EXAMINATIONS OF PLACENTAL THREE-DIMENSIONAL POWER DOPPLER INDICES IN PREGNANCIES COMPLICATED BY DIABETES MELLITUS AND INTRAUTERINE GROWTH RESTRICTION

Ph.D. Thesis

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1. Abbreviations:

2-D	two-dimensional
2-DPD	two-dimensional power Doppler
2-DCD	two-dimensional color Doppler
3-D	three-dimensional
3-DPD	three-dimensional power Doppler
AC	abdominal circumference
AD	abdominal diameter
AFI	amniotic fluid index
AOR	adjusted odds ratio
BMI	body mass index (kg/m2)
BPD	biparietal diameter
CI	confidence interval
D	minimum telediastolic velocity (cm/s)
DM	diabetes mellitus
EFW	estimated fetal weight
FGF-2	fibroblast growth factor-2
FI	flow index
FL	femur length
GA	gestational age
GDM	gestational diabetes mellitus
HC	head circumference
HIV	human immunodeficiency virus
HRE	hypoxia-inducible factor responsive element
ICC	intra-class correlation coefficient
IGF	insulin-like growth factors
IGF-1	insulin-like growth factor 1
IGF-2	insulin-like growth factor 2
IUGR	intrauterine growth restriction
EDTA-Na_2	disodium ethylenediaminetetraacetate dihydrate
OGTT	oral glucose tolerance test
PD	power Doppler
PVS	placental volume sampling

GDM	gestational diabetes mellitus
PI	pulsatility index
RI	resistance index
S	maximum systolic velocity (cm/s)
S.D.	standard deviation
SGA	small for gestational age
T1DM	diabetes mellitus type 1
VEGF	vascular endothelial growth factor
VFI	vascularisation flow index
VI	vascularisation index (%)
VOCAL	virtual organ computer-aided analysis
vs	versus
wk	week
ys	years
WHO	World Health Organisation

2. Summary

A prospective study was carried out in order to examine placental vascularisation using 3-dimensional power Doppler (3-DPD) technique with Virtual Organ Computer-aided Analysis (VOCAL) program in the second and third trimester of pregnancies complicated by diabetes mellitus (DM) and intrauterine growth restriction (IUGR) and to compare them with those of the normal controls.

Conventional two-dimensional (2-D) ultrasound has been widely used for the evaluation of the placenta during pregnancy. This 2-D ultrasound evaluation includes the morphology, anatomy, location, implantation, anomaly, size, color/power and pulsed Doppler ultrasound assessment of the placenta. The 2-D ultrasound is useful to assess normal and abnormal placentas in most pregnancies. The three-dimensional (3-D) reconstruction of the placenta gives information about 3-D placental vasculature and placental blood flow. The quantitative 3-DPD histogram analysis by VOCAL program provides more details concerning qualitative assessments of the vascularisation and blood flow of the placenta. 3-DPD ultrasound examination can depict intraplacental vessel characteristics such as coiling of vessels, branching and changes in lumen by subjective assessment. We analyzed the alteration in vascularisation by an objective evaluation method using the VOCAL program.

A consecutive series of pregnant women was recruited at the maternity outpatient clinic between 2011 and 2013 at Department of Obstetrics and Gynecology, Szeged, Hungary.
a, In the GDM/T1DM study: pregnancies complicated by gestational diabetes mellitus (GDM) (n=56) and diabetes mellitus type-I (T1DM) (n=43) were compared to uncomplicated pregnancies (n=113).
b, In the IUGR study pregnancies were divided into two groups: non-pathological control group (n=171) and IUGR group (n=52).

For 2-D, 3-D and color Doppler examinations Voluson 730 ultrasound equipment (GE Medical System, Kretztechnik, Austria) and RAB 2-5 MHz convex transducer were used. The vascularisation of placentas was assessed in the second and third trimester of pregnancies complicated by GDM/T1DM and IUGR as well as of normal pregnancies using 3-DPD technique. For „placental vascular biopsy" we applied the *„Mercé-type sonobiopsy"* at insertion of the umbilical cord, the most vascularised part of the placenta. The stored 3-D volume images were analyzed with VOCAL program pertaining to the computer software 4-D View (GE Medical Systems, Austria, version 10.4). The VOCAL program calculates automatically the indices from gray-scale and color values of the acquired spherical sample. The calculated indices were: a) vascularisation index (VI), b) flow index (FI) and c)

vascularisation flow index (VFI) of the placenta. The statistical analysis was performed with the help of SPSS for Windows 17.0 program.

In case of IUGR and diabetic patients, significant deterioration of VI, FI and VFI occurred compared to the control group. The 3-DPD indices were not significantly different between the two diabetic subgroups. It can be established that all 3-DPD indices were constant during the progress of normal pregnancies. Placental vascularisation in pregnancies complicated by IUGR is lower even in the first trimester of gestation. In diabetic pregnancies despite of the hypervascularisation of villi low placental vascularisation is measured because of placental edema.

In conclusion, 3-DPD assessment of placental vascularisation may provide new insights into normal and abnormal fetoplacental hemodynamics.

3. Introduction

The placenta is the organ responsible for the transfer of almost all the nutrients and gases between mother and fetus. Thus, the placenta plays a fundamental role. It allows the transfer of nutrients and oxygen from the mother to the fetus and the transfer of waste products and carbon dioxide back from the fetus to the maternal blood system. The placenta plays a pivotal role in excretion of urea, uric acid and creatinine from the fetus to the maternal blood. It takes part in immunity, it has endocrine function during pregnancy as well and provides a reservoir of blood for the fetus. The human placenta is a highly invasive and proliferative structure during the first half of pregnancy. The development and maturation of placenta and the maintenance of successful pregnancy are dependent on the proper proliferation and differentiation of the villous cytotrophoblast cells into maternal deciduas and myometrium in early pregnancy. This process leads to transformation of the spiral arteries supplying the intervillous space. These physiological changes alter the vascular supply to a low-pressure high-flow system, allowing adequate flow to the developing villous circulation, which undergoes progressive arborisation until late pregnancy in the third trimester. Consequently, all of the respiratory gases, nutrients, and waste products that are exchanged between the material and fetal system are transported via placenta. The importance of transplacental exchange in supplying metabolic substrates required for fetal growth is apparent and has long been recognized [1, 2]. Fetal growth is the result of complex cascade of processes which requires coordination of components within the maternal, placental and fetal compartments (see *Table 1*)**.** Placental dysfunction is known to be a major cause of complications during pregnancy, such as intrauterine growth restriction [3, 4]. The alteration of the uteroplacental circulation leads to fetal hypoxia. It is followed by circulatory redistribution for the sake of substantial organs such as brain, heart and adrenal glands. The fetus stops or reduces weight-gain.

In diabetes mellitus, the placenta undergoes a variety of structural and functional changes [5-7]. Their nature and extent depend on a range of variables including the quality of glycemic control achieved during the critical periods in placental development, the modality of treatment, and the time period of severe deviation from excellent metabolic control of a non-diabetic environment. In pregnancies with diabetes, the concentration of insulin in the fetoplacental circulation is increased due to hyperglycemia. High concentrations of insulin and insulin-like growth factors (IGFs) in maternal serum in pregnancy have been associated with high birth-weight in offspring of women both with and without diabetes [8, 9]. In pregnancy these factors are synthesized in the placenta and they also regulate placental

growth. It is conceivable that there is a relatively larger growth of the placenta than of the offspring.

Pregnancy is a state of requirement of higher amount of oxygen. Women with diabetes have an increased prevalence of cardiovascular disease and atherosclerosis [10, 11]. Atherosclerosis may impair the oxygenation of the tissues and, in pregnancy, may increase the risk of uteroplacental hypoxia. Hypoxia stimulates angiogenesis [12, 13] and it is conceivable that uteroplacental hypoxia initiates placental angiogenesis and thereby placental growth [12]. The severity of maternal vascular dysfunction is likely to be associated with the duration of diabetes. The presence of atherosclerosis is associated with the duration of diabetes [10]. Diabetes, independent of type, may also cause low grade inflammation with micro- and macrovascular complications associated with hypoxia [11]. Inflammation not only on the maternal site but also in the placenta has been reported in diabetic pregnancies, and such inflammation may cause placental dysfunction and activate angiogenesis [14]. Large offspring may have increased need for oxygen and nutrition and such a need may initiate compensatory growth of the placenta. Also, a large placenta in itself may have an increased need for oxygen and nutrition. Thus a large placenta is dysfunctional relative to the fetal needs, leading to a compensatory placental growth. Histological abnormalities with immature villi are mentioned in pregnancies with diabetes [15]. Placental villous immaturity and large placentas have been seen in well controlled diabetic pregnancies [16], suggesting that there may be a compensatory growth of placenta with immature villi, independent on the maternal glucose concentrations.

