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TITLE PAGE

Evaluation of placental vascularization indices in monochorionic diamniotic and dichorionic diamniotic twin pregnancies

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ABSTRACT

Objectives We aimed to investigate and compare placental vascularization indices between monochorionic-diamniotic, dichorionic-diamniotic normal twin pregnancies, and normal singular pregnancies. We hypothesized that there is correlation between placental three-dimensional power Doppler vascularization indices and birth weight in case of twin pregnancies, and that normal singular pregnancies have higher placental vascularization indices than normal twin pregnancies.

Study design Placental three-dimensional power Doppler vascularization indices, such as vascularization index, flow index, and vascularization-flow index were measured in monochorionic-diamniotic (N=15) and dichorionic-diamniotic (N=36) normal twin pregnancies, and in normal singular (N=109) pregnancies. Correlations were analyzed between vascularization indices, and birth weight, APGAR score, umbilical pH, umbilical venous bicarbonate, lactate, and base excess.

Results Vascularization indices and birth weight were significantly ($p<0.01$) higher in normal singular gestations (vascularization index=10.36, flow index=46.08, vascularization-flow index=4.08, average birth weight=3377g at 38.2 weeks average gestational age) compared to monochorionic-diamniotic and dichorionic-diamniotic normal twin pregnancies. No significant differences were found in vascularization indices between monochorionic-diamniotic and dichorionic-diamniotic normal twins. There were no significant differences in APGAR score, umbilical pH, umbilical venous bicarbonate, lactate, and base excess between groups examined ($p<0.01$). We found strong linear correlations between placental vascularization indices and birth weight in both twin groups.

Conclusion Placental three-dimensional power Doppler vascularization indices seem appropriate for predicting birth weight in monochorionic-diamniotic and dichorionic-diamniotic normal twin pregnancies. Our pilot study revealed reference values for vascularization indices in case of twin pregnancies examined.

ABBREVIATIONS:
There are no abbreviations.

Key words placental vascularization indices, twin pregnancy,

INTRODUCTION

In recent decades, the incidence of twin pregnancies has increased as a result of assisted reproduction technique, widely used oral contraception (1), and advanced maternal age (2). According to Hellin's law, the frequency of twin pregnancies is one in 85 births (3), but the actual frequency of twin pregnancies has approximately tripled.

In case of assisted reproduction techniques, the risk of multiple fetuses increases with the number of embryos transferred (4,5,6,7,8). Although the overall twin pregnancy rate has increased during recent decades, assisted reproduction technique multiple delivery rates started to decline gradually in the most developed countries during the 2000s (9).

The study by Murphy et al. provided some support for the hypothesis that there is an increased risk for monozygotic twins within a year of ceasing oral contraception use (1). As for dizygotic twins, the risk is three times as high in women over 35 (with four or more children), then in women under 20, at their first pregnancy (2,10).

According to the literature, different placentation may occur in monozygotic twin pregnancies (11,12). See *Table 1.*

In twin pregnancies, maternal complications may include preeclampsia, anemia, cervical incompetence, placental failure, abruption of the placenta, and placenta previa, while increased perinatal morbidity and mortality rates are observed compared to singular pregnancies (13). It is known that perinatal morbidity and mortality of monochorionic-diamniotic are higher than those of singular or dichorionic-diamniotic pregnancies (14). The reasons for the higher mortality rate of monochorionic-diamniotic can be found in placental morphology. In monochorionic-diamniotic vascular anastomoses increase the risk of complications such as twin-to-twin transfusion syndrome, selective intrauterine growth restriction, twin anemia polycythemia sequence, twin reverse arterial perfusion sequence and intrauterine demise.

The natural rate of twin pregnancy is low in East Asia and Latin America (about 2–4 per 1,000), high in sub-Saharan Africa (about 15 per 1,000), and at an intermediate level in Europe (about 7 per 1,000) (10). The rate has steadily increased in the United States from 9.5 twin deliveries per 1,000 in 1980, to 33.5 twin deliveries per 1,000 in 2015 (6,15). It has roughly doubled in many other developed countries over the same time period.

