

Original Research

The Relationship between First Trimester Serum Progesterone Levels and Spiral Artery Doppler Findings with Adverse Perinatal Outcomes

Ramazan Adan^{1,*}, Hicran Acar Şirinoğlu², Fatih Şahin¹, Savaş Özdemir¹¹Department of Obstetrics and Gynecology, Prof. Dr. Cemil Taşcıoğlu City Hospital, 34384 Istanbul, Turkey²Department of Perinatology, Prof. Dr. Cemil Taşcıoğlu City Hospital, 34384 Istanbul, Turkey*Correspondence: r.adan187.ra@gmail.com (Ramazan Adan)

Academic Editor: Michael H. Dahan

Submitted: 5 February 2024 Revised: 14 March 2024 Accepted: 29 March 2024 Published: 27 May 2024

Abstract

Background: Progesterone is essential for maintaining pregnancy. Spiral arteries, providing blood flow to the placenta, undergo changes in early pregnancy, reducing uteroplacental resistance. Our study was aimed at investigating the relationship between the first trimester serum progesterone levels, spiral artery Doppler findings and pregnancy outcome. **Methods:** This observational retrospective cohort study included 126 pregnant women at 11–14 weeks of gestation, confirmed by last menstrual period and ultrasonography. Between 11–14 weeks, serum progesterone levels were measured and spiral artery pulsatility index (PI), resistive index (RI), peak systolic velocity (PSV) and systolic/diastolic ratio (S/D) values were recorded. Pregnant women were followed up until delivery, and serum progesterone levels and spiral artery Doppler findings were compared between patients with poor perinatal outcomes and others. **Results:** This study included 126 pregnancies, of which 13 patients were lost to follow-up. Miscarriage occurred in 2 of the pregnant women, stillbirth in 1, intrauterine growth retardation in 10 and preeclampsia in 5 of them. 6 infants developed respiratory problems and 3 infants developed jaundice. Seventeen of the infants were hospitalized in the neonatal intensive care unit. There was no statistically significant difference between serum progesterone, spiral artery PI, RI and S/D values of 86 uncomplicated and 27 complicated pregnant women. **Conclusions:** It is known that there is a strong relationship between the first trimester serum levels of progesterone, which is an important hormone for the healthy continuation of pregnancy, and the risk of miscarriage. The quantity and quality of placental and fetal circulation is determined by the changes that occur in the spiral arteries with pregnancy. Although a close relationship is known between both variables and pregnancy outcomes, no correlation could be established between them in terms of early detection of poor perinatal outcomes.

Keywords: progesterone; spiral artery; Doppler; perinatal outcomes

1. Introduction

Progesterone is secreted from the corpus luteum in the luteal phase and prepares the endometrium for blastocyst implantation, reduces and inhibits maternal immune response against fetal semi-allograft, maintains decidualization [1–3]. Circulating levels of progesterone rise during pregnancy; the major origin is the corpus luteum until just about week 8 of pregnancy, and the placenta thereafter. One of the major mechanisms of progesterone action in maintaining pregnancy is inhibition of the contractions of the myometrium [4]. Sufficient oxygen and nutrient supply are another important factor that determines proper embryo development [5]. Efficient blood transport depends on an adaptive capacity of the uterine vascular system. Arterial blood is transported to the uterus by way of the uterine and ovarian arteries. The ascending branch of the uterine artery is an essential source of the blood supply to the embryo. The spiral arteries compose the terminal portions of the uterine vasculature and penetrate the implantation and placental site [6]. The spiral artery, which provides endometrial blood supply, undergoes remodeling in the early stages of pregnancy and thus uteroplacental resistance is significantly reduced. As a result, blood flow to the placenta

increases significantly [7,8]. These changes in the spiral artery can be evaluated by ultrasonography, and normal or abnormal findings can be recognized [9,10]. Detection of the relationship between abnormal spiral artery findings and negative perinatal outcomes may create a chance for close follow-up and timely medical intervention for pregnancies at risk [11]. Various studies have been conducted to determine how valuable progesterone serum levels are in terms of predicting perinatal outcomes, and normal progesterone levels have been tried to be determined. As a result, it has been revealed that serum progesterone levels differ according to trimester and have wide ranges that cannot be clinically useful in predicting perinatal outcomes [12,13]. Maternal factors that may affect serum progesterone levels such as body mass index (BMI), age, parity, smoking and ethnicity may also be effective in this [14]. It has been suggested that pregnancies with low serum progesterone levels and a high risk of miscarriage may benefit from progesterone therapy [15]. Progesterone can increase uteroplacental circulation by decreasing the pulsatility index and resistive index of spiral arteries with its effects on vascular structures. When Roberts *et al.* [16] detected maternal blood flow to the placenta at the 6th ges-