Table 1. ***Maternal***, ***fetal*** *and* ***uteroplacental factors*** *responsible for development of* ***IUGR***

I. **Maternal factors**:

1. ***General and constitutional factors****:*

-age (<20 and >30 years)
-mother's weight before pregnancy (<50 kg)-mother's height (<150cm)
-mother's birth weight
-ethnic/racial characteristics (e.g. in case of Roma ethnicity in our country)

2. ***Social and economic situation***:

-no education
-dangerous physical work
-single mother
-maternal malnutrition

3. ***Geographical and climatic relations***:

-sea level (the more its value is, the more frequently IUGR occurs)
-climatic conditions

4. ***Toxic substances***:

-smoking, alcohol, caffeine (7-8 coffee/day), drugs

5. ***Medical treatment of the pregnant woman***:

-tetracyclines
-anticoagulants (Warfarin)
-folic acid antagonist (aminopterin)
-hydantoin anticonvulsants
-lithium

6. ***Maternal diseases*****:**

-chronic kidney disease
-preeclampsia
-chronic hypertension
-maternal hyperinsulinemia
-chronic cardiopulmonary disease
-anaemia
-urinary infection
-autoimmune diseases (Systemic Lupus Erythematosus)

II. **Fetal factors**:

-fetal sex
-chromosomal abnormality
(autosomal genetic disorders: Down, Edwards, Patau syndromes)
(gender dependant genetic disorders: Turner, Multiple X syndrome)
-genetic syndromes: (bone dysplasia)
-intrauterine infection
-multiple pregnancy
-immunological factor
-time between pregnancies (weight loss <2 or >6 years)

III. **Uteroplacental factors:**

-uterine malformations
-placental anomalies
-abnormal localisation of placenta (placenta previa)
-tumor (haemangioma)
-single umbilical artery (SUA)
-placental mosaicism

3.1. GDM/T1DM

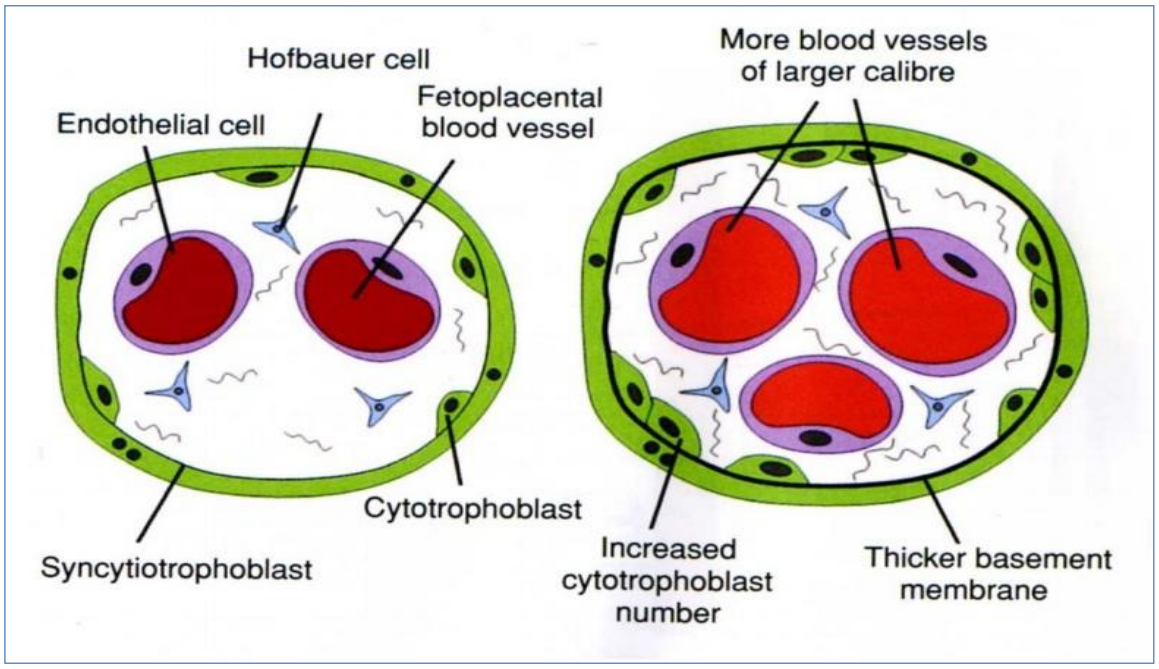
The placenta fulfils a fundamental role sustaining adequate fetal growth, and it has been implicated in fetal growth and has been associated with maternal diabetes. Various morphological and functional placental changes can be observed in these pregnancies as well. They depend on period in gestation when the diabetic insult manifests itself [17]. In our study diabetes mellitus type 1 (T1DM) has been already diagnosed before pregnancy and GDM is discovered for the first time during pregnancy. GDM is a pathological condition, in which women without previously diagnosed diabetes exhibit high blood glucose levels during pregnancy, irrespective of whether it is treated with diet or insulin, which disappears or maintains after pregnancy [18, 19]. In addition to these facts, a transient self-limiting state of hyperglycemia may occur in pregnancy as a result of maternal endocrine changes. Glucose homeostasis is maintained by the balance between insulin, which reduces glucose levels by increasing cellular uptake, and other hormones such as glucagon and cortisol, which increase glucose production. Carbohydrate intolerance develops often during pregnancy. The placenta produces additional cortisol as well as other insulin antagonists such as human placental lactogen, progesterone, human chorionic gonadotropin, insulysin, all of which tend to increase the maternal glucose level [20]. If the pancreatic B islet cells are unable to produce sufficient insulin to balance this increase or if there is maternal insulin resistance, the mother may develop a state of hyperglycemia referred to as gestational diabetes [21]. Several risk factors are associated with the development of GDM (see *Table 2*).

*Table 2. The most common **risk factors** which are associated with the **development of GDM***

-non-Caucasian women,	-former fetal malformation
-high maternal age (>35 years)	-intrauterine death
-obesity (BMI>30kg/m^2)	-habitual abortion
-familial diabetes	-hypertension
-glycosuria during pregnancy	
-previous birth of a macrosomic (birth weight at the term is ≥4000 grams) or a large for gestational age neonate (above the 90 percentile)	

Excess placental weight in diabetes correlates with antenatal glucose control. The increase in tissue mass results from overproduction of extracellular matrix worsened by interstitial edema [17]. Villous immaturity and cytotrophoblasts that are more apparent by histological investigation, for any given gestational age (GA) are typical in the diabetic placenta [17]. The etiology of this cytotrophoblast prominence is unclear, but may relate to cytotrophoblast fusion to form syncytiotrophoblast. Basement membranes of the syncytiotrophoblast and endothelium are thickened in pre-gestational and GDM and this likely reduces villous diffusion capacity for oxygen. Hypervascularisation of the villi is also a common finding. In T1DM this secondary feature leads to enhanced longitudinal growth of vessels, whereas in GDM vascular branching is increased. The underlying mechanism for stimulation of branching or non-branching angiogenesis is unknown (see *Figure 2*) [17].

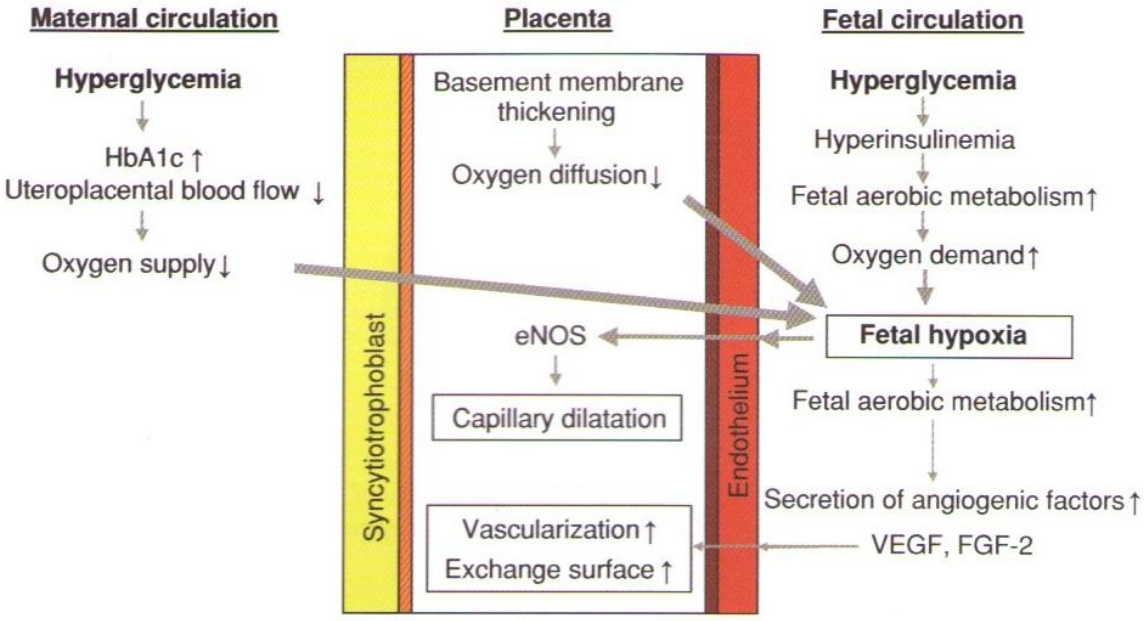
Figure 2. ***Characteristic changes*** *associated with* ***maternal diabetes*** *in* ***placental villi*** *at term of pregnancy [17]*



The enlargement of the vascular surface and also the placental surface area of exchange may be counterintuitive in a situation of maternal nutritional oversupply. However this may reflect to a response to inadequate oxygen supply to the fetus where fetal hypoxia is linked to polycythemia and elevated cord blood erythropoietin levels. Elevated maternal HbA1c levels with higher oxygen affinity, reduced uteroplacental blood flow, and a longer

maternal-to-fetal diffusion distance may diminish oxygen supply. At the same time fetal hyperinsulinemia stimulates aerobic metabolism, thus increasing oxygen demand. Low-fetal-oxygen levels may induce placental expression of pro-angiogenic factors, such as fibroblast growth factor-2 (FGF-2) and vascular endothelial growth factor (VEGF) [17]. The FGF-2, VEGF, leptin, insulin and insulin-like growth factors 1 and 2 (IGF1 and 2) stimulate mitogenic, angiogenic and invasive process [17]. The expansion of the vascular tree is also paralleled by an increase in capillary volume and diameter. This dilatation of the placental capillaries may again result from fetal hypoxia, because low oxygen is known to up-regulate eNOS expression via hypoxia-inducible factor responsive elements (HREs) enabling the production of higher levels of vasodilator NO (see *Figure 3*) [17].

Figure 3. ***Causes of fetal hypoxia*** *and* ***consequences in regard to placental morphology,*** *such as enlargement of vascular surface and capillary dilatation [17]*



Gestational diabetes results in variances in uteroplacental circulation [17] and in contrast with normal pregnancy it leads to fetal vascular redistribution, alterations in uteroplacental circulation can be detected in case of pregnancies complicated by IUGR as well, which can be demonstrated by blood flow indices using 3-D technology [22, 23].

