ORIGINAL ARTICLE

UDC: 618.177-089.888.11:618.39-037
https://doi.org/10.2298/VSP170313061P

Twin pregnancies conceived by assisted reproduction – Early prediction of preterm birth

Blizanačke trudnoće nastale tehnikom asistirane reprodukcije – rano predviđanje prevremenog porođaja

Igor Pilić, Darko Plećaš, Jelena Dotlić, Snežana Plešinac

Clinical Center of Serbia, Clinic of Obstetrics and Gynecology, Belgrade, Serbia; University of Belgrade, Faculty of Medicine, Belgrade, Serbia

Abstract

Background/Aim. Twins conceived by assisted reproduction techniques (ART) are the most susceptible for perinatal complications. The aim of this study was to examine the role of prenatal noninvasive fetal screening of the first and second trimester in prediction of delivery time of ART conceived twins. **Methods.** Prospective cohort study of all ART conceived twin pregnancies was conducted at the Clinic for Obstetrics and Gynecology, Clinical Center of Serbia, during the period from January 1, 2016 to December 31, 2017. In the 12th gestational week (GW) twins crown-rump lenght (CRL) and thickness nuchal translucency (NT) were measured ultrasonographically. Moreover, serum levels of beta subunit of human chorionic gonadotropin (β hCG) and pregnancy-associated plasma protein A (PAPPA) were assessed. In the 17th GW twins biparietal diameter (BPD), abdominal circumference (AC) and femur length (FL) were determined ultrasonografically. Additionally, β hCG, alpha-fetoprotein (AFP), estriol (E3) and inhibin (INH) were measured in mothers serum. The GW of delivery was noted for each pregnancy. **Results.** Study included 100 pregnant women with mean age 35.44 ± 5.82. In the examined sample of ART conceived twins significantly more (51%) were delivered in term (≥ 35 GW) ($p = 0.001$). Delivery time correlated negatively with NT and first trimester β hCG serum levels, while it correlated positively with FL of the smaller twin, second trimester β hCG, AFP and E3 concentrations. According to obtained model for prediction of delivery time in ART conceived twin pregnancies based on first trimester diagnostic tests the significant predictors were PAPPA and β hCG in the 12th GW as well as NT of the first larger twin. Nevertheless, reliability (sensitivity 50%–75%, specificity 30%–40%) of these diagnostic tests was moderate. **Conclusion.** Prenatal noninvasive fetal screening of the first and second trimester (ultrasonography and laboratory testing) can be used for prediction of delivery time of ART conceived twins.

Key words:
premature births; prognosis; reproductive techniques, assisted; twins; ultrasonography.

Apstrakt

Uvod/Cilj. Blizanci nastali tehnikama asistirane reprodukcije (ART) su najosetljiviji na pojavu perinatalnih komplikacija. Cilj rada bio je da se ispita uloga prenatalnog neinvazivnog fetalnog skrininga prvog i drugog trimestra u predviđanju vremena porođaja blizanaca nastalih metodama ART. **Metode.** Prospektivna kohortna studija kojom su obuhvaćene sve blizanačke trudnoće nastale ART metodama sprovedena je na Klinici za ginekologiju i akušerstvo Kliničkog centra Srbije u periodu od 01.01.2016. do 31.12.2017. U 12. gestacionoj nedelji (GN) ultrasonografski su blizancima izmereni razmak teme-trtica (CRL) i nuhalno zadebljanje (NT). Takođe, određeni su serumski nivoi beta podjedinice humanog horionskog gonadotropina (β hCG) i plazma proteina A povezanog sa trudnoćom (PAPPA). U 17. GN ultrasonografski su blizancima izmereni biparietalni dijametar (BPD), obim abdomena (AC) i dužine femura (FL). Pored toga, β hCG, alfa fetoprotein (AFP), estriol (E3) i inhibin (INH) su izmereni u serumu majki. Za svaku trudnoću zabeležena je GN porođaja. **Rezultati.** Studija je obuhvatila 100 trudnica koje su u proseku imale 35,44 ± 5,82 godina života. U ispitivanom uzorku blizanaca, nastalih tehnikama ART, značajno više (51%) je bilo porođeno u terminu (≥ 35 GN) ($p = 0,001$). Vreme porođaja je negativno korelisalo sa NT i nivoima serumskog β hCG u prvom trimestru, dok je pozitivno korelisalo sa FL manjeg blizanca, kao i koncentracijama β hCG, AFP i E3 u drugom trimestru. Prema dobijenom modelu za predviđanje vremena porođaja blizanaca nastalih tehnikom ART, na osnovu dijagnostičkih testova prvog i drugog trimestra, značajni prediktori bili su PAPPA i β hCG u 12. GN, kao i NT prvog većeg blizanca. Ipak, pouzdanost (senzitivnost 50%–75%, specifičnost 30%–40%) tih dijagnostičkih testova bila je umerena. **Zaključak.**

Correspondence to: Igor Pilić, Clinical Center of Serbia, Clinic of Obstetrics and Gynecology, Koste Todorovića 26, 11 000 Belgrade, Serbia. E-mail: pilic.igor@yahoo.com

Prenatalni neinvazivni fetalni skrining prvog i drugog trimestra (ultrasonografija i laboratorijsko testiranje) može se koristiti za predviđanje vremena porođaja blizanaca nastalih metodama ART.