tational week, they revealed that this flow did not increase significantly until the 13th week. As a result of the significant increase observed in the 13th week, changes were also detected in spiral artery Doppler findings. Changes in the spiral artery are extremely important for the course of pregnancy. As a result of a deficiency or disorder that may occur in this remodeling, high-speed maternal blood flow and oxidative load may occur to the placenta. The spiral arteries of the multiparous uterus are partially replaced by the connective tissue of the smooth muscle layer, with fragmentation and duplication of the elastica. Therefore, these persistent changes are thought to reduce uteroplacental resistance [17]. Mechanical-biochemical trophoblastic damage and increased apoptosis are observed in the placenta [18]. Placenta is a temporary organ connecting the fetus and her mother. The trophoblast is the main cellular component of the placenta. The normally functioning trophoblast is crucial for the establishment and maintenance of pregnancy, development of the placenta, remodeling of the spiral artery, and subsequently fetal growth and maturation [17]. Dysregulation of trophoblasts is associated with pregnancy complications including early pregnancy loss (EPL) and preeclampsia [18]. The function of trophoblasts is regulated by a group of genes, especially placenta-specific genes including placenta-specific protein 1 (PLAC1), syncytin and galectins. These genes regulate trophoblastic migration, invasion, proliferation, apoptosis, and metabolism [19].

There is no study in the literature that specifically examines the relationship between first trimester progesterone levels, endometrial flow and pregnancy outcomes. In this study, we aim to analyse the relationship between serum progesterone levels, endometrial flow and pregnancy outcome.

2. Materials and Methods

This observational retrospective study included singleton pregnant women aged 18–39 years, who underwent routine first-trimester prenatal examination (11–13 weeks + 6 days) between May 2019 and December 2019 at a tertiary hospital. In this study, historical records of progesterone levels, spiral artery Doppler findings and pregnancy outcomes of normal pregnant women who came to the clinic for the first trimester screening test and had no complaints were evaluated. Multiple pregnancies, pregnancies with fetal anomalies, pregnancies using hormone replacement therapy or assisted reproductive techniques were excluded from the study. Ethical documents, including protocols in accordance with the Declaration of Helsinki, were approved by the Istanbul Prof. Dr. Cemil Taşcıoğlu City Hospital Clinical Research Ethics Committee (Approval number: 2021/386) and written informed consent was obtained from all patients. In the hospital laboratory progesterone levels individual blood samples are collected into plain tubes and centrifuged for 10 minutes at 3000

g within 2 hours of collection. Samples are stored at -4°C before testing. The serum progesterone level is then measured in the laboratory on the Cobas® 8000 Modular Analyzer (<https://diagnostics.roche.com/tr/tr/products/systems/cobas-8000-analyzer-series-sys-128.html>) according to the manufacturer's instructions. The analytical (linear) detection range was 0.05–60 ng/mL for progesterone. The minimum detection limit was 0.05 ng/mL. The reported intra-assay and inter-assay coefficients of variability (CVs) were 2.3% and 3.2% respectively. All spiral artery Doppler examinations were performed with a 1.2–6 MHz convex abdominal probe with Mindray Resona 7 device (<https://www.mindray.com/na/products/ultrasound/womens-health/resona-7/>) and by Perinatologist Hicran Acar Sirinoglu. The measurements were made similarly in each case, between 11–14 weeks, in a quiet room, with the patient in the supine position and breathing levels were kept steady to prevent noise and artifacts. The methods described by Stevenson *et al.* [20] were used for spiral artery detection and measurements.

Statistical Methods

Statistica 13.3.1 (TIBCO Software Inc., Palo Alto, CA, USA) was used for the statistical evaluation of the data obtained in this study. The Shapiro-Wilk test was used to determine whether the data was distributed in accordance with the normal distribution. Normally distributed numerical variables were summarized as mean $\pm$ standard deviation, and non-normally distributed numerical variables were summarized with median [min.–max.] values. Categorical variables were summarized as numbers and percentages. The relationship between continuous variables was examined using the Spearman correlation coefficient. In the comparison of two groups, Student's *t* test was used when the distribution assumption was met, and Mann Whitney U test was used if the assumption was not met, $p < 0.05$ was accepted as statistical significance level. In the comparison of more than two groups, analysis of variance (ANOVA) was used when the assumption was met, and the Kruskal Wallis test was used if it was not.