In Hungary, the prevalence of twin pregnancies was 22.2‰ in 1990, while in 2012 it was 32.2‰ in relation to all live births (16,17).

Proper uterine and placental vascularization is important for the normal development of pregnancies (18,19). Three-dimensional power Doppler has enabled us to study the morphology of the vascular tree *in vivo* and to quantify the direct blood flow of the placenta (20,21,22,23).

It is unclear whether there is difference in placental three-dimensional power Doppler vascularization indices depending on the chorionicity of twin pregnancies (monochorionic or dichorionic), and it is unknown whether there are correlations between placental vascularization indices and birth weight when comparing twins. There are no published reports comparing three-dimensional power Doppler placental vascularization indices between singular and twin pregnancies.

We expected that placental three-dimensional power Doppler vascularization will differ between normal twin pregnancies whether they are monochorionic or dichorionic. We also hypothesized that there are correlations between placental three-dimensional power Doppler vascularization indices and birth weight, APGAR score, umbilical pH or acid-base balance. Our additional hypothesis was that normal singular pregnancies have higher three-dimensional power Doppler placental vascularization indices than normal twin pregnancies.

The main purpose of the present pilot study was to clarify the potential roles of three-dimensional ultrasound and placental three-dimensional power Doppler vascularization indices in normal monochorionic-diamniotic and dichorionic-diamniotic twin pregnancies. Our second goal was to determine reference values for further investigations.

MATERIALS AND METHODS

We performed a prospective three-dimensional power Doppler study of placental vascularization indices. We included women with singular, and twin pregnancies seen once in the second or third trimester at our outpatient clinic at University of Szeged, Faculty of Medicine, Department of Obstetrics and Gynecology, in Szeged, Hungary. Our study was carried out between 1 March 2014 and 31 March 2016 in accordance with the Code of Ethics of the Declaration of Helsinki for scientific research involving humans, and our study was approved by the University of Szeged Committee for Regional Scientific and Research Ethics of Szent-Gyorgyi Albert Medical and Pharmacy Centre (No.: SZTE 32/2014). Informed consent was signed by the observed patient after a detailed and clear explanation about the conditions and aims of the study.

Selection of Patients for the Study

Inclusion criteria for twin pregnancies

In our study we analyzed twin pregnancies between 20 and 36 weeks of gestation, which were divided into two groups (*Table 1*). Gestational age was determined on the basis of the first day of the last menstrual period and on the basis of the first trimester ultrasound biometry of cases examined.

Chorionicity was based on the first-trimester ultrasound (between 11week+0day and 13week+6day) and was assigned according to the number of placental masses and T-, or λ-signs in the case of a single placental mass (24).

All twin pregnancies were spontaneously conceived.

Inclusion criteria for singular pregnancies

In our study we analyzed singular pregnancies between 20 and 36 weeks of gestation as well. See *Table 2*. Gestational age was determined on the basis of the first day of the last menstrual period and on the basis of the first trimester ultrasound biometry of cases examined.

Exclusion criteria

Exclusion criteria included; pregnancy hypertension, gestational-, and pre-gestational diabetes mellitus, thrombophilia, molar pregnancy, structural or chromosomal anomaly and fetal abnormalities (with 1-month follow-up after delivery); placenta previa; self-reported drug, alcohol, caffeine or nicotine abuse; exposure to circulatory medications (calcium dobesilate); systemic diseases (such as diabetes mellitus, chronic hypertension, vasculitis).

Methods

Ultrasound examination

Pregnant women who visited our outpatient clinic had been prospectively enrolled into 3 groups, based on 11th-13th week ultrasound examination (25), as you can see in *Table 1*. Those who did not meet the criteria of the study design during the study period were excluded (N=3) because of developing twin-to-twin transfusion syndrome, intrauterine death from placental abruption, and loss to follow up.