Ključne reči:
porođaj, prevremeni; prognoza; reprodukcija, asistirana, tehnike; blizanci; ultrasonografija.

Introduction

Application of assisted reproduction technologies (ART) as infertility treatment, especially in-vitro fertilisation (IVF), intracytoplasmic sperm injection (ICSI) and oocyte donation (DO), has lead to increase in the number of twin pregnancies 1, 2.

Despite advances in obstetrical and neonatal care, it is well kown that twin pregnancies still carry a high risk for numerous gestational complications and adverse pregnancy outcomes [1–3]. Twin pregnancies are associated with low birth weight of fetuses, caesarean section, prematurity and associated perinatal morbidity and mortality. Literature data show that in twin pregnancies there is also an increased risk of gestational hypertensive disorders of the mother [2, 3]. Nevertheless, there are conflicting reports on whether conception by ART further increases these risks when compared to spontaneously conceived pregnancies [4, 5]. Pregnancies concieved by ART are found to be more often complicated with preterm delivery and fetal low birth weigh, placental abruption, gestational diabetes and hypertension [2–4]. Consequently, ART conceived twins are the most susceptible for various perinatal complications among which the most severe is premature pregnancy ending [6].

One of the most important issues in perinatology is early and noninvasive prediction of adverse pregnacy outcomes [1, 6]. Therefore, the aim of this study was to examine the role of prenatal noninvasive fetal screening of the first and second trimester in prediction of delivery time of ART conceived twins.

Methods

A prospective cohort study of all pregnant women with ART conceived twin pregnancies was conducted at the Clinic for Gynecology and Obstetrics, Clinical Center of Serbia in Belgrade, during the period from January 1st 2016, to December 31, 2017.

Exclusion criteria for this study were miscarriage before 8th gestational week (GW), genetic disorders of twins, monochorionicity and monoamnionicity of twins and existence of chronic ilnesses in mothers that could influence the pregnancy course and outcome. The study was approved by the Institutional Review Board. All patients signed a written informed consent form.

Already during the first examination for pregnancy confirmation, a complete history regarding mothers age, chronic ilnesses, previous parity and ART type (IVF, ICS, DO) was taken from each patient. Investigated women were regularly checked-up at least once per trimester in our Clinic.

In the first trimester, during the 12th gestational week, for each twin, the crown-rump lenght (CRL) and the thickness of nuchal translucency (NT) were measured and chorionicity determined ultrasonographically. Moreover, biochemical markers that are incorporated in Double test screening such as beta subunit of human chronic gonadotropin (β hCG) and pregnancy-associated plasma protein A (PAPPA) were assessed. In the 17th GW, ultrasound examination for both twins determined their biometric parameters such as biparietal diameter (BPD), abdominal circumference (AC) and femur length (FL). Additionally, in the second trimester we evaluated markers used for Triple testing i.e. β hCG, alpha fetoprotein (AFP), estriol (E3) and inhibin (INH).

Monitoring of twins was performed by ultrasound examinations. Ultrasound biometry and monitoring were performed through ACCUVIX device (Samsung Medison, Seoul, Sought Korea), with 3.75 MHz abdominal and vaginal probe.

For laboratory diagnostic tests 5 to 10 mL of blood was drawn by venipuncture into nonheparinized tubes and centrifuged for 15 min. After serum separation concentrations of investigated biochemical markers were measured using BRAHMS KRYPTOR analyzer and applying fluorocytometric immunoassay with SsdwLab 5 software. The measured serum concentrations (IU/L) of biomarkers were then converted into multiples of median (MoM) and adjusted for GW for easier comparison and analysis. The standard referral range of all tested pregnancy markers, most widely used in literature and adopted in this study as well, was from 0.5 to 2 MoM.

All complications of pregnancy were registered regularly throughout pregnancy. The GW in which delivery occurred as well as the delivary type [vaginal or cesarean section (SC)] were noted for each pregnancy. Time of delivery was classified as miscarriage (before 12 GW), early preterm (before 25th GW), late preterm (before 35 GW) and term delivery (in and after 35th GW).

Upon delivery, all data collected throughout pregnancy were correlated with delivery time and analyzed by methods of descriptive statistics (number, percent, mean, standard deviation) and analytical statistics using the SPSS 20 software. Correlations of biochemical parameters and occurrence of hypertension were tested using Spearman's correlation analysis. Significance of differences between categories of assessed parameters was examined by χ^2 test.