3. Results

This study included 113 pregnancies, of which 13 patients were lost to follow-up. It was observed that 27 out of 113 pregnant women experienced problems in the perinatal and prenatal periods. 2 pregnancies were resulted in miscarriage. In addition, intrauterine growth retardation was found in 10 pregnant women, stillbirth in 1 pregnant, and preeclampsia in 5 pregnant women during pregnancy follow-up and perinatal period. Respiratory problems developed in 6 infants and jaundice in 3 infants. As a result of these, 17 of the newborns were hospitalized in the intensive care unit (1–60 days). Miscarriage is defined as pregnancy losses within 20 weeks of gestation. Intrauterine growth retardation was diagnosed in cases with a fetal weight that is below the 10th percentile for gestational age

Table 1. 11–14 weeks measurements, perinatal results and demographic finding.

	Complicated				Uncomplicated				<i>p</i>
	N Valid	Median	Minimum	Maximum	N Valid	Median	Minimum	Maximum	
Age (years)	86	28.00	19.00	39.00	27	32.00	19.00	39.00	0.122
Pregnancy week (weeks)	86	12.00	11.00	14.00	27	12.00	11.00	14.00	0.015
Progesteron (ng/mL)	86	29,000.00	14.80	52.90	27	28,000.00	16.80	40.80	0.527
Spiral artery RI	86	0.78	0.48	0.96	27	0.82	0.57	1.70	0.531
Spiral artery PI	86	1.53	0.99	2.44	27	1.58	0.97	2.42	0.749
S/D	86	3.07	2.08	4.14	27	3.12	2.15	4.13	0.721
Birth weight (Gr)	86	3300.00	2500.00	5180.00	25	2600.00	930.00	4000.00	0.001
Birth week (weeks)	86	39.00	34.00	41.00	27	37.00	11.00	41.00	0.001
Mother height (cm)	86	160.00	145.00	173.00	27	158.00	146.00	170.00	0.075
Mother weight (kg)	86	75.00	55.00	105.00	27	69.00	55.00	89.00	0.018
Gravide	86	2.00	1.00	6.00	27	2.00	1.00	7.00	0.365
Parite	86	1.00	0.00	3.00	27	1.00	0.00	3.00	0.314
Apgar 1	86	8.00	6.00	8.00	25	5.00	3.00	8.00	<0.001
Apgar 5	86	9.00	7.00	9.00	25	8.00	1.00	9.00	<0.001

RI, resistive index; PI, pulsatility index; S/D, systolic/diastolic ratio. There is a statistically significant difference between the groups for the *p* values I have colored in bold. Although the values for the week of pregnancy are the same, the reason for the difference is that the statistics we call mean rank are used when making comparisons. Mean rank in the complicated group for gestational age = 60.85; In the non-complicated group, mean rank = 44.

Table 2. The relationship between serum progesterone levels, spiral artery Doppler measurement values and perinatal outcomes.

Variables	Perinatal Outcome		<i>p</i>
	Complicated (n = 27)	Noncomplicated (n = 86)	
	Median [min.–max.]	Median [min.–max.]	
Progesterone (ng/mL)	28.61 [16.80–40.80]	29.68 [14.80–52.90]	0.492
RI spiral artery	0.82 [0.56–1.70]	0.78 [0.48–0.96]	0.606
PI spiral artery	1.58 [0.97–2.42]	1.52 [0.99–2.44]	0.714
S/D	3.12 [2.15–4.13]	3.07 [2.08–4.14]	0.709

RI, resistive index; PI, pulsatility index; S/D, systolic/diastolic ratio.

as determined through an ultrasound [21]. Pregnancies with high blood pressure (140/90 mmHg) that develops during pregnancy or during the postpartum period that is associated with overt protein in the urine or the new development of decreased blood platelets [22]. No problem was encountered in 86 of 113 pregnant women and they were recorded as non-complicated pregnancies with normal results. The study was conducted in accordance with the Principles of the Declaration of Helsinki.

The characteristics of the patients, including pregnancy outcomes and the results of serum progesterone levels, spiral artery Doppler finding are shown in Table 1. Serum progesterone and spiral artery Doppler (resistive index (RI) spiral artery, pulsatility index (PI) spiral artery and systolic/diastolic ratio (S/D)) findings of 27 complicated and 86 non-complicated pregnancies were compared. There is no statistically significant difference between serum progesterone levels and spiral artery Doppler measurement values and perinatal outcomes (Table 2).