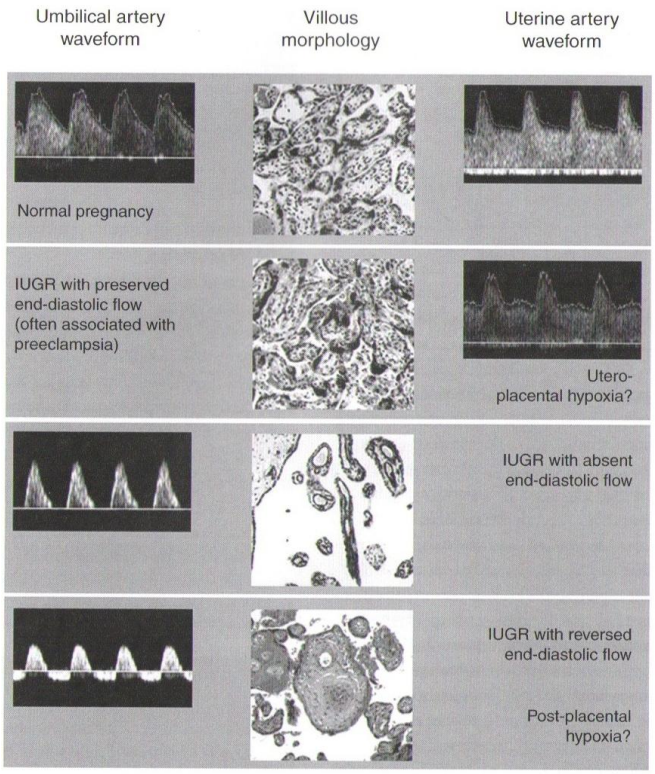
3.2. IUGR

The estimated fetal weight below the 10th percentile for gestational age is considered growth restricted [24, 25]. The frequency of intrauterine restriction is 8-10% in Hungary [24]. This ratio indicates similar ascending tendency in our country, such as in other parts of the world [26]. Perinatal complications happen 3-4 times more frequently than in case of eutrophic newborns at the same gestational age. Nearly 50% of premature infants are retarded as well [24]. 30% of still-born have IUGR [7]. Therefore IUGR is a clinically significant perinatal problem [28, 29].

Clinical appearance of IUGR primarily depends on the GA of the pregnancy when the different inducing factors are present as well as on the duration of damage to the fetus. Reasons for development of IUGR can be maternal, fetal, uteroplacental or any other reasons [29] (see *Table 1*). The decline of adaptive capacity of the fetus causes circulatory redistribution for the good of substantial organs such as brain, heart and adrenal glands *(„heart and brain sparing effect")* [30]. If the fetal circulation worsens further during pregnancy, the physiological compensatory mechanisms will be exhausted and hypoxic stress will be emerged eventuating in hypoglycemia, acidosis, thrombocytopenia and oliguria. The alteration of the uteroplacental circulation leads to fetal hypoxia, in consequence of which not only the pO2, but also the depot of glucose and glycogen will be reduced. Thus the fetal energy sparingly adapts to hypoxia and hypoglycaemia. The fetus stops or reduces weight-gain, the fetal activity is impaired and the IUGR comes into existence.

The main entity of IUGR development is characterized by the damage of the function of fetoplacental unit, which can have several effects including decrease of uteroplacental flow, insufficient metabolic process of the intervillous space and damage to the umbilical cord and fetal blood flow [29]. Reductions in uteroplacental blood flow and alterations in intervillous hemodynamics result from retained spiral arteriole contractility originating from inadequate remodelling by trophoblasts. Impairment in the development of placental vessels leads to diminution of fetal growth. The reduction in umbilical blood flow can be due to a decrease in uteroplacental blood flow, abnormal villous structure at the interface between the maternal and fetal circulation and primary abnormality in the umbilical-placental perfusion (see *Figure 4*) [30]. A decline in the number and surface area of the arterioles of the tertiary stem villi, a reduction in terminal capillary loops with elongated villi and a reduction in villous tree elaboration have been shown in IUGR [31, 32].

*Figure 4. The **relationship** between **Doppler flow velocities** of **umbilical** and **uterine artery** and **placental morphology of villi** [30]*



4. Aims of the investigation

The aims of our study were as follows:

4.1. GDM/T1DM

1. To evaluate the placental 3-DPD indices in pregnancies complicated by the alteration of carbohydrate metabolism (GDM and T1DM pregnancies) in the second and third trimester.
2. To observe the changes of 3-DPD indices by gestational age.
3. To test the hypothesis whether there is an association between 2-D color Doppler indices (2-DCD) of umbilical/uterine arterial flow imaging and placental 3-DPD indices both in T1DM and GDM as well as in the control group.

4.2. IUGR

1. To evaluate the placental 3-DPD indices in pregnancies complicated by IUGR in the second and third trimester.
2. To analyze the correlation between 2-DCD indices determined for conventional uterine/umbilical arterial flow imaging and 3-DPD indices in pregnancies complicated by IUGR.

3. To survey the effect of parity, gravidity and pregestational BMI on the alteration of vascularisation indices.

4. To examine perinatal outcomes in pregnancies complicated by IUGR.

5. Materials and methods

A consecutive series of pregnant women was recruited for a case-control study at the Department of Obstetrics and Gynecology, Szeged, Hungary from 2011-2013. We received informed written consent from all participants.

5.1. GDM/T1DM

The pregnancies were divided into two groups: I. non-pathological control group (n=113) and II. case group comprising pregnancies complicated by two subgroups of diabetes mellitus (DM) (n=99): II.a) DM (n=43) and II.b) GDM (N=56). GDM cases complied with White's class A1-A2, while DM cases belonged to DM type I (T1DM) with a good glycemic control (HbA1c: 20-42 mmol/mol) corresponding to White's class B-D [33]. No patient was classified in White's class R, F, RF, G, H and T [33]. *Table 3* contains the exclusion criteria.

*Table 3. **Exclusion criteria** of **GDM/T1DM** and **IUGR***

- multiple pregnancy
- enlarged (≥3mm) nuchal translucency from 11^{+0} to 13^{+6} weeks of gestation
- fetal or neonatal structural or chromosomal anomaly
- inadequate localization of the placenta (placenta previa)
- posterior placenta (did not belong to the exclusion criteria of IUGR)
- self-reported drugs, alcohol, caffeine or nicotine abuse
- exposure to circulatory medication (oxerutins, calcium dobesilate)
- not signing the consent form.
- diabetes accompanied by another systemic disease (autoimmune disease, vasculitis, haemophylia, thrombophylia, hypertension, HIV infection, etc.)
- diabetes mellitus (belonged to exclusion criteria of IUGR), abnormal HbA1c value

5.1.1. Diagnosis of GDM/T1DM

According to the recommendation of American Diabetes Association, GDM is a pathological condition, in which women without previously diagnosed diabetes exhibit high blood glucose levels during pregnancy, irrespective of whether it is treated with diet or insulin, which disappears or maintains after pregnancy [17, 18]. Diagnostic criteria for DM and GDM are based on World Health Organisation (WHO)'s guidelines, which correlates with the guideline of Hungarian College of Obstetricians and Gynecologists [18].

In our study oral glucose tolerance test (OGTT) was used between 24-28th weeks of gestation for diagnosing diabetes. 75 grams of glucose solution was consumed by the pregnant woman after a fasting period of 8 hours.

The glucose level in maternal serum (sample tube contains potassium oxalate and sodium fluoride/Na_2 EDTA) samples was measured at the start (0 min) and after 2 hours [17, 18]. Diagnostic criteria for GDM are set up, if a) the fasting blood glucose level is ≥ 7 mmol/l, or b) the fasting glucose is normal, but the postprandial 120-minute value is ≥7.8 mmol/l, or c) the random glucose level is ≥11.1 mmol/l which are measured twice [18, 34-36]. If women before subsequent pregnancy had been affected by DM, the OGTT was not performed, but dietary treatment was administered. High risk pregnancies for GDM (see *Table 2*) [33] were screened with OGTT between 12-16th weeks of gestation, and if the result was below the limit, then OGTT was repeated between 24-28th weeks of gestation. In our country the patients who do not have high risk for GDM are screened with OGTT when they are 24-28th weeks pregnant [17, 18, 37].

5.2. IUGR

The pregnancies were divided into two groups: non-pathological control group (n=171) and IUGR group (n=52) [24]. The exclusion criteria can be found in *Table 3*.

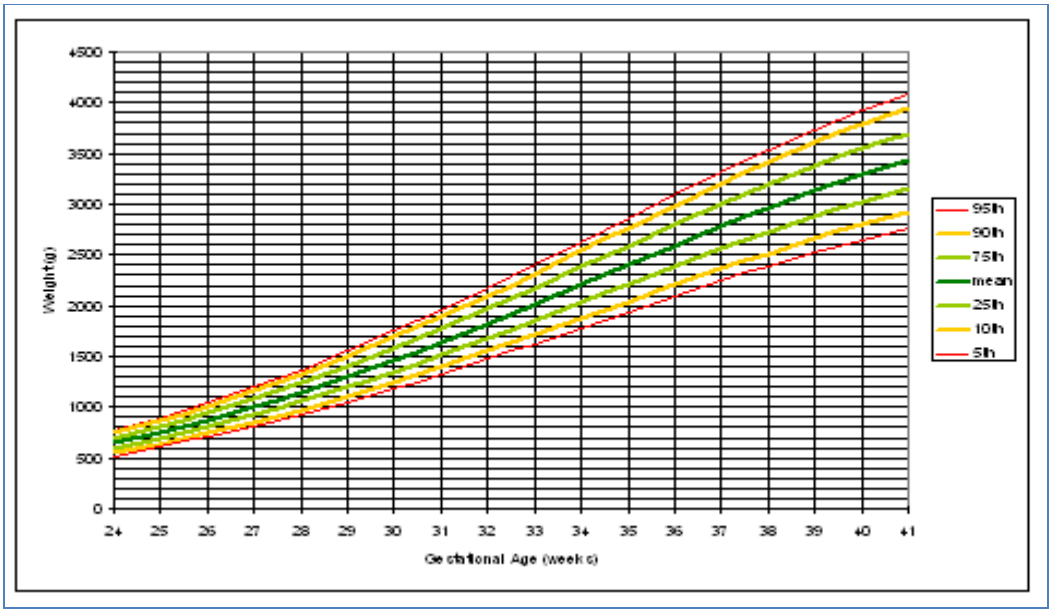
5.2.1. Diagnosis of IUGR

A fetus whose estimated weight is below the 10th percentile for its gestational age is considered as a fetus with IUGR *(Figure 5, 6)* [24, 38].