Enter method of binary logistic regression was applied to construct models for prediction of delivery time of ART conceived twins based on investigated ultrasonographic and biochemical markers of the first and second pregnancy trimester. All models were adjusted for potential confoundings (mothers' age, and parity).

Finaly, study authors performed the Receiver Operating Characteristics (ROC) analysis to set the cut-off values of ul-

trasonographic and biochemical parameters of the first and second trimester that could imply the delivery time in ART conceived twin pregnancies.

Results

Study included 100 pregnat women with mean age 35.44 ± 5.82 (range 21 to 45). Eighty eight percent of investigated women did not have previous deliveries, while 29% of them had previous miscarriages and/or abortions. Significantly more (59%) women had no gestational illnesses and pregnancy complications ($p = 0.001$). The most common pregnancy complication was gestational hypertension (21% of mothers). In our sample there were no significant differences in frequency of different ART types (53% had only IVF, while 42% had IVF/ICSI; $p = 0.259$). Still, only five women had DO.

In the examined sample of ART conceived twins significantly more (51%) were delivered in term (after 35 GW) ($p = 0.001$). We registered 8 cases of miscarriage before 12th GW, 8 early preterm births before 24th GW, while 33 twins were born between 24th and 33th GW ($p = 0.001$). Majority of twins were liveborn (86% of first larger and 87% of second smaller twins). Delivery was mostly by SC ($p = 0.001$).

Diagnostic parameters (ultrasonografy and laboratory) of the first and second pregnancy trimester are shown in Table 1. Majority of twins had all values in the refferal range for GW.

Correlations of delivery time with general patients data and the first trimester diagnostic testing are presented in Table 2, while correlations with the second trimester ultrasound and laboratory findings are presented in Table 3.

Delivery time correlated positively with mothers age and negatively with previous parity. Out of all assessed ultrasonographic fetal measures in the first trimester only the thickness of NT for both twins was negatively correlated with the delivery time. Moreover, FL of the smaller second twin measured ultrasonographically in the second trimester correlated positively with the delivery time, but was not correlated with the exact GW of delivery.

When laboratory analyses were assessed it was determined that β hCG serum levels in the first trimester correlated positively with gestational week of delivery. Contrary, β hCG as well as AFP concentrations in the second trimester correlated negatively with the exact GW of delivery. Serum levels of E3 in the second trimester correlated positively with term delivery.

We obtained a significant model for prediction of delivery time in ART conceived twin pregnancies based on the first trimester diagnostic tests – ultrasound and laboratory (B = 0.041; Wald = 0.140; Nagelkerke $R^2 = 0.628$; $\chi^2 = 38.664$; $p = 0.001$; explained variance = 73.0%). The significant predictors were PAPPA and β hCG in 12th GW as well as NT of the first larger twin. Mothers age and previous parity were confoundings in this model.

ART twins term delivery = 3.054 – 1.142 × PAPPA 12 GW– 1.263 × β hCG 12 GW – 0.940 × NT gemellus I – 2.128 x previous parity + 0.104 x mothers age

Table 1
Ultrasound and laboratory diagnostic findings of first and second trimester

Parameters	Min–Max	Mean ± SD
I trimester-12 gestational weeks		
CRL twin I	17.00–87.00	58.51 ± 11.21
NT twin I	1.40–3.70	2.21 ± 0.54
CRL twin II	16.00–85.90	60.69 ± 11.88
NT twin II	1.40–3.70	2.21 ± 0.52
PAPPA	0.40–4.10	1.33 ± 0.72
Beta-hCG	0.65–2.56	1.21 ± 0.57
II trimester-17 gestational weeks		
AC twin I	112.00–126.00	117.46 ± 4.72
AC twin II	112.00–126.00	117.17 ± 4.42
BPD twin I	31.00–39.00	34.83 ± 2.67
BPD twin II	31.00–39.00	34.84 ± 2.60
FL twin I	2.00–29.00	20.73 ± 7.64
FL twin II	2.00–29.00	20.77 ± 7.64
Beta-hCG	0.51–2.80	1.30 ± 0.64
Estriol	0.51–2.80	1.31 ± 0.69
Alpha fetoprotein	0.51–2.80	1.32 ± 0.70
Inhibin	0.51–2.80	1.22 ± 0.66

CRL – crown-rump lenght; NT – nuchal translucence; HCG – human chorionic gonadotropin; AC – abdominal circumference; BPD – biparietal diameter; FL – femur lenght; PAPPA – pregnancy-associated plasma protein A; min – minimum; max –maximum; SD – standard deviation.