4. Discussion

Our study was aimed to investigate the relationship between the first trimester (11–14 weeks) serum progesterone levels, spiral artery Doppler values and pregnancy outcomes. There was no relationship detected between first trimester progesterone levels, spiral artery Doppler values and pregnancy outcomes.

Several studies have revealed that the decrease in progesterone levels in the first trimester poses a risk for a healthy pregnancy. There is currently no definitive value for diagnosing progesterone deficiency and there is no consensus on treatment in pregnant women with progesterone deficiency [23]. Some studies indicate that low serum progesterone was associated with miscarriage [24–28]. For this reason, exogenous progesterone supplements are often used to treat the threat of abortion [25]. However, the benefit of progesterone therapy is currently very controversial [26–28].

In the studies conducted by Özkan *et al.* [11] and Özkaya *et al.* [29] comparing spiral artery Doppler findings with fetal perinatal outcomes, no significant correlation was found between spiral artery PI and RI values and fetal perinatal outcomes. In a study by Makikallio *et al.* [30] they compared the results of 10 women with preeclampsia and preterm labor and 31 control group pregnant women (6th, 8th, 9th and 11th week values), and they could not find a significant difference between the spiral artery RI values between the two groups [30]. In another study first-trimester uterine artery pulsatility index (UtA-PI) and uterine artery resistive index (UtA-RI) trajectories were not significantly different between pregnancies after frozen-thawed embryo transfer (ET), fresh ET and after natural conception in both models [31]. A study by Pillai *et al.* [32] also suggests that the most commonly used biomarkers of serum human chorionic gonadotropin (HCG) and progesterone are not useful in predicting the outcome of a pregnancy with a live fetus. The fact that normal first trimester progesterone levels do not predict negative pregnancy outcomes may be related to the higher blood progesterone level of the therapeutic aspect of progesterone. But it does not explain the different results in patients who received progesterone therapy. A study is related to the fact that normal first trimester blood progesterone levels do not differ between positive and negative pregnancy outcomes. Low serum levels such as dual of estradiol and progesterone or estradiol alone at 7–9 weeks, β -HCG or progesterone combining estradiol at 5–6 weeks of gestation can be used better to predict miscarriage in first trimester [33]. Therefore, other factors should not be forgotten in predicting negative pregnancy outcomes.

Theoretically, first trimester serum progesterone levels and spiral artery Doppler findings were critical for the course of pregnancy, but they could not predict risks in terms of miscarriage, preeclampsia, intrauterine growth retardation, jaundice and respiratory problems. Complicated pregnancies requiring intensive care were unpredictable. Remodeling in the spiral artery takes place between 12–13 weeks and our pregnant women were evaluated between 11–14 weeks. Studies performed after an estimated 14 weeks after the completion of remodeling may be more useful in terms of evaluating spiral arteries.

This study has many strengths. All measurements were performed by one perinatology specialist. Contrary to other studies, the progesterone levels of pregnant women between 11–14 weeks who applied for the first trimester screening test were measured and their effects on pregnancy outcomes were examined. Our study had limitation that the number of patients is small.

Predicting the adverse perinatal outcomes and the risks that may be encountered during pregnancy will be beneficial both in terms of preventive medicine and in terms of preventing complications with early treatment. However, no method currently provides definitive results in predicting poor perinatal outcomes. In our study, serum pro-

gesterone levels and spiral artery Doppler findings which were measured between 11–14 weeks did not provide statistical benefit in terms of early detection of poor perinatal outcomes. We think that using more specific markers and longer follow-ups would be beneficial in this regard.

5. Conclusions

It is known that there is a strong relationship between the first trimester serum levels of progesterone, which is an important hormone for the healthy continuation of pregnancy, and the risk of miscarriage. The quantity and quality of placental and fetal circulation is determined by the changes that occur in the spiral arteries with pregnancy. Although a close relationship is known between both variables and pregnancy outcomes, no correlation could be established between them in terms of early detection of poor perinatal outcomes.

Availability of Data and Materials

All data points generated or analyzed during this study are included in this article and there are no further underlying data necessary to reproduce the results.

Author Contributions

RA designed the research study. FŞ, HAŞ, SÖ performed the research and analyzed the data. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

The retrospective study received ethical approval from the Istanbul Prof. Dr. Cemil Taşcıoğlu City Hospital Clinical Research Ethics Committee, with approval number: 2021/386. Written permission has also been obtained from the institutions where the research was conducted, and informed consent has been obtained from the patients. The study was conducted in accordance with the Principles of the Declaration of Helsinki.