Figure 5. WHO: ***Weight percentile chart*** *(data).* The weight percentile illustration is based on fetal weight equation proposed by Hadlock et al. [42].

Weight percentile chart for the local population											
Gestational age	Percentile										
	99th	97th	95th	90th	75th	mean	25th	10th	5th	3rd	1st
24	820	786	768	741	695	644	593	547	520	502	468
25	957	918	897	865	812	752	692	639	607	586	547
26	1110	1064	1040	1003	941	872	803	741	703	679	634
27	1278	1225	1198	1155	1083	1004	924	853	810	782	730
28	1461	1401	1369	1320	1238	1147	1057	975	926	894	834
29	1658	1590	1554	1498	1405	1302	1199	1106	1051	1015	947
30	1869	1792	1751	1689	1584	1468	1352	1247	1184	1144	1067
31	2091	2005	1960	1890	1773	1643	1513	1395	1325	1280	1194
32	2324	2228	2178	2100	1970	1825	1681	1551	1473	1422	1327
33	2564	2459	2403	2317	2173	2014	1854	1711	1625	1569	1464
34	2809	2694	2632	2538	2381	2206	2032	1874	1780	1719	1604
35	3056	2930	2864	2761	2590	2400	2210	2039	1937	1870	1745
36	3301	3165	3093	2983	2798	2593	2387	2203	2092	2020	1885
37	3540	3395	3318	3199	3001	2781	2561	2362	2244	2167	2021
38	3770	3615	3533	3407	3196	2961	2727	2516	2390	2308	2153
39	3987	3823	3736	3603	3380	3132	2884	2660	2527	2440	2276
40	4186	4014	3923	3783	3549	3288	3028	2794	2653	2562	2390
41	4365	4185	4090	3944	3700	3428	3157	2913	2766	2671	2492

Figure 6. WHO: ***Weight percentile chart*** *(diagram).* The weight percentile illustration is based on fetal weight equation introduced by Hadlock et al. [42].



The term small for gestational age (SGA) defines an infant that has failed to achieve a weight threshold (10^{th} percentile). Using this threshold, 10 % of the normal population will be included by statistical definition and this group will comprise constitutionally small but

healthy babies. IUGR fetuses represent a subgroup of fetuses that have failed to reach their growth potential for various reasons. In other words, IUGR is the pathological counterpart of SGA [39].

The oldest of the clinical methods, namely abdominal palpation, is accomplished using the Leopold manoeuvres [40], but its ability to predict fetal weight is limited [41]. Abdominal palpation (measuring symphysis-fundal height) is a tool which raises suspicion of low birth weight. The most valuable method to define IUGR is the ultrasound examination. Parameters most commonly used for determining estimated fetal weight (EFW) by ultrasonography include biparietal diameter (BPD), head circumference (HC), abdominal circumference (AC) and femur length (FL) [42].

5.3. Conventional two-dimensional (2-D) and color Doppler investigations

The determination of GA was based on the first day of the last menstrual period and/or on ultrasound biometry (crown-rump length and biparietal diameter) at 10^{th} week of pregnancy. All patients were scanned in a semirecumbent position. An initial 2-D conventional study provided data about fetal position and presentation, body movements and fetal heart rate, placental localization and umbilical cord insertion. The factorial default setting "Obstetrics/2-3 trimester" was used in 2-D mode. The examination was followed by a fetal biometry to assess biparietal diameter, head circumference, abdominal circumference and femur length. Fetal weight was calculated by the formula B of Hadlock [42]. A conventional color Doppler study of umbilical and uterine arteries [43] were also performed and the flow S/D ratio and resistance index (RI) were calculated according to the formula RI=S–D/S, where S is the maximum systolic velocity and D is the minimum telediastolic velocity. Besides, we measured the pulsatility index (PI) with the help of the formula: PI=S-D/mean velocity. The indices were read from the reports of the display of ultrasound machine.

5.4. Three –dimensional (3-D) ultrasound evaluation of the placenta

3-DPD ultrasound as well as quantitative 3-DPD histogram analysis, quantitative and qualitative assessments of the vascularisation and blood flow of the placenta have become feasible as compared to 2-DCD imaging.

5.4.1. Three-dimensional power Doppler (3-DPD) ultrasound

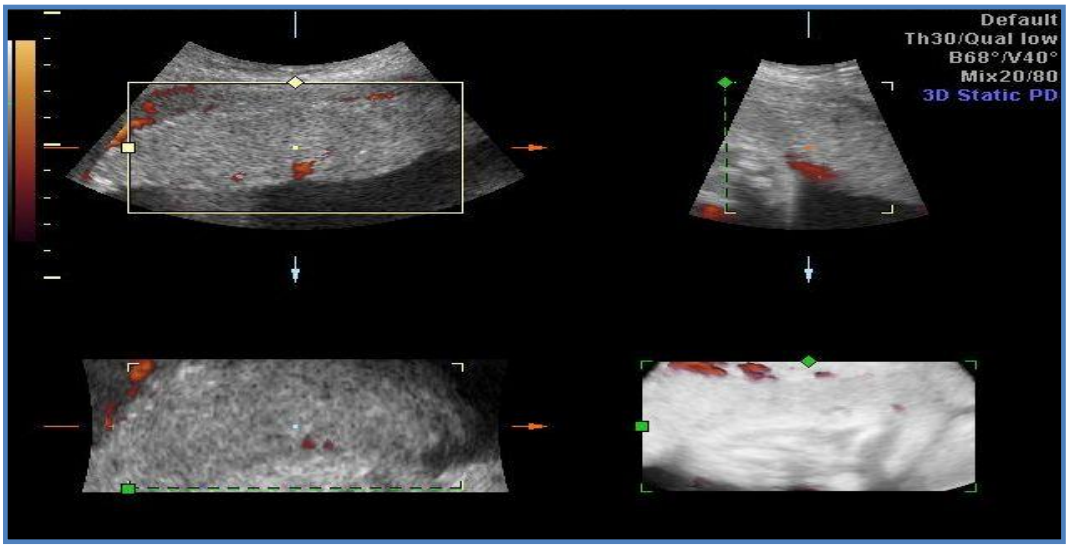
3-DPD ultrasound can depict intraplacental vessel characteristics such as density of vessels, branching and changes in lumen [1, 44-47]. 3-DPD ultrasound examination was

found to be more accurate than 2-D power Doppler (2-DPD) ultrasound examination for the detection of secondary and tertiary stem vessels in the placenta [46].

Total reduced placental vascularity and impaired budding of the villous circulation are predictive of those cases of IUGR not identified by either uterine or umbilical artery blood „flow velocity waveforms" [1]. This should be relevant in clinical practice, because incomplete placental development is generally associated with IUGR [44]. The sonographic presence of thick heterogeneous placenta is strongly associated with perinatal death, hypertensive disorders, IUGR, and preterm delivery [48]. 3-DPD ultrasound clearly shows reduced placental vascularity and impaired budding of the villous circulation in severe IUGR pregnancy with a thick heterogeneous placenta *(Figure 7)*. 3-DPD ultrasound may be useful in the future for diagnosing impaired vascularity associated with IUGR pregnancies.

3-DPD ultrasound plays an important role in the evaluation of vasa previa, it shows clearly a highly visualized placental chorioangioma and it is a useful technique to diagnose placenta accreta. Furthermore in monochorionic placenta vascular anastomosis (arterio-arterial, arteriovenous) can be visualized in detail as well [49].

Figure 7. ***Multiplanar display*** *of the* ***thick heterogeneous placenta*** *in a case of severe* ***fetal growth restriction*** *(<3 percentile) at 29 weeks of gestation*



5.4.2. Quantitative three-dimensional power Doppler (3-DPD) histogram analysis

3-D volume is constituted of small units of volume-„voxels". Voxels contain all the information about grey and color intensity scale ranging from 0 to 100. According to these values, three power Doppler indices suitable for evaluating vessels and blood flow can be derived from this measurement system. These 3-DPD indices are utilized to assess placental perfusion, and it has been accepted that these indices potentially reflect to both uteroplacental and fetoplacental blood flow [45, 50, 51].

The vascularisation index (VI), which refers to the color voxel/total voxel ratio, measures the number of color voxels in the studied volume and represents the blood vessels within the volume of interest, and expresses it as a percentage (vascularity). The flow index (FI) is the average color value of all the color voxels and it shows the average blood flow intensity from 0 to 100 (no unit). The vascularisation flow index (VFI), which refers to the weighted colour voxel/total voxel ratio, combines the information on vessel presence (vascularity) and amount of blood cells transported (no unit). The value is 0-100. [52, 53].

The assessment of placental perfusion using 3-DPD ultrasound examination is called 'placental vascular biopsy' [52], 'placental vascular sonobiopsy' (PVS) [54], or 'virtual placental biopsy' [51]. There are different kinds of methods applying PVS. Vascularisation indexes can be determined in the whole placenta [55-57] *(Figure 8)*, in 5 [58] *(Figure 9)* or 9-12 [54] *(Figure 10)* different parts of the placenta and in a 3x3x3mm volume of the placenta at insertion of umbilical cord [52] *(Figure 11)*. The VOCAL program calculates automatically the indices (VI, FI, VFI) from the acquired sample *(Figure 12)*.