All patients were scanned in a semi-recumbent position. The same pre-established instrument power settings were used in all cases ("Obstetrics/2–3 trimester" in two-dimensional mode). A conventional two-dimensional ultrasound study provided data about the position and presentation of the fetuses, fetal heart rate, localization of the placenta(s), insertion points of the umbilical cords, and volume of amniotic fluid.

Two-dimensional ultrasound was followed by fatal biometry of fetus(es) ("A" and "B") to assess biparietal diameter, head circumference, abdominal circumference, femur length in order to calculate estimated fetal weight using formula B of Hadlock (26). Fetuses from twin pregnancies were matched to their prenatal identification, but it often meant that we had to break with the obstetric tradition of calling the first-born: Baby "A". That is why we introduced the terms "smaller fetus" and "larger fetus" instead of fetus "A" and "B".

The next step was the three-dimensional scan of the placenta(s) fitted with power Doppler method at the insertion points of the umbilical cords of each fetus separately. We used three-dimensional rendering mode, in which the color and gray value information were processed and combined to give a three-dimensional image (27,28,29). Power Doppler window (pulse repetition frequency at 900Hz and wall filter of 50Hz) was placed over the placenta(s), mapping the vascular tree from basal to chorionic plates at both umbilical cord insertion(s). This technique shows higher sensitivity as it is based on amplitude instead of mean frequencies to depict the vascular tree. Moreover, color mapping is independent of the angle of insonation and does not show 'aliasing'. However, it is more sensitive to patient movements, so the volumes should be acquired while avoiding any probe or patient movements; otherwise artifacts could be present. (30,31). The sweep angle was set at maximum 70°, and we used fast-low resolution acquisition to avoid any kind of artifacts.

All two-dimensional and three-dimensional ultrasound scans were performed by the same experienced sonographer using the Voluson 730 system (RAB 2-5 MHz transducer, GE Healthcare, Kretztechnik, Zipf, Austria).

Calculation of three-dimensional power Doppler indices

Volume files were analyzed using the Virtual Organ Computer-aided AnaLyses software pertaining to the computer software 4D VIEW (GE Medical Systems, Zipf Austria, version 10.4) by an expert in three dimensional-analysis.

We used Mercé-type sono-biopsy (31), a reproducible, valid alternative for evaluation of the vascular tree of the entire placenta (30,32). See *Figure 1*. The spherical sample volume was 28 mL constantly, and the Virtual Organ Computer-aided AnaLyses software automatically calculated the vascularization indices (vascularization index, flow index and vascularization-flow index) from the acquired spherical sample volume in all cases (31).

The two-dimensional and three-dimensional ultrasound acquisitions were performed at the same time, and three-dimensional volume files were analyzed by Virtual Organ Computer-aided AnaLyses software at a later time. The ultrasound images of two-dimensional and three-dimensional scans were stored on a hard disk.

Data collection after delivery

Data about neonatal outcome and mode of delivery were collected and classified after delivery. We collected data on umbilical cord pH, APGAR score, umbilical venous bicarbonate, lactate, base excess, birth weight, prolonged neonatal jaundice, and days spent in the neonatal intensive care unit.

Statistical analysis

Statistical analyses were performed with IBM SPSS Statistics 21.0 for Windows program (IBM, New York, USA). One-way variance analyses test results were significant for our database demonstrating that our study samples were not normally distributed. Continuous variables were expressed as mean ± standard deviation.

Comparisons of maternal age, gestational age, and birth weight were analyzed by Bonferroni correction ($p<0.05$).

The Kruskal-Wallis test was used for the comparisons of continuous variables in the three groups examined, and comparison between the three groups was performed with analyses of variance in case of vascularization indices, (level of significance was set at $p<0.01$). Univariate comparisons for categorical variables were assessed with Bartlett's test for equal variances $\chi 2$ tests. Linear regression coefficient values and equations depending on gestational age were also calculated for vascularization-, flow-, and vascularization-flow index for the groups.

Two-sample t test was applied to analyze the influence of maternal body mass index on placental three-dimensional power Doppler vascularization indices.

