sato Y, Fujiwara H, Konishi I. Role of platelets in placentation. *Med Mol Morphol.* 2010, 43 (3), 129-133.
23. Harper MJ. Platelet-activating factor: a paracrine factor in preimplantation stages of reproduction? *Biol Reprod.* 1989, 40 (5), 907-913.
24. Saito S. Cytokine cross-talk between mother and the embryo/placenta. *J Reprod Immunol.* 2001, 52 (1-2), 15–33.
25. Goffin F, Munaut C, Malassiné A, [et al.]. Evidence of a limited contribution of feto-maternal interactions to trophoblast differentiation along the invasive pathway. *Tissue Antigens.* 2003, 62 (2), 104-116.