Figure 8. ***Determination of placental indexes*** *in the* ***entire placenta*** *by Rizzo et al., de Paula et al. and Pomorski et al.[55-57]*

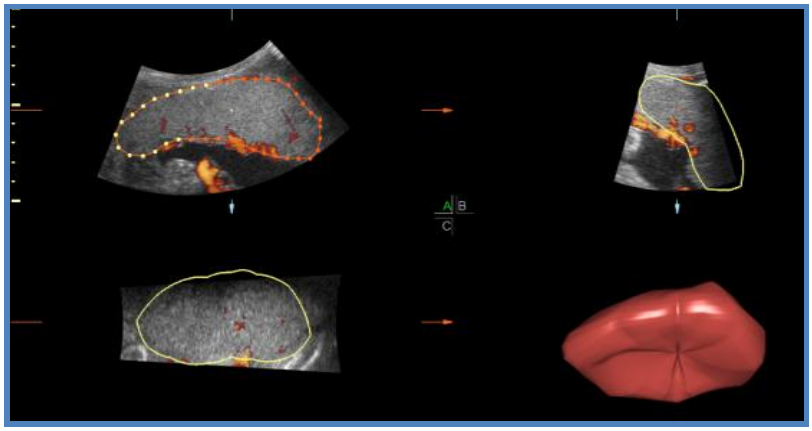


Figure 9. ***Determination of placental indexes*** *in* ***5 different parts of the placenta*** *by Guiot et al. [58]*

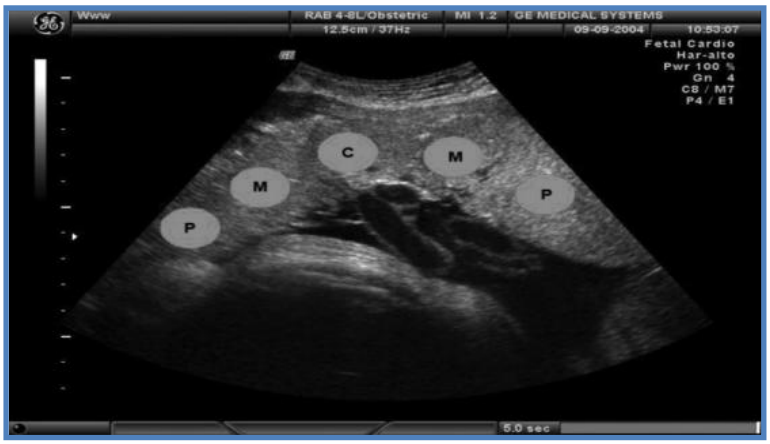


Figure 10. ***Determination of placental indexes*** *in* ***9-12 different parts of the placenta*** *by Noguchi et al. [54]*

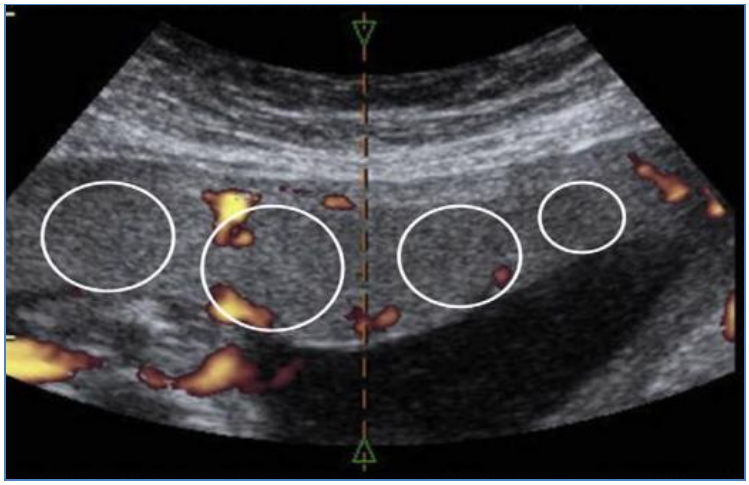


Figure 11. ***Determination of placental indices*** *by „****Mercé-type sonobiopsy****” [52]*

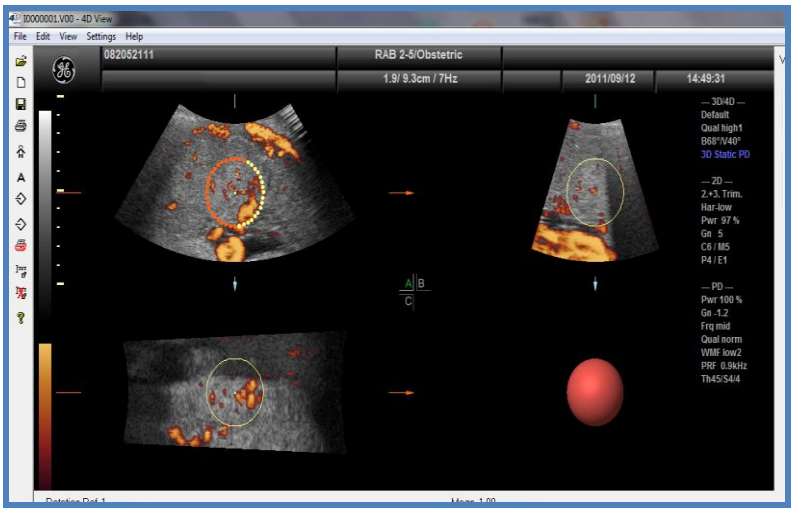
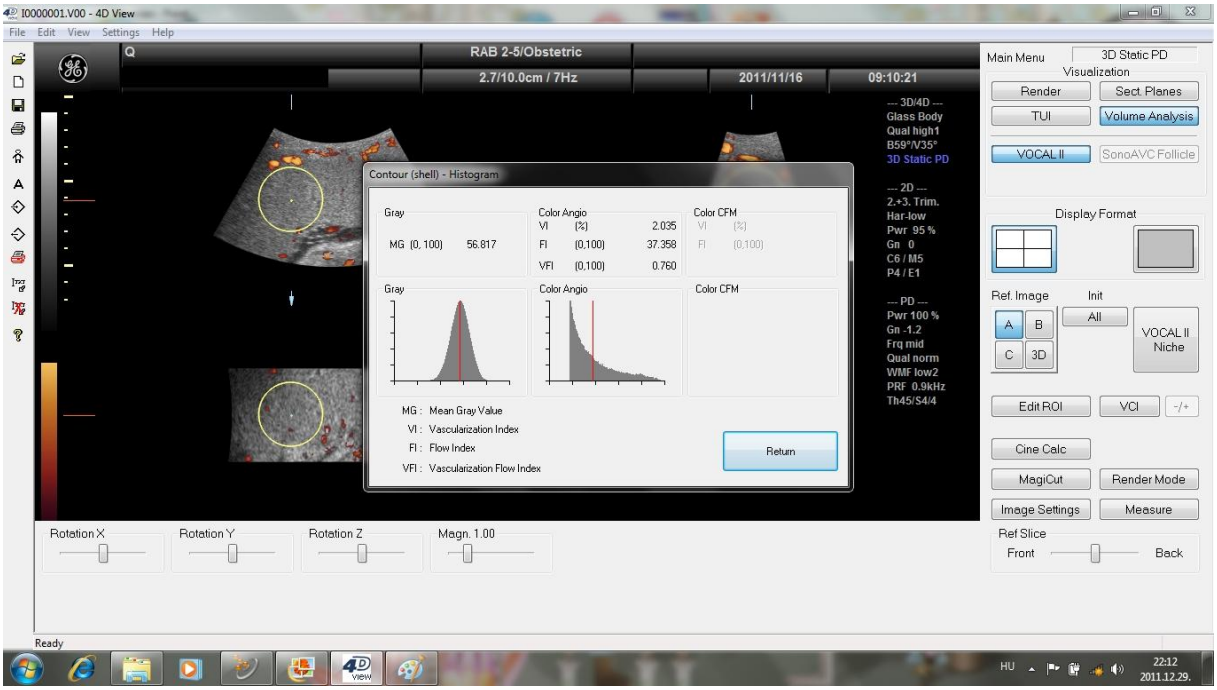


Figure 12. ***The results of histogram analysis*** *of the sphere volume obtained are shown automatically on the screen (Mercé-type sonobiopsy)*



Despite its usefulness, one study has focused on 3-D examination of the placenta in DM so far [57] and only quite a few studies in IUGR [54, 56, 58]. In 2012, *Rizzo et al*. performed 3-DPD ultrasonography of the placenta at 11+0-13+6 weeks in 32 pregnancies complicated by T1DM. Placental volume and vascularisation indices were calculated in the whole placenta [55]. In 2008, *Guiot et al* [58] performed examinations on 45 gravidas between 23-37th weeks of gestation, among which 15 pregnant women were enlisted in the group of physiological pregnancies and 30 pregnant women in the IUGR group. Placental vascularisation indices were assayed in 5 different parts of the placenta. In 2009, *Noguchi et al* [54] examined 221

placentas (normal group: 12-40 weeks of gestation, 208 pregnancies; IUGR group: 29-39 weeks of gestation, 13 pregnancies). Placental indices were calculated in 9-12 parts of the placenta. In 2011, *Pomorski et al* [56] involved 120 pregnant women in their studies between 22-42 gestational weeks (normal group: 100 gravidas, IUGR group: 20 gravidas). They measured the volume of the placenta and determined vascularisation indices in the whole placenta.