The associations between placental three-dimensional power Doppler indices, neonatal birth weight and pregnancy outcome were determined by Spearman’s rank correlations and multiple regression correlation analysis ANOVA.

RESULTS

Placental vascularization indices

The analysis of three-dimensional volume acquisition demonstrated that placental vascularization indices are significantly lower in monochorionic-diamniotic and dichorionic-diamniotic normal twins compared to normal singular pregnancies. See *Table 3.*:

- For vascularization index, level of significance was *p=0.00* in monochorionic-diamniotic or dichorionic-diamniotic normal twins compared to normal singular pregnancies.
- For flow index, level of significance was *p=0.00* in monochorionic-diamniotic or dichorionic-diamniotic normal twins compared to normal singular pregnancies.
- For vascularization-flow index, level of significance was *p=0.00* in monochorionic-diamniotic or dichorionic-diamniotic normal twins compared to normal singular pregnancies.

There were no significant differences in three-dimensional power Doppler vascularization indices between smaller and larger fetuses within the groups.

All placental vascular indices estimated by three-dimensional power Doppler ultrasonography presented a constant distribution throughout gestation in cases of normal singular pregnancies. See *Figure 2.*

Maternal characteristics

There was no significant difference in mean maternal age between the groups examined. For mean gestational age at the time of the three-dimensional scan and mean gestational age at delivery, the differences were statistically significant (*p<0.00*). See *Table 4.*

Statistical analyses of maternal pregestational body mass index discovered a strong influence on placental vascularization indices (vascularization index *p=0.00*; flow index *p=0.00*; vascularization-flow index *p= 0.00*).

Mode of delivery is demonstrated in *Figure 3*.

Cesarean sections of monochorionic-diamniotic cases were performed under spinal anesthesia in 76.9% (10/13), epidural anesthesia in 15% (2/13), and intubation anesthesia in 7% (1/13) of patients. Cesarean sections of dichorionic-diamniotic cases were performed under spinal anesthesia in 97% (33/34) and intubation anesthesia in 3% (1/33) of patients.

Neonatal characteristics

Neonatal characteristics are shown in *Table 5*.

No statistically significant differences were found ($p>0.05$) in umbilical cord pH, APGAR score, umbilical venous bicarbonate, lactate and base excess between the groups examined. There were no significant differences in birth weight between smaller and larger fetuses in monochorionic-diamniotic and dichorionic-diamniotic normal twin pregnancies.

There were no characteristic differences in prolonged neonatal jaundice or transportation to the neonate intensive care unit between the groups examined.

COMMENT

In our prospective study of vascularization analysis, we examined *in-vivo* placental function in monochorionic-diamniotic and dichorionic-diamniotic normal twins, and compared it to that in normal singular pregnancies.

This is the first study that compare~~s~~ three-dimensional power Doppler placental vascularization indices between normal singular and twin pregnancies. It was unclear whether or not there would be differences in placental vascularization indices depending on the chorionicity of twin pregnancies, and it was unknown whether there would be correlations between placental vascularization indices and birth weight when comparing monochorionic-diamniotic and dichorionic-diamniotic normal twins.

We hypothesized, but found no significant differences in umbilical pH, APGAR score, and acid-base balance such as umbilical venous bicarbonate, lactate and base excess between normal singular and normal monochorionic-diamniotic, or dichorionic-diamniotic twin pregnancies. There were no characteristic differences in prolonged neonatal jaundice and transportation to the neonate intensive care unit between the groups examined, as all neonates were born from normal, non-pathologic singular or twin pregnancies.

We found that placental vascularization indices are significantly lower in monochorionic-diamniotic and dichorionic-diamniotic normal twins compared to normal singular pregnancies as hypothesized. Our findings are in accordance with those of Papageorghiou et al., as the effect of fetal number is independent of chorionicity (33). We expected that placental vascularization will differ between normal twin pregnancies whether they are monochorionic-diamniotic or dichorionic-diamniotic, but we found that there are no statistical differences between the groups.