Acknowledgment

We would like to express our gratitude to all those who helped us during the writing of this manuscript. Thanks to all the peer reviewers for their opinions and suggestions.

Funding

This research received no external funding.

Conflict of Interest

The authors declare no conflict of interest.

References

- [1] Yalçın I, Taşkın S, Pabuçcu EG, Söylemez F. The value of placental protein 13, β -human chorionic gonadotropin and progesterone in the prediction of miscarriages in threatened miscarriage

- patients. *Journal of Obstetrics and Gynaecology*. 2015; 35: 283–286.
- [2] Wang XX, Luo Q, Bai WP. Efficacy of progesterone on threatened miscarriage: Difference in drug types. *The Journal of Obstetrics and Gynaecology Research*. 2019; 45: 794–802.
- [3] Ku CW, Allen JC, Jr, Malhotra R, Chong HC, Tan NS, Østbye T, *et al.* How can we better predict the risk of spontaneous miscarriage among women experiencing threatened miscarriage? *Gynecological Endocrinology*. 2015; 31: 647–651.
- [4] Arrowsmith S, Neilson J, Bricker L, Wray S. Differing In Vitro Potencies of Tocolytics and Progesterone in Myometrium From Singleton and Twin Pregnancies. *Reproductive Sciences*. 2016; 23: 98–111.
- [5] Mendes S, Timóteo-Ferreira F, Almeida H, Silva E. New Insights into the Process of Placentation and the Role of Oxidative Uterine Microenvironment. *Oxidative Medicine and Cellular Longevity*. 2019; 2019: 9174521.
- [6] Degner K, Magness RR, Shah DM. Establishment of the Human Uteroplacental Circulation: A Historical Perspective. *Reproductive Sciences*. 2017; 24: 753–761.
- [7] Gebb J, Dar P. Colour Doppler ultrasound of spiral artery blood flow in the prediction of pre-eclampsia and intrauterine growth restriction. *Best Practice & Research. Clinical Obstetrics & Gynaecology*. 2011; 25: 355–366.
- [8] Burton GJ, Jauniaux E. Pathophysiology of placental-derived fetal growth restriction. *American Journal of Obstetrics and Gynecology*. 2018; 218: S745–S761.
- [9] Schiffer V, Evers L, de Haas S, Ghossein-Doha C, Al-Nasiry S, Spaanderman M. Spiral artery blood flow during pregnancy: a systematic review and meta-analysis. *BMC Pregnancy and Childbirth*. 2020; 20: 680.
- [10] Odibo AO, Kayisli U, Lu Y, Kayisli O, Schatz F, Odibo L, *et al.* Longitudinal assessment of spiral artery and intravillous arteriole blood flow and adverse pregnancy outcome. *Ultrasound in Obstetrics & Gynecology*. 2022; 59: 350–357.
- [11] Özkan MB, Ozyazici E, Emiroglu B, Özkara E. Can we measure the spiral and uterine artery blood flow by real-time sonography and Doppler indices to predict spontaneous miscarriage in a normal-risk population? *Australasian Journal of Ultrasound in Medicine*. 2015; 18: 60–66.
- [12] Schock H, Zeleniuch-Jacquotte A, Lundin E, Grankvist K, Lakso HÅ, Idahl A, *et al.* Hormone concentrations throughout uncomplicated pregnancies: a longitudinal study. *BMC Pregnancy and Childbirth*. 2016; 16: 146.
- [13] Ghosh S, Chattopadhyay R, Goswami S, Chaudhury K, Chakravarty B, Ganesh A. Assessment of sub-endometrial blood flow parameters following dydrogesterone and micronized vaginal progesterone administration in women with idiopathic recurrent miscarriage: a pilot study. *The Journal of Obstetrics and Gynaecology Research*. 2014; 40: 1871–1876.
- [14] Goh JY, He S, Allen JC, Malhotra R, Tan TC. Maternal obesity is associated with a low serum progesterone level in early pregnancy. *Hormone Molecular Biology and Clinical Investigation*. 2016; 27: 97–100.
- [15] Xie X, Kong B, Duan T. Ninth edition: obstetrics and gynecology. People's Health Publishing House: Beijing. 2018. (In Chinese)