5.5. Volume acquisition

Acquisition of the images used for the determination of placental volume and 3-DPD indices was realized at the time of visit. All 3-D scans and the 2-D ultrasound measurements were achieved by Voluson 730 Expert ultrasound machine (GE Medical Systems, Kretztechnik GmbH&Co OHG, Austria) equipped with a multifrequency probe (2-5 MHz). Each sample was examined using 3-D rendering mode, in which the color and gray value information was processed and combined to give 3-D image (mode cent; smooth: 4/5; FRQ: low; quality: 16; density: 6; enhance: 16; balance: 150; filter: 2; actual power: 2 dB; pulse repetition frequency: 0.9) [37]. For laterally located placentas slight lateral inclination of the transducer was positioned to reach proper images. Power Doppler window (pulse repetition frequency at 900 Hz and wall filter of 50 Hz) was placed over the placenta mapping the vascular tree from basal to chorionic plates. We used fast low resolution acquisition to avoid any kind of artefacts. The 3-D static volume box was placed over the highest villous vascular density zone at umbilical cord insertion [53, 59]. The sweep angle was set at maximum 70 degrees. Volume acquisition was made during a time interval varying from 5 to 15 seconds in the absence of fetal movements and with mother being as motionless as possible. Each image was recovered from the disk in succession for processing [53, 59]. During gestation we recorded one sample from each patient.

5.6. Calculation of power Doppler (PD) Indices

The stored volumes were further analyzed using the VOCAL program pertaining to the computer software 4-DView (GE Medical Systems, Austria, version 10.4). Each volume was recovered from the hard disk in succession and processed using the multiplanar system. The three planes of acquired placental volume were explored to localize the zone where the highest vascular density was found by PD mode [59]. The type of sonobiopsy (called *"Mercé-type sonobiopsy*") that we used is a reproducible, validated method [53, 54, 57], and by obtaining a representative sample of the placental tree it is applicable throughout the whole

pregnancy in contrary to other methods, in which the entire placenta needs to be visualized [55].

PD technique shows high sensitivity, because it is based on amplitude instead of mean frequencies to depict the vascular tree [59]. Moreover, the colour mapping is independent of the angle of insonation and does not show 'aliasing'. However, it is more sensible to patient movements, so the volumes should be acquired avoiding any probe or patient movements, otherwise artefacts could be present. Due to shortest possible time that was applied to acquire a placental volume (5 to 15 seconds), that sort of artefacts affecting visualisation was absolutely minimized [59]. We did not take the 3-DPD volume into consideration if motion artefact appeared and in these cases we repeated the acquisition again and again until we have obtained a clear view of 3-DPD volume, besides 4-D view program is not able to calculate the 3-DPD indices (VI, FI, VFI) when an artefact is recorded.

5.7. Statistics

5.7.1. Statistical analysis methods in groups GDM/T1DM

The statistical analysis was conducted with SPSS for Windows version 17.0 (SPSS Inc, Chicago, IL, USA). The continuous variables were expressed as median±standard deviation. Kruskal-Wallis tests were used for comparison of continuous variables depending on the three subgroups (T1DM versus (vs.) GDM vs. controls), whereas comparison between two subgroups (T1DM vs. GDM) was assessed with Mann-Whitney-U test. Univariate comparisons for categorical variables were assessed by $\chi 2$-tests. Linear regression coefficient values and equations depending on gestational age were also calculated for VI, FI, VFI indices both for diabetic and control groups. The distributions of placental indices (25th, 50th and 75th percentiles) according to GA were plotted and predictive values were also calculated for diabetic pregnancies. The association between placental 3-DPD indices and 2-DCD indices (RI and PI of umbilical and uterine arteries) was determined by Spearman's rank correlations.

5.7.2. Statistical analysis methods in group IUGR

The statistical analysis was conducted with SPSS for Windows version 17.0 (SPSS Inc, Chicago, IL, USA). An analysis of variance (ANOVA) was carried out to examine the association of the 3-DPD indices with the gravidity, parity, and pregestational body mass index (BMI). The Mann–Whitney *U* test, the *t* test, and the z-score were used to compare 3-DPD indices (VI, FI, VFI); estimated fetal weight, birth weight, and birth length; the mode of

delivery; the occurrence of intrauterine complications; the necessity of transfer to the neonatal intensive care unit; the Apgar scores at 1, 5, and 10 minutes; and the umbilical cord arterial pH between the IUGR group and the control group. $p \leq 0.01$ was considered statistically significant. A quantile regression method was used to investigate the relationship between 3-DPD indices (VI, FI, VFI) and gravidity, parity, and pregestational BMI. The association between placental 3-DPD indices and 2-DCD indices (RI and PI of umbilical and uterine arteries) was determined by Spearman's rank correlations.

6. Results

6.1. GDM/T1DM

The characteristics of pregnancies are found in *Table 4*, which show no significant difference in control, T1DM and GDM groups by Kruskal-Wallis test ($p>0.05$). The ultrasound-derived estimated fetal weight was not distinguishable between the two diabetic subgroups in any logistic regression analysis in relation to GA and placental 3-DPD indices ($p>0.05$).

Table 4. ***Pregnancy characteristics*** *of each study group at ultrasound examination*

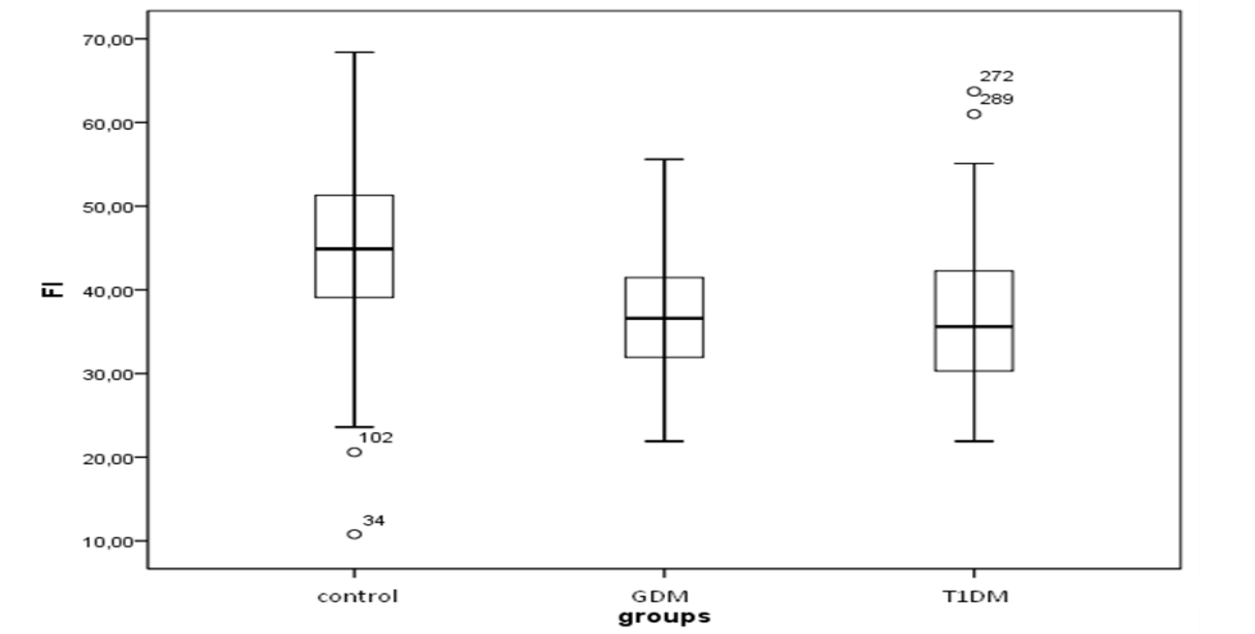
	GDM (n=56)	T1DM (n=43)	Control (n=113)
Maternal age (years) (mean±S.D.)	33±5,1	32±5	30,7±5,4
Weeks of gestation (mean±S.D.)	$30^{+7}\pm6^{+4}$	$31 \pm7^{+4}$	$28^{+4} \pm5^{+5}$
Estimated fetal weight (gram) (mean±S.D.)	1723±1007	1737±1222	1223±195,1

GDM: *gestational diabetes mellitus*
T1DM: *diabetes mellitus type I*
S.D.: *standard deviation*

Figure 13 a-c and *Table 5* display the placental indices in the three study groups. Kruskal-Wallis tests showed that all three placental indices were significantly reduced among

diabetic pregnant women as compared to the control group (p<0.001 for each index). None of placental indices indicated significant difference between T1DM and GDM pregnancies (p>0.05).

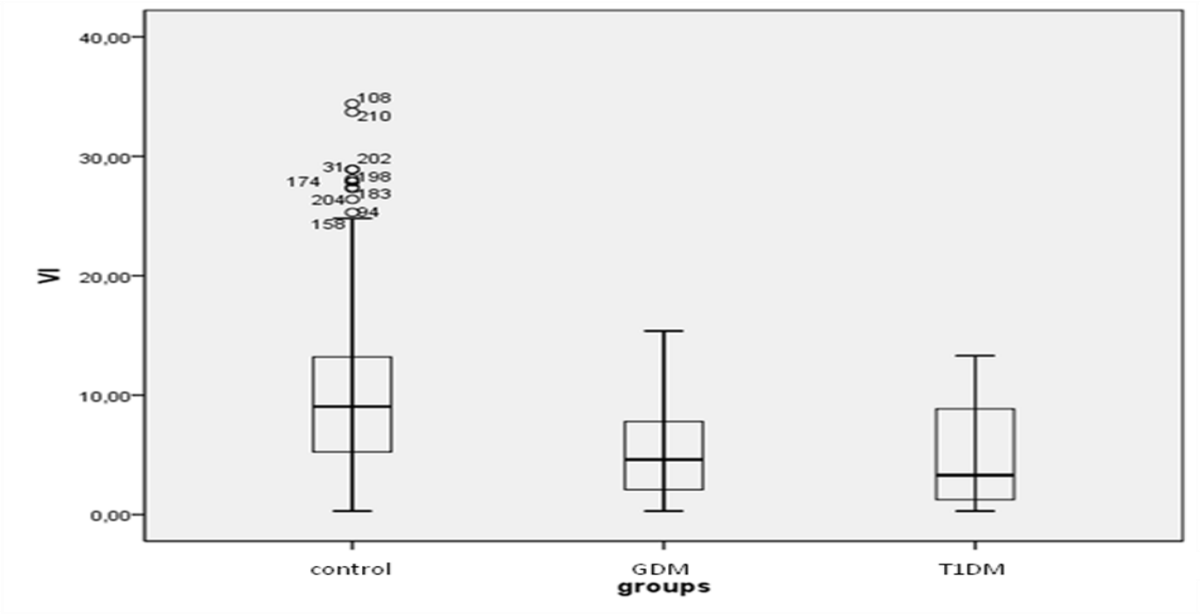
Figure 13.a. ***Flow index (FI)*** *in* ***GDM/T1DM*** *and* ***control*** *group. Horizontal lines represent interquartile ranges with medians, whiskers represent smallest and largest values and º represents an outlier (control: 113 cases, GDM: 56 cases, T1DM: 43 cases)*