From our point of view the reason for this is that all twin pregnancies were non-pathologic. No statistically significant differences were observed in vascularization indices between smaller and larger fetuses or fetus “A” and “B” of monochorionic-diamniotic and dichorionic-diamniotic normal twin pregnancies.

Previous studies already reported, there was no statistically significant difference for *in-vivo* placental function at mean gestational age when the three-dimensional performed (29,34).

We found strong linear correlations between placental vascularization indices and birth weight because when the placental vascularization is lower, the birth weight will be smaller. We also found strong correlations between maternal pregestational body mass index and placental vascularization indices, therefore estimated fetal weight should also have a correlation with maternal pregestational body mass index.

From our point of view the differences in mean gestational age at delivery was mainly because of the common placenta. The common placenta may have different developmental potential, as the embryos separate from each other after or around implantation, thus implant to the same location (same side of the uterine wall) of the uterine cavity.

The main limitation of our study is the small number of patients in the case groups examined, especially in case of monochorionic-diamniotic twins. We could not take into account the differences in gender in case of dichorionic-diamniotic twins. Male and female fetuses were not being separated in case of singular pregnancies either, although that may have some influence on birth weight.

Limitations of the used method are that the measurements are time-consuming, and patients should lay still during volume acquisition. Pulse repetition frequency is crucial, thus same pre-established instrument power settings should be used during all examinations.

Our goal was to examine placental vascularization of monochorionic-diamniotic and dichorionic-diamniotic normal twin pregnancies in the 2nd and 3rd trimesters, and to provide reference ranges for further investigations.

Our results demonstrated that placental vascularization indices are significantly lower in monochorionic-diamniotic and dichorionic-diamniotic normal twins compared to normal singular pregnancies, and that there are strong correlations between placental vascularization indices and birth weight in cases of monochorionic-diamniotic, and dichorionic-diamniotic normal twin pregnancies.

We found that for early detection of discordance between twin fetuses, three-dimensional power Doppler indices may be useful for observing the most frequent fetal complications, and thus can help us make decisions about management and monitoring strategy.

CONFLICTS OF INTEREST NOTIFICATION

The authors report no conflicts of interest.

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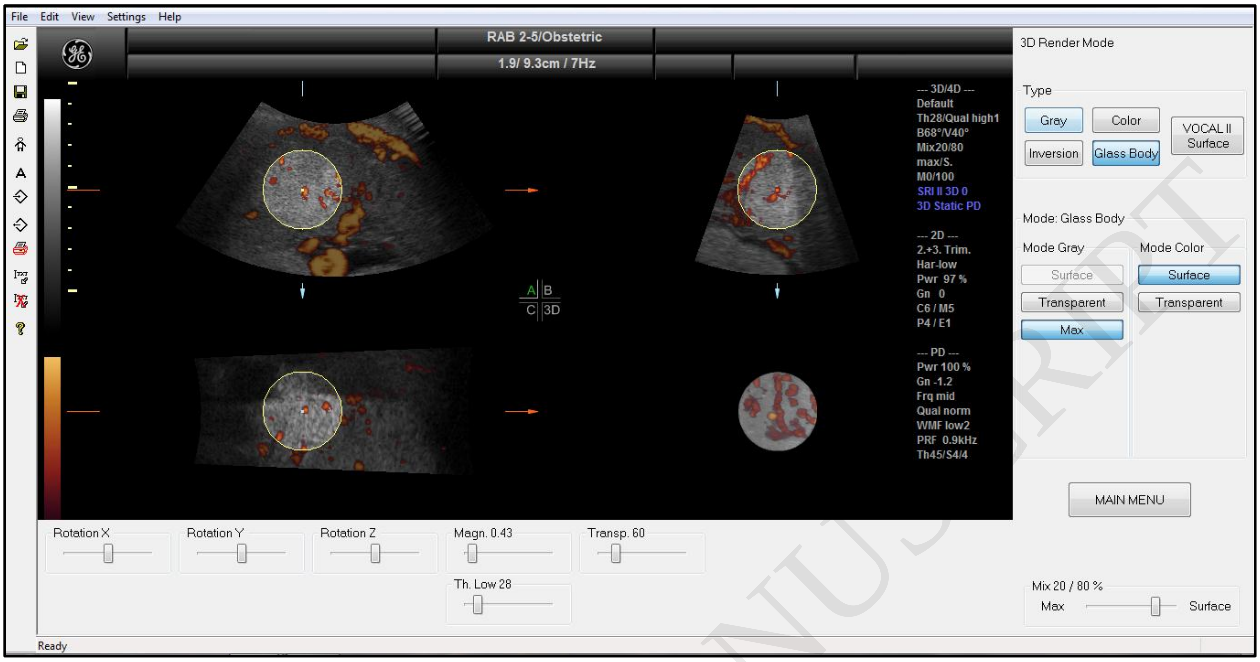