- [16] Roberts VHJ, Morgan TK, Bednarek P, Morita M, Burton GJ, Lo JO, *et al.* Early first trimester uteroplacental flow and the progressive disintegration of spiral artery plugs: new insights from contrast-enhanced ultrasound and tissue histopathology. *Human Reproduction*. 2017; 32: 2382–2393.
- [17] Jensen OE, Chernyavsky IL. Blood flow and transport in the human placenta. *Annual Review of Fluid Mechanics*. 2019; 51: 25–47.
- [18] Jauniaux E, Burton GJ. The role of oxidative stress in placental-related diseases of pregnancy. *Journal De Gynecologie, Obstetrique et Biologie De La Reproduction*. 2016; 45: 775–785.
- [19] Özdemir S, Şahin F, Doğan GÖ, Baytekin Ö. Evaluation of maternal serum galectin-9 levels in pregnancies with the threat of preterm birth. *Annals of Clinical and Analytical Medicine*. 2023; 14: 651–654.
- [20] Stevenson GN, Noble JA, Welsh AW, Impey L, Collins SL. Automated Visualization and Quantification of Spiral Artery Blood Flow Entering the First-Trimester Placenta, Using 3-D Power Doppler Ultrasound. *Ultrasound in Medicine & Biology*. 2018; 44: 522–531.
- [21] Sharma D, Shastri S, Farahbakhsh N, Sharma P. Intrauterine growth restriction - part 1. *The Journal of Maternal-Fetal & Neonatal Medicine*. 2016; 29: 3977–3987.
- [22] Rana S, Lemoine E, Granger JP, Karumanchi SA. Preeclampsia: Pathophysiology, Challenges, and Perspectives. *Circulation Research*. 2019; 124: 1094–1112.
- [23] Carp HJA. Progesterogens and pregnancy loss. *Climacteric*. 2018; 21: 380–384.
- [24] Siew JYS, Allen JC, Hui CYY, Ku CW, Malhotra R, Østbye T, *et al.* The randomised controlled trial of micronised progesterone and dydrogesterone (TRoMaD) for threatened miscarriage. *European Journal of Obstetrics, Gynecology, and Reproductive Biology*. 2018; 228: 319–324.
- [25] Ku CW, Allen JC, Jr, Lek SM, Chia ML, Tan NS, Tan TC. Serum progesterone distribution in normal pregnancies compared to pregnancies complicated by threatened miscarriage from 5 to 13 weeks gestation: a prospective cohort study. *BMC Pregnancy and Childbirth*. 2018; 18: 360.
- [26] Deng Y, Chen C, Chen S, Mai G, Liao X, Tian H, *et al.* Baseline Levels of Serum Progesterone and the First Trimester Pregnancy Outcome in Women with Threatened Abortion: A Retrospective Cohort Study. *BioMed Research International*. 2020; 2020: 8780253.
- [27] Rabiee S, Hemmati M, Fallah N. Comparison of serum progesterone in non-viable pregnancy and ectopic pregnancy. *Acta Medica Iranica*. 2013; 51: 693–696.
- [28] Lek SM, Ku CW, Allen JC Jr, Malhotra R, Tan NS, Østbye T, *et al.* Validation of serum progesterone <35nmol/L as a predictor of miscarriage among women with threatened miscarriage. *BMC Pregnancy Childbirth*. 2017; 17: 78.
- [29] Özkaya U, Ozkan S, Ozeren S, Corakci A. Doppler examination of uteroplacental circulation in early pregnancy: can it predict adverse outcome? *Journal of Clinical Ultrasound*. 2007; 35: 382–386.
- [30] Makikallio K, Jouppila P, Tekay A. First trimester uterine, placental and yolk sac haemodynamics in pre-eclampsia and preterm labour. *Human Reproduction*. 2004; 19: 729–733.
- [31] van Duijn L, Rousian M, Reijnders IF, Willemsen SP, Baart EB, Laven JSE, *et al.* The influence of frozen-thawed and fresh embryo transfer on utero-placental (vascular) development: the Rotterdam Periconception cohort. *Human Reproduction*. 2021; 36: 2091–2100.
- [32] Pillai RN, Konje JC, Tincello DG, Potdar N. Role of serum biomarkers in the prediction of outcome in women with threatened miscarriage: a systematic review and diagnostic accuracy meta-analysis. *Human Reproduction Update*. 2016; 22: 228–239.
- [33] Deng W, Sun R, Du J, Wu X, Ma L, Wang M, *et al.* Prediction of miscarriage in first trimester by serum estradiol, progesterone and β -human chorionic gonadotropin within 9 weeks of gestation. *BMC Pregnancy and Childbirth*. 2022; 22: 112.