Table 2

Correlation of delivery time with general patients data and first trimester diagnostic testing

Parameters	Term delivery	Delivery time		Gestational week (GW)	
		twin I	twin II	twin I	twin II
Mothers age					
Ro	**0.208**	**0.273**	**0.273**	**0.280**	**0.279**
p	**0.038**	**0.006**	**0.006**	**0.005**	**0.005**
Parity					
Ro	**-0.256**	**-0.348**	**-0.348**	**-0.355**	**-0.355**
p	**0.010**	**0.001**	**0.001**	**0.001**	**0.001**
ART type					
Ro	0.009	-0.084	-0.084	-0.131	-0.132
p	0.926	0.408	0.408	0.194	0.191
CRL twin I					
Ro	-0.031	0.020	0.020	-0.022	-0.024
p	0.760	0.840	0.840	0.827	0.816
NT twin I					
Ro	**-0.288**	**-0.354**	**-0.354**	**-0.373**	**-0.373**
p	**0.004**	**0.001**	**0.001**	**0.001**	**0.001**
CRL twin I					
Ro	-0.106	-0.056	-0.056	-0.054	-0.054
p	0.294	0.580	0.580	0.590	0.592
NT twin II					
Ro	**-0.218**	**-0.248**	**-0.248**	**-0.230**	**-0.231**
p	**0.047**	**0.013**	**0.013**	**0.021**	**0.021**
PAPPA					
Ro	0.041	0.023	0.023	-0.002	-0.003
p	0.683	0.823	0.823	0.982	0.977
Beta-hCG					
Ro	**0.253**	**0.264**	**0.264**	**0.234**	**0.235**
p	**0.011**	**0.008**	**0.008**	**0.019**	**0.019**

ART – assisted reproduction technique; term delivery (yes/no) – before or after 35 GWs; delivery time – before 12 GWs, from 12 to 25, from 25 to 35, 35 and more GWs; CRL – crown-rump lenght; NT – nuchal translucence; HCG – human chorionic gonadotropin; PAPPA – pregnancy associated plazma protein A; ART type – *in vitro* fetrilisation (IVF), intracytoplasmic sperm injection (ICSI), oocyte donation (DO); Ro – coefficient of correlation.
Nore: Statisticaly significant values are bolded.

Table 3

Correlation of delivery time with second trimester diagnostic testing

Parameters	Term delivery	Delivery time		Gestational week (GW)	
		twin I	twin II	twin II	twin II
AC twin I					
Ro	0.027	0.045	0.045	-0.002	-0.002
p	0.798	0.673	0.673	0.982	0.986
AC twin II					
Ro	0.002	-0.023	-0.023	0.040	0.040
p	0.997	0.826	0.826	0.706	0.706
BPD twin I					
Ro	0.003	-0.014	-0.014	-0.036	-0.035
p	0.981	0.898	0.898	0.735	0.743
BPD twin II					
Ro	0.117	0.116	0.116	0.027	0.026
p	0.266	0.269	0.269	0.798	0.803
FL twin I					
Ro	0.134	0.153	0.153	0.081	0.080
p	0.202	0.145	0.145	0.444	0.447

Table 3 – *continued*

Parameters	Term delivery	Delivery time		Gestational week (GW)	
		twin I	twin II	twin II	twin II
FL twin II					
Ro	**0.219**	**0.222**	**0.222**	0.121	0.121
p	**0.036**	**0.033**	**0.033**	0.249	0.252
Beta HCG					
Ro	-0.135	-0.194	-0.194	**-0.221**	**-0.220**
p	0.200	0.064	0.064	**0.035**	**0.035**
Estriol (E3)					
Ro	**0.215**	0.184	0.184	0.099	0.098
p	**0.039**	0.079	0.079	0.349	0.354
Alpha feto protein (AFP)					
Ro	-0.166	-0.201	-0.201	**-0.245**	**-0.246**
p	0.114	0.055	0.055	**0.019**	**0.018**
Inhibin					
Ro	0.077	0.066	0.066	0.101	0.101
p	0.467	0.531	0.531	0.337	0.338

Term delivery (yes/no) – before or after 35 GWs; delivery time – before 12 GWs, from 12 to 25, from 25 to 35, 35 and more GWs; AC – abdominal circumference; BPD – biparietal diameter; FL – femur lenght; HCG – human chorionic gonadotropin.
Ro – coefficient of correlation.
Nore: Statisticaly significant values are bolded.