Figure 1. Interface of Virtual Organ Computer-aided AnaLyses (VOCAL) software presenting placental sono-biopsy at umbilical cord insertion (32^{nd} week of gestation).

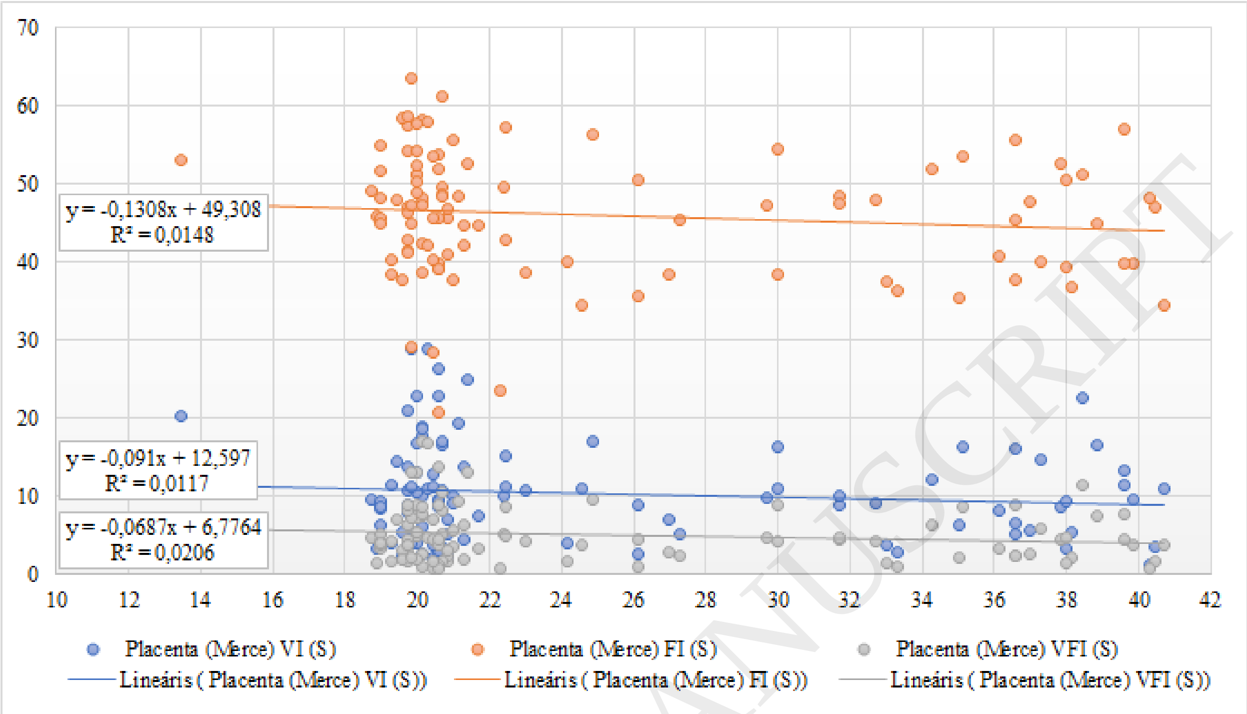

Figure 2. Placental vascular indices (vascularization index [VI], flow index [FI], and vascularization-flow index [VFI]), estimated by 3-Dimensional Power Doppler and Mercé sono-biopsy, calculated by Virtual Organ Computer-aided AnaLyses software, from 20 weeks of gestation until term in case of uncomplicated singular (S) pregnancies. These indices presented a constant distribution throughout the entire pregnancy.