GDM: *gestational diabetes mellitus*

T1DM: *diabetes mellitus type I*

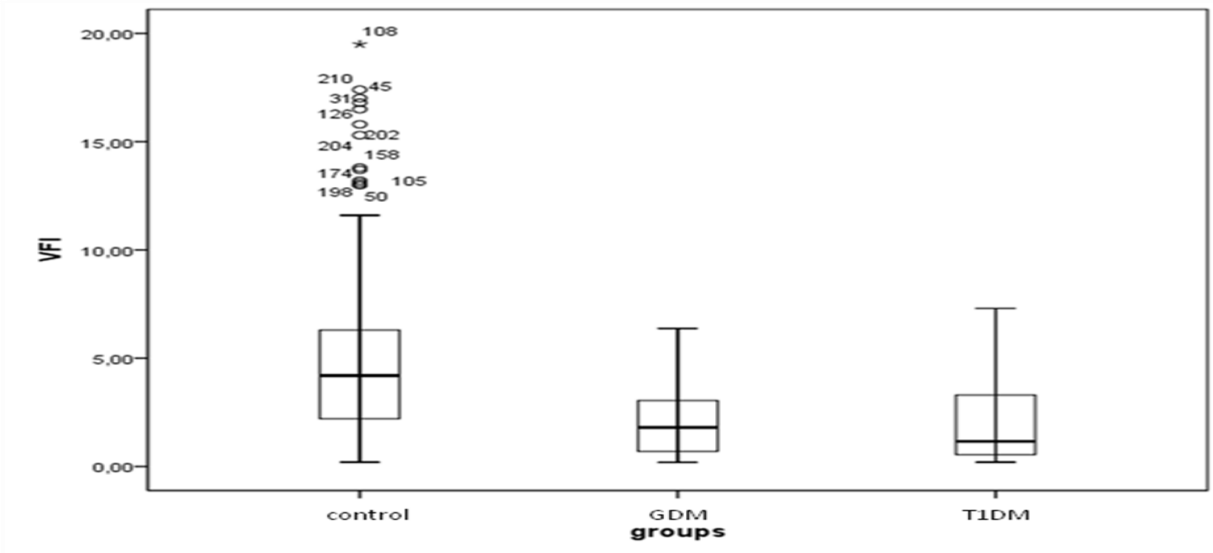
Figure 13.b. ***Vascularisation index (VI)*** *in* ***GDM/T1DM*** *and* ***control*** *group. Horizontal lines represent interquartile ranges with medians, whiskers represent smallest and largest values and ° represents an outlier (control: 113 cases, GDM: 56 cases, T1DM: 43 cases)*



GDM: *gestational diabetes mellitus*

T1DM: *diabetes mellitus type I*

Figure 13.c. ***Vascularisation flow index (VFI)*** *in* ***GDM/T1DM*** *and* ***control*** *group. Horizontal lines represent interquartile ranges with medians, whiskers represent smallest and largest values and ° represents an outlier (control: 113 cases, GDM: 56 cases, T1DM: 43 cases)*



GDM: *gestational diabetes mellitus*

T1DM: *diabetes mellitus type I*

Multiple logistic regression analysis showed that the odds of GDM are increased at a higher GA [p<0.001, adjusted odds ratio (AOR): 1.1 (95% Confidence Interval (CI): 1.07-1.16)] and a lower FI [p<0.001, AOR: 0.88 (95%CI: 0.85-0.93)], or at a higher GA and a lower VI [p<0.001, AOR: 0.83 (95%CI: 0.77-0.94)], or at a higher GA and a lower VFI [p<0.001, AOR: 0.63 (95%CI: 0.51-0.77)]. If all power Doppler indices are low [FI: p<0.001, AOR: 0.91 (95% CI: 0.87-0.94)]; [VFI: p<0.001, AOR: 0.64 (95% CI: 0.55-0.75)]; [VI: p<0.001, AOR: 0.83 (95% CI: 0.77-0.89)] during late pregnancy, then the odds of the fact that the pregnancy is complicated by any type of diabetes [p<0.001, AOR: 1.10 (95% CI: 1.06-1.14)] is also significantly high.

Table 5. ***Placental Doppler indices*** *of each study group*

	GDM (n=56)	T1DM (n=43)	Control (n=113)	p-value
Vascularisation index (mean±S.D.)	5,30±3,88	4,81±3,72	10,26±6,73	<0,001
Flow index (mean±S.D.)	36,71±7,18	36,86±10,44	44,83±8,47	<0,001
Vascularisation flow index (mean±S.D.)	2,12±1,58	2,02±1,87	4,94±3,73	<0,001

GDM: *gestational diabetes mellitus*
T1DM: *diabetes mellitus type I*
S.D.: *standard deviation*

The positive predictive reference values for VI, FI, and VFI in pregnancies complicated by diabetes are shown in *Table 6.*

Table 6. ***Reference values*** *for* ***Flow Index, Vascularisation Flow Index and Vascularisation Index*** *among mothers with* ***(gestational) diabetes mellitus***

Gestational age (weeks)	Flow Index			Vascularisation Flow Index			Vascularisation Index		
	-S.D.	Predicted value	+S.D.	-S.D.	Predicted value	+S.D.	-S.D.	Predicted value	+S.D.
12	39.06	40.65	42.24	2.32	2.76	3.2	5.93	6.21	6.49
13	38.83	40.42	42.01	2.28	2.72	3.16	5.87	6.15	6.43
14	38.6	40.19	41.78	2.24	2.68	3.12	5.81	6.09	6.37
15	38.37	39.96	41.55	2.2	2.64	3.08	5.75	6.03	6.31
16	38.14	39.73	41.32	2.16	2.6	3.04	5.69	5.97	6.25
17	37.91	39.5	41.09	2.12	2.56	3	5.63	5.91	6.19
18	37.68	39.27	40.86	2.08	2.52	2.96	5.57	5.85	6.13
19	37.45	39.04	40.63	2.04	2.48	2.92	5.51	5.79	6.07
20	37.22	38.81	40.4	2	2.44	2.88	5.45	5.73	6.01
21	36.99	38.58	40.17	1.96	2.4	2.84	5.39	5.67	5.95
22	36.76	38.35	39.94	1.92	2.36	2.8	5.33	5.61	5.89
23	36.53	38.12	39.71	1.88	2.32	2.76	5.27	5.55	5.83
24	36.3	37.89	39.48	1.84	2.28	2.72	5.21	5.49	5.77
25	36.07	37.66	39.25	1.8	2.24	2.68	5.15	5.43	5.71
26	35.84	37.43	39.02	1.76	2.2	2.64	5.09	5.37	5.65
27	35.61	37.2	38.79	1.72	2.16	2.6	5.03	5.31	5.59
28	35.38	36.97	38.56	1.68	2.12	2.56	4.97	5.25	5.53
29	35.15	36.74	38.33	1.64	2.08	2.52	4.91	5.19	5.47
30	34.92	36.51	38.1	1.6	2.04	2.48	4.85	5.13	5.41
31	34.69	36.28	37.87	1.56	2	2.44	4.79	5.07	5.35
32	34.46	36.05	37.64	1.52	1.96	2.4	4.73	5.01	5.29
33	34.23	35.82	37.41	1.48	1.92	2.36	4.67	4.95	5.23
34	34	35.59	37.18	1.44	1.88	2.32	4.61	4.89	5.17
35	33.77	35.36	36.95	1.4	1.84	2.28	4.55	4.83	5.11
36	33.54	35.13	36.72	1.36	1.8	2.24	4.49	4.77	5.05
37	33.31	34.9	36.49	1.32	1.76	2.2	4.43	4.71	4.99
38	33.08	34.67	36.26	1.28	1.72	2.16	4.37	4.65	4.93
39	32.85	34.44	36.03	1.24	1.68	2.12	4.31	4.59	4.87
40	32.62	34.21	35.8	1.2	1.64	2.08	4.25	4.53	4.81
41	32.39	33.98	35.57	1.16	1.6	2.04	4.19	4.47	4.75

Figure 14. a-c present the placental 3-D Doppler indices in relation to GA in the control group versus diabetic cases. The linear regression equations for VI, FI, and VFI vs. GA among the control cases were as follows: FI=47.82+(-0.13*GA); r=0.123, p<0.001; VI=14.67+(-0.20*GA); r=0.288, p<0.001; VFI=7.44+(-0.11*GA); r=0.233, p<0.001. All 3-DPD indices decreased slightly by GA. The linear regression equations were FI=43.41+(-0.23*GA); r=0.182, p=0.07; VI=6.93+(-0.06*GA); r=0.012, p<0.25; VFI=3.24+(-0.04*GA); r=0.164, p=0.107 among diabetic pregnant women.