Table 4
ROC analysis of potential predictors for assisted reproduction technique (ART) conceived twins delivery time

Parameters	Area under the curve	*p*	Cut-off value	Sensitivity (%)	Specificity (%)
CRL twin I	0.440	0.322	56.50	53.00	32.70
NT twin I	0.381	**0.051**	1.95	53.00	30.30
CRL twin II	0.378	**0.045**	57.50	60.80	32.80
NT twin II	0.433	0.268	1.86	60.80	30.00
AC twin I	0.516	0.798	115.00	62.70	45.00
AC twin II	0.500	0.997	115.00	64.70	41.50
BPD twin I	0.501	0.981	33.50	68.60	44.30
BPD twin II	0.576	0.210	22.00	63.00	42.50
FL twin I	0.567	0.270	33.50	70.60	45.10
FL twin II	0.626	**0.039**	22.00	68.60	46.30
PAPPA 12 GW	0.546	0.446	0.81	74.50	40.10
Beta-hCG 12 GW	0.630	**0.033**	0.81	76.50	41.50
Beta-hCG 17 GW	0.503	0.956	0.82	64.70	31.70
Estriol (E3) 17 GW	0.491	0.884	0.85	55.00	30.20
AFP 17 GW	0.404	0.114	0.87	54.90	41.50
Inhibin 17 GW	0.544	0.465	0.80	64.70	40.90

CRL – crown-rump lenght; NT – nuchal translucence; HCG – human chorionic gonadotropin; AC – abdominal circumference; BPD – biparietal diameter; FL – femur lenght; HCG – human chorionic gonadotropin; PAPPA – pregnancy associated plazma protein A; AFP – alpha fetoprotein; GW – gestational week.
Note: Statistically significant values are bolded.

We did not manage to obtain the significant binary logistic regression for prediction of delivery time in ART conceived twin pregnancies based on the second trimester diagnostic tests ($\chi^2 = 22.355$; $p = 0.217$).

ROC analysis of ultrasonografic diagnostic tests is presented on Figure 1 while on the Figure 2 we presented laboratory tests of the first and second trimester. Newly established cut-off values with their sensitivity and specificity for potential ultrasonographic and laboratory predictors of twins delivery time are presented in Table 4. The significant predictors were β hCG in 12th GW, NT of the first larger twin in 12th GW as well as CRL in 12th GW and FL in 17th GW of the second smaller twin.

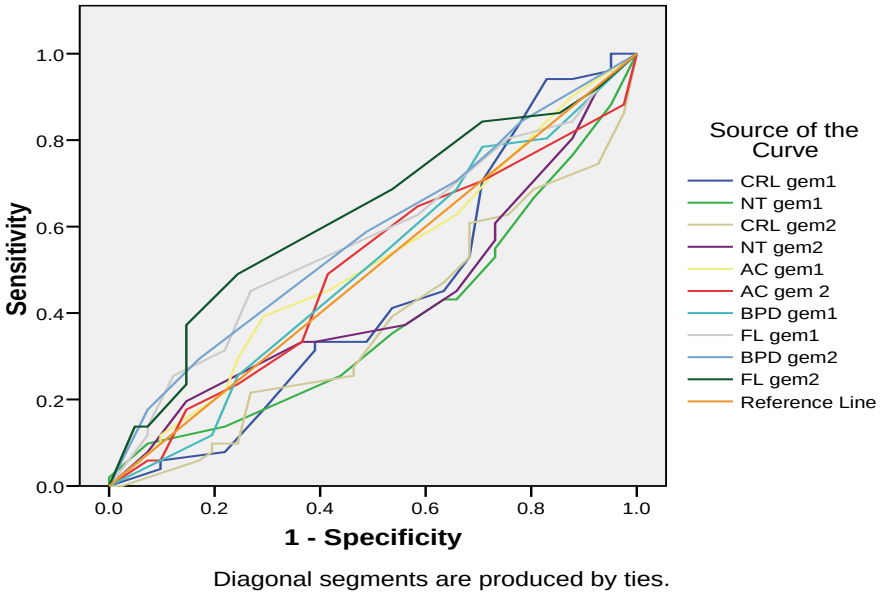

Fig. 1 – Ultrasonographic predictors for assisted reproduction technique (ART) conceived twins delivery time.
CRL – crown-rump length; NT – nuchal translucence; AC – abdominal circumference; BDP – biparietal diameter; FL – femur length.