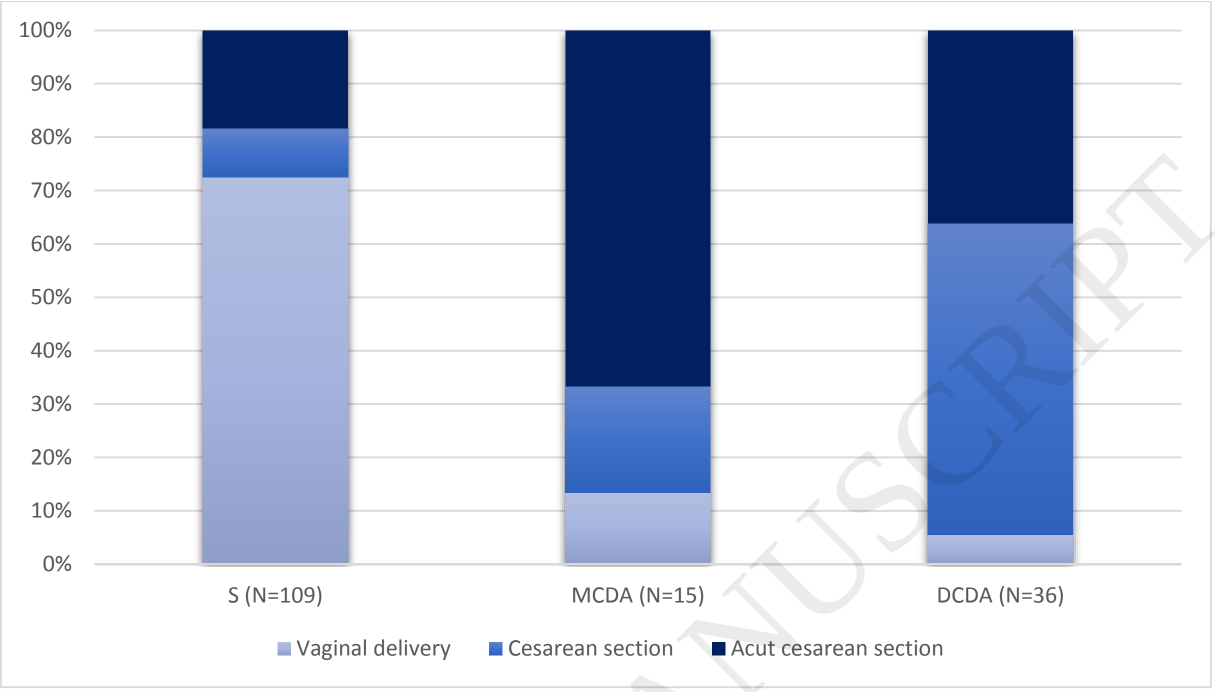

Figure 3. Mode of delivery of normal singular (S) pregnancies and of monochorionic (MCDA) or dichorionic (DCDA) diamniotic normal twin pregnancies.

Table 1. Placentation in monozygotic twins	
Diplacental dichorionic diamniotic	Separation takes place within a few hours after the zygote has formed.
Monoplacental dichorionic diamniotic	As a result of partial separation.
Monoplacental monochorionic diamniotic	Separation takes place after differentiation of the trophoblast.
Monoplacental monochorionic monoamniotic	The embryoblast separates immediately before or after implantation

Table 1. Placentation in monozygotic twin pregnancies (13,14)

Table 2. Groups of pregnancies examined		No. of cases
Singular	group of normal singular pregnancies	109
Monochorionic diamniotic	group of monochorionic diamniotic twin pregnancies	15
Dichorionic diamniotic	group of dichorionic diamniotic twin pregnancies	36

Table 2. Groups of pregnancies and number of cases examined: normal singular pregnancies and monochorionic or dichorionic diamniotic normal twin pregnancies.