Figure 14.a. ***Placental 3-D power Doppler indices*** *in* ***GDM/T1DM*** *as well as in* ***control*** *group compared to* ***gestational age****: vascularisation index (♦: control group n=113, ■: GDM/T1DM group n=99)*

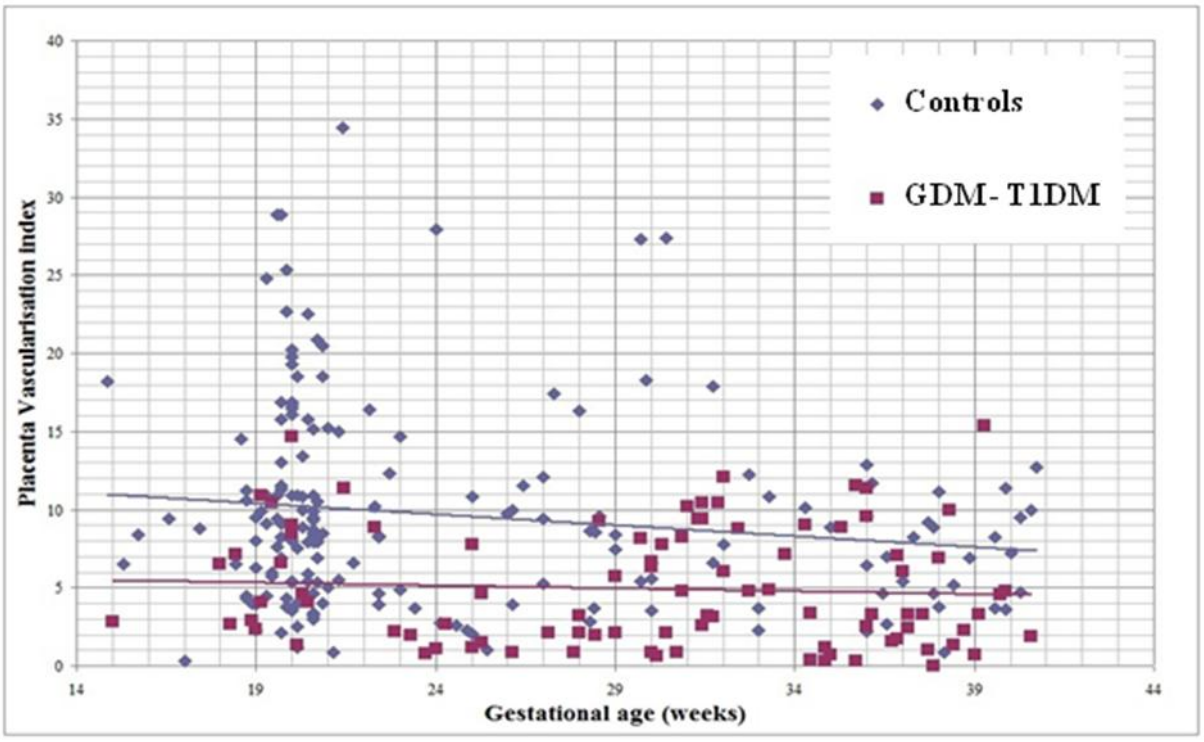


Figure 14.b. ***Placental 3-D power Doppler indices*** *in* ***GDM/T1DM*** *and* ***control*** *group compared to* ***gestational age****: flow index (♦: control group n=113,* ■*: GDM/T1DM group n=99)*

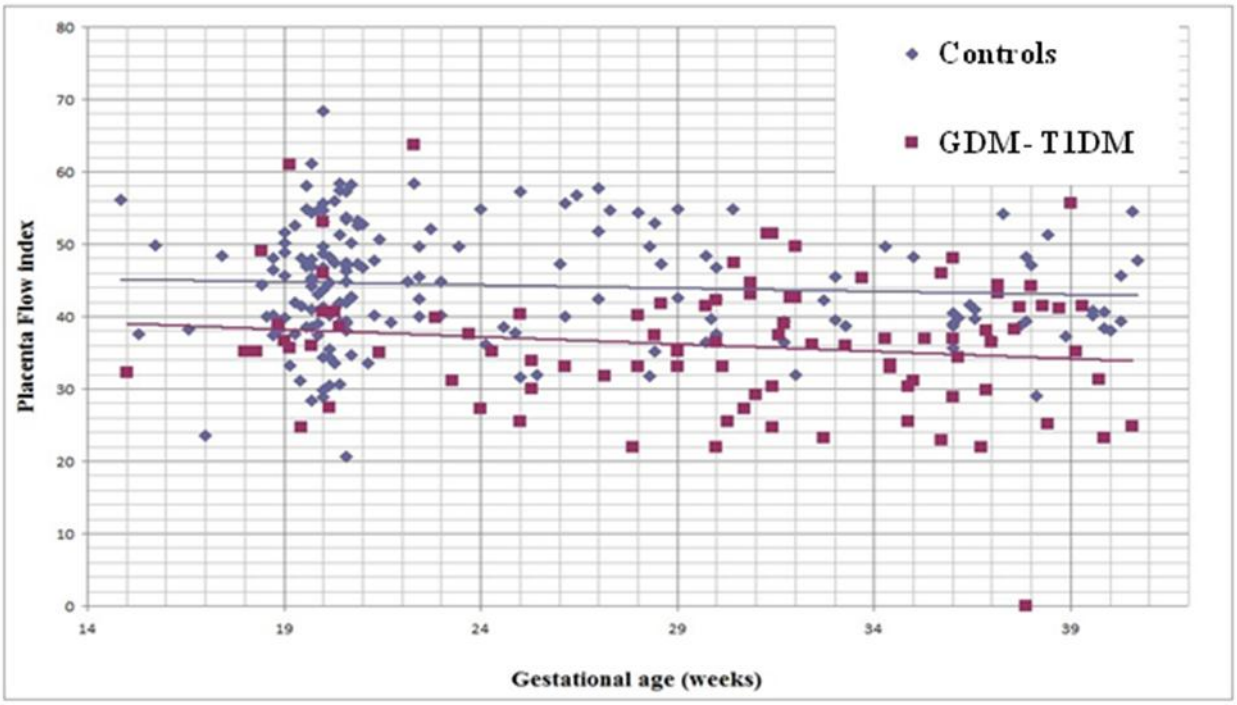
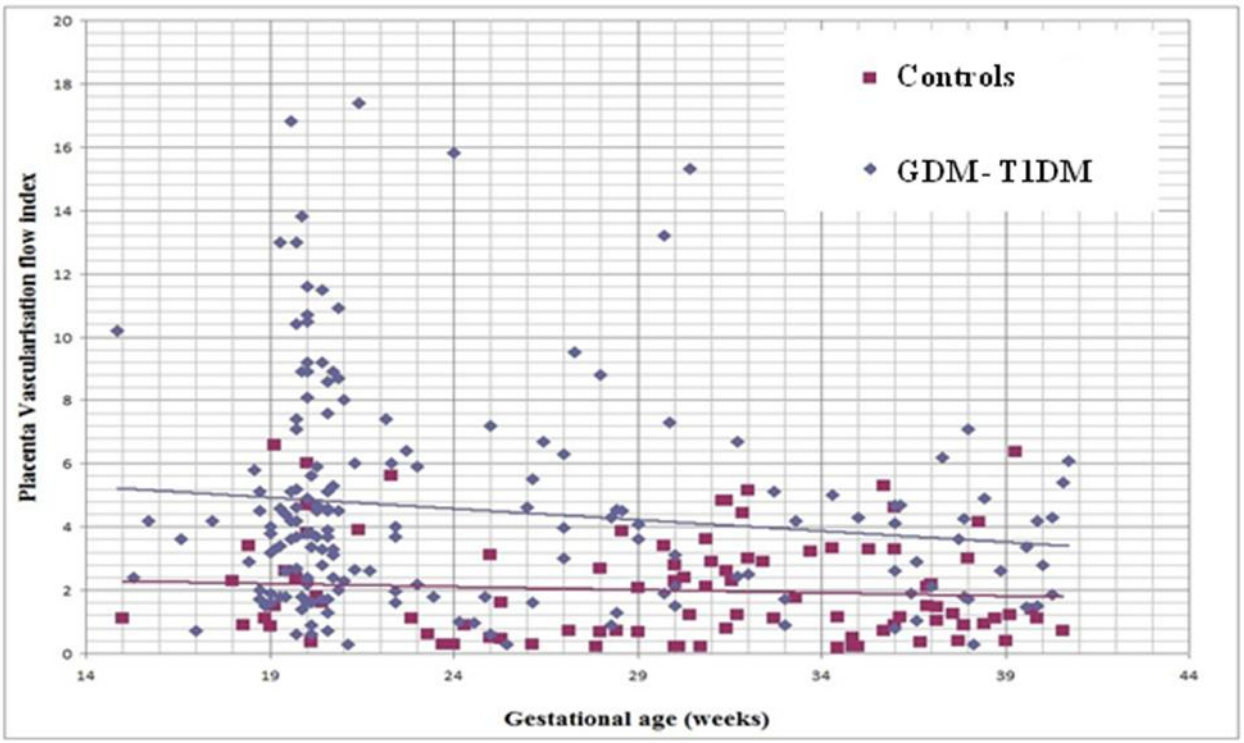


Figure 14.c. ***Placental 3-D power Doppler indices*** *in* ***GDM/T1DM*** *and* ***control*** *group compared to* ***gestational age****: vascularisation flow index. (♦: control group n=113,* ■*: GDM/T1DM group n=99)*



The equations were not significantly different from each other in T1DM and GDM group and the parameters of equations were significantly different from the parameters of the control group.

There were no associations between placental 3-DPD indices and 2-DCD indices (PI, RI of umbilical and uterine arteries) which were determined by Spearman's rank correlations (p<0.01). The intraobserver errors were evaluated by repeated measurements of the 3-DPD indices at the initiation of the study. The intra-class correlation coefficients for all Doppler indices were excellent (0.99) for all indices. The difference between the mean difference (VI: 0.04, FI: 0.09, VFI: -0.008) and zero was not significant for any 3-DPD index (p>0.05).

6.2. IUGR

A total of 223 women were enrolled: 171 were in the control group and 52 were in the IUGR group. Demographic and obstetric characteristics are demonstrated in *Table 7*.

Table 7. ***Demographic*** *and* ***obstetric characteristics*** [a]