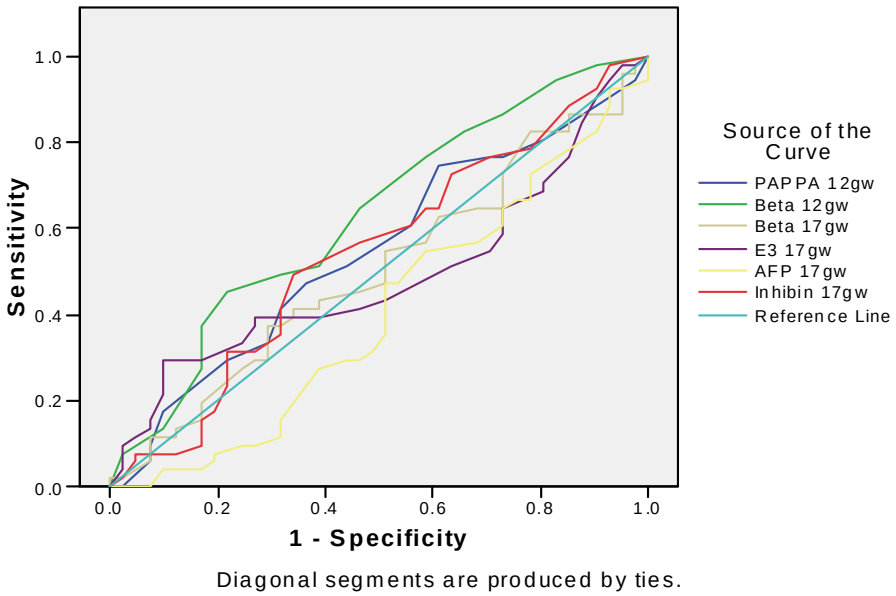

Fig. 2 – Laboratory predictors for assisted reproduction technique (ART) conceived twins delivery time.
PAPPA – pregnancy-associated plasma protein A; GW – gestational week; E_3 – estriol; AFP – alpha fetoprotein.

Discussion

Twin pregnancies, in general, are at higher risk of numerous pregnancy complications including miscarriage or preterm birth caused by a development of two fetuses instead of one [1, 2]. Adverse obstetric outcome after ART might be caused by the very procedure [4]. Moreover, ART conceived twins can be at even higher risk of pregnancy complications and adverse obstetric outcomes due to maternal age, pre-existing maternal medical conditions such as polycystic ovary syndrome or thyroid diseases that are associated with miscarriages and aneuploidies [7–9].

Studies show that the mean gestational age at delivery was significantly lower in the ART group compared with the spontaneous conception group regardless of the cerclage application [3]. ART conceived twins are at significantly increased risks of preterm birth both early and late, althouhg according to majority of studes ART twins were mostly de-

livered moderately preterm (32–34 GW) [2, 10]. However, the rate of preterm birth of ART conceived twins in several investigations was similar to that of spontaneously conceived ones or that very preterm deliveries even occurred more frequently in spontaneous twin pregnancies [4]. In our study, similarly, more women delivered at term than preterm (51% vs. 49%, respectively), while late preterm birth was predominant time of delivery of preterm twins (33%). Moreover, although somewhat unexpectedly, older mothers delivered at term more often. A possible explanation could be that older women who had ART as infertility treatment are more concerened for the pregnancy sucess and therefore comply better to their therapy and have more regular check-ups that could have prevented preterm labor.

Consequently, ART twin pregnancies require additional antenatal care [7]. Potential early prediction of adverse outcomes could enable adequate and timely management of pregnancy complications allowing preventing premature pregnancy ending [11]. Prenatal noninvasive fetal screening in patients with ART conceived twin pregnancies is essential part of appropriate control of these high-risk pregnancies. It is performed at the time of standard fetal screening for chromosomal anomalies (Double, Triple or Q test) by ultrasound examination of both fetuses and the analysis of mothers biomarkers (hormones) serum levels has been established during the first (11 to 14 GWs) and/or second (16 to 18 gestational weeks) pregnancy trimester. Literature data show that regular and thorough check-ups of twins can show, apart from potential risk of aneuploidies, also the risk of adverse pregnancy outcomes [11].

Data from current literature are still contradictory regarding the strength of association between pregnancy biochemical markers and twin pregnancy outcomes [12, 13]. Biochemical markers are associated with a variety of external factors, such as patient's and partner's age, demographic data, course and outcome of previous pregnancies, especially premature births, spontaneous abortion, perinatal morbidity and mortality, type of performed ART (IVF, ICSI, DO), number of previous unsuccessful ART attempts, the course of current pregnancy and the presence of symptoms that indicate imminent abortion or premature delivery [12, 13]. There is still an on going debate how could serum biomarkers be used in the prediction of pregnancy course and outcome. It has been found that in the case of twin pregnancies the values of biomarkers depend on the horionicity as well as the gestational age [6]. Studies that evaluated serum β hCG levels for prediction of twins preterm birth found that reliability and predictive performance of high β hCG levels were low [11, 14]. Similar findings were reported for PAPPA serum levels (senisitivity mostly below 50%). Nevertheless, specificity of PAPPA as well as the first and second trimester β hCG serum concentrations were significantly higher reaching above 90% in some cases for prediction of twins premature birth [11, 14]. Although several studies found a negative correlation between elevated maternal serum AFP levels and twins gestational age at birth, predictive ability of elevated maternal AFP levels was poor (sensitivity of 30%, specificity of 88%) [11, 14].