Table 3.	Singular (N=109)	Monochorionic diamniotic (N=15)		Dichorionic diamniotic (N=36)	
		smaller fetus	*larger fetus*	*smaller fetus*	*larger fetus*
VI (mean ± SD)	10.36±6.19	5.80±3.07	6.60±3.52	6.73±4.67	7.49±4.91
FI (mean ± SD)	46.08±7.75	38.37±6.80	39.32±8.93	38.87±8.33	41.56±4.91
VFI (mean ± SD)	5.07±3.08	2.37±1.60	2.75±1.70	3.02±3.50	3.10±1.90

Table 3. Vascularization index (VI), flow index (FI), and vascularization-flow index (VFI) in normal singular pregnancies and in monochorionic or dichorionic diamniotic normal twin pregnancies. For VI, FI and VFI, level of significance was *p=0.00* in monochorionic or dichorionic normal twins compared to normal singular pregnancies.

Table 4.	Singular (N=109)	Monochorionic diamniotic (N=15)	Dichorionic diamniotic (N=36)
Mean maternal age (*years; mean±S/D*)	30.73±4.78	30.64±5.22	32.20±3.95
Mean gestational age at the time of 3D scan (*weeks^{+days}; mean±S/D*)	24^{+6}±7^{+2}	30^{+1}±10^{+6}	27^{+3}±7^{+1}
Mean gestational age at the time of delivery (*weeks^{+days}; mean±S/D*)	38^{+4}±1^{+6}	34^{+1}±2^{+4} *	36^{+5}±1^{+4} *
Mean pregestational body mass index *(kg/m^2; mean±S/D)*	30.72±5.19	22.52±2.39 *	25.45±4.14 *

Table 4. Examination of maternal characteristics of normal singular pregnancies and of monochorionic or dichorionic diamniotic normal twin pregnancies. * level of significance: p=0.00

Table 5.	Singular (N=109)	Monochorionic diamniotic		Dichorionic diamniotic	
		smaller fetus (N=15)	*larger fetus* (N=15)	*smaller fetus* (N=36)	*larger fetus* (N=36)
1' APGAR score	9.07±1.48	6.87±1.65	7.00±1.88	9.00±0.97	9.07±1.04
5' APGAR score	9.81±0.31	8.57±0.75	8.71±1.20	9.59±0.53	9.68±0.62
10' APGAR score	9.93±0.21	8.92±0.73	9.07±0.82	9.95±0.19	9.89±0.21
Umbilical pH	7.26±0.07	7.25±0.06	7.25±0.03	7.27±0.10	7.27±0.09
Umbilical venous bicarbonate (mEq/L)	20.2±2.67	17.90±3.06	18.08±2.56	18.45±2.66	18.62±2.61
Lactate (mmol/L)	1.85±0.64	1.90±0.77	1.72±0.53	2.01±0.44	1.73±0.55
Base excess	-5.4±3.51	-7.12±3.21	-6.92±3.53	-7.04±4.83	-6.20±3.75
Birth weight (g); (percentile): at average gestational age at birth	3357±555 (50-75)	1911±542 (10-25)	2087±505 (25-50)	2262±422 (10-25)	2555±429 (25-50)
Mean gestational age at birth (*weeks^{+days}; mean±S/D*)	38^{+4}±1^{+6}	34^{+1}±2^{+4}		36^{+5}±1^{+4}	

Table 5. Examination of neonatal characteristics of normal singular pregnancies and of monochorionic or dichorionic diamniotic normal twin pregnancies. Birth weight percentile refers to the average gestational age at the time of delivery /Singular: 38 weeks, Monochorionic diamniotic: 34 weeks, Dichorionic diamniotic: 36 weeks/.