The role of ultrasound in control of ART conceived twin pregnancies is essential as it for diagnosing chorionicity and placentation, discordinant growth, twin-to-twin transfusion syndrome [15]. Both early the first and second trimester sonographic examinations are investigated in recent studies for predicting adverse perinatal outcomes [16]. It is postulated that fetuses suffering from reduced oxygenation and/or nutrient limitation caused by progressed pregnancy complications during early pregnancy tend to be smaller when their biometrical measurements are evaluated [17, 18]. Several studies have examined the relationship between the parameters obtained during ultrasonographic surveillance of twins with the course, delivery time and type as well as the outcome of both dichorionic and monochorionic twin pregnancies [5, 19]. According to literature data significant correlations of parameters measured and determined during the ultrasound screening such as intertwin differences in CRL and NT, chorionicity, amniotic fluid quantity, AC of both fetuses in the second trimester with the pregnancy outcome and numerous gestational complications are commonly found [15, 18]. According to some investigations abnormal measurements of CRL in the first trimester were found to be associated with early fetal loss [10, 20]. A meta-analysis showed that twin pregnancies with CRL discordance $\geq 10\%$ were at significantly higher risk of fetal loss and premature delivery after 24th until 34th GW, but not before 24 weeks of gestation [21]. Only few studies have assessed NT in predicting preterm birth in twin gestations. Still, they reported sensitivity of 75% and specificity of 94% in prediction of preterm birth of twins [11, 22]. Intertwin AC discordances were proven by different authors implying on adverse obstetric outcomes. A single biometric assessment of twins at 16 weeks could adequately predict subsequent pregnancy complications and premature pregnancy ending in almost 50% of cases [23, 24]. On the other hand, high reliability of biometrical measurements of the first and second trimester was not confirmed in all available studeis. Moreover, cut-off values of ultrasonographically determined twins biomety vary among studies. Further studies on this matter are still needed [20, 21].

In our study we found that out of assessed ultrasonographic fetal measures the thickness of NT of both twins and femur lenght of the smaller second twin in the second trimester could be used for prediction of preterm birth of ART conceived twins. When laboratory analyses were assessed β hCG serum levels measured in the first and the second trimester, as well as PAPPA and AFP concentrations could imply on GW of ART concieved twins delivery. We obtained a significant model for prediction of delivery time in ART concieved twin pregnancies based on the first trimester diagnostic tests – ultrasound and laboratory. Moreover, we set the cut-off levels for ultrasonografic and laboratory diagnostic tests of the first and second trimester. Performed ROC anlysis showed that sensitivity was better than specificity for prediction of delivery time of ART conceived twins. Nevertheless, reliability of these diagnostic testst in our study was not high (sensitivity 50% to 75%, specificity 30 to 40%), similarly as in the literature.

Conclusion

It can be seen that in the examined sample of one hundred ART concieved twins significantly more (51%) were delivered in term (after 35 GW), while more than 80% of them were liveborn. Based on the results of our study it can be concluded that prenatal noninvasive fetal screening of the first and second trimester (ultrasonography and laboratory testing) can be used for prediction of delivery time of ART conceived twins. The significant predictors were PAPPA and β hCG of the first trimester, larger twin NT as well as smaller twin CRL and FL. Nevertheless, reliability of these diagnostic testst in our study was moderate. Therefore, further studies are needed to more thoroughly investigate how could we potentially increase the reliability of these predictors as well as to test the obtained prediction model for delivery time of ART conceived twins based on ultrasonography and laboratory diagnostic tests of the first and second trimester.

Conflict of interest statement

Authors declare no conflict of interest. This study received no funding.

R E F E R E N C E S

1. *Domingues AP, Dinis SR, Belo A, Couto D, Fonseca E, Moura P.* Impact of induced pregnancies in the obstetrical outcome of twin pregnancies. Fertil Steril 2014; 101(1): 172–7.
2. *Geisler ME, O'Mahony A, Meaney S, Waterstone JJ, O'Donoghue K.* Obstetric and perinatal outcomes of twin pregnancies conceived following IVF/ICSI treatment compared with spontaneously conceived twin pregnancies. Eur J Obstet Gynecol Reprod Biol 2014; 181: 78–83.
3. *Caserta D, Bordi G, Stegagno M, Filippini F, Podagrosi M, Roselli D,* et al. Maternal and perinatal outcomes in spontaneous versus assisted conception twin pregnancies. Eur J Obstet Gynecol Reprod Biol 2014; 174: 64–9.
4. *Andrijasevic S, Dotlic J, Aksam S, Micic J, Terzic M.* Impact of Conception Method on Twin Pregnancy Course and Outcome. Geburtshilfe Frauenheilkd 2014; 74(10): 933–9.
5. *Murray SR, Norman JE.* Multiple pregnancies following assisted reproductive technologies - A happy consequence or double trouble? Semin Fetal Neonatal Med 2014; 19(4): 222–7.
6. *Dolgun ZN, Inan C, Altintas AS, Okten SB, Sayin NC.* Preterm birth in twin pregnancies: Clinical outcomes and predictive parameters. Pak J Med Sci 2016; 32: 922–6.
7. *Jauniaux E, Ben-Ami I, Maymon R.* Do assisted-reproduction twin pregnancies require additional antenatal care? Reprod Biomed Online 2013; 26(2): 107–19.
8. *Anbazhagan A, Hunter A, Breathnach FM, Mcauliffe FM, Geary MP, Daly S*, et al. Comparison of outcomes of twins conceived spontaneously and by artificial reproductive therapy. J Matern Fetal Neonatal Med 2014; 27: 458–62.
9. *Hack KE, Vereycken ME, Torrance HL, Koopman-Esseboom C, Derks JB.* Perinatal outcome of monochorionic and dichorionic twins after spontaneous and assisted conception: a retrospective cohort study. Acta Obstet Gynecol Scand 2018; 97(6): 717–26.
10. *McDonald SD, Han Z, Mulla S, Ohlsson A, Beyene J, Murphy KE*, et al. Preterm birth and low birth weight among in vitro fertilization twins: a systemic review and meta-analyses. Eur J Obstet Gynecol Reprod Biol 2010; 148(2): 105–13.
11. *Conde-Agudelo A, Romero R.* Prediction of preterm birth in twin gestations using biophysical and biochemical tests. Am J Obstet Gynecol 2014; 211(6): 583–95
12. *Chan RL.* Biochemical markers of spontaneous preterm birth in asymptomatic women. Biomed Res Int 2014; 2014: 164081.
13. *Iskender C, Tarim E, Cok T, Yalcinkaya C, Kalayci H, Yanik FB.* Obstetrical complications associated with first-trimester screening markers in twin pregnancies. J Obstet Gynaecol Res 2013; 39: 1495–9.
14. *Bergh E, Rebarber A, Oppal S, Saltzman DH, Klauser CK, Gupta S*, et al. The association between maternal biomarkers and pathways to preterm birth in twin pregnancies. J Matern Fetal Neonatal Med 2015; 28(5): 504–8.
15. *Fajardo-Exposito MA, Hervias B, Gonzalez FB, Melero-Jimenez V, Quintero-Prado R, Facio-Fernandez MC,* et al. First trimester fetal head and trunk volume predict growth disturbance in twin pregnancy. Prenat Diagn 2011; 31(6): 543–7.
16. *Khalil AA, Khan N, Bowe S, Familiari A, Papageorghiou A, Bhide A*, et al. Discordance in fetal biometry and Doppler are independent predictors of the risk of perinatal loss in twin pregnancies. Am J Obstet Gynecol 2015; 213(2): 222.e1–222.e10.
17. *Johansen ML, Oldenburg A, Rosthoj S, Cohn Maxild J, Rode L, Tabor A.* Crown-rump length discordance in the first trimester: a predictor of adverse outcome in twin pregnancies? Ultrasound Obstet Gynecol 2014; 43(3): 277–83.
18. *Aksam S, Plesinac S, Dotlic J, Tadic J, Vrzic-Petronijevic S, Petronijevic M*, et al. First trimester ultrasonographic parameters in prediction of the course and outcome of monochorionic twin pregnancies. Turk J Med Sci 2017; 47: 934–41.
19. *Kaponis A, Thanatsis N, Papadopoulos V, Decavalas G.* Intertwin estimated fetal weight or crown rump length discordance and adverse perinatal outcome. J Perinat Med 2016; 44(8): 863–9.
20. *Grande M, Gonce A, Stergiotou I, Bennasar M, Borrell A.* Intertwin crown-rump length discordance in the prediction of fetal anomalies, fetal loss and adverse perinatal outcome. J Matern Fetal Neonatal Med 2016; 29(17): 2883–8.
21. *D'Antonio F, Khalil A, Pagani G, Papageorghiou AT, Bhide A, Thilaganathan B.* Crown-rump length discordance and adverse perinatal outcome in twin pregnancies: systematic review and meta-analysis. Ultrasound Obstet Gynecol 2014; 44(2): 138–46.
22. *Prats P, Rodriguez I, Comas C, Puerto B.* First trimester risk assessment for trisomy 21 in twin pregnancies combining nuchal translucency and first trimester biochemical markers. Prenat Diagn 2012; 32: 927–32.
23. *O'Connor C, McAuliffe FM, Breathnach FM, Geary M, Daly S, Higgins JR, et al.* Prediction of outcome in twin pregnancy with first and early second trimester ultrasound. J Matern Fetal Neonatal Med 2013; 26(10): 1030–5.
24. *Allaf MB, Campbell WA, Vintzileos AM, Haeri S, Javadian P, Shamshirsaz AA,* et al. Does early second-trimester sonography predict adverse perinatal outcomes in monochorionic diamniotic twin pregnancies? J Ultrasound Med 2014; 33(9): 1573–8.

Received on December 12, 2017.
Revised on March 6, 2018.
Accepted on March 26, 2018.
Online First April, 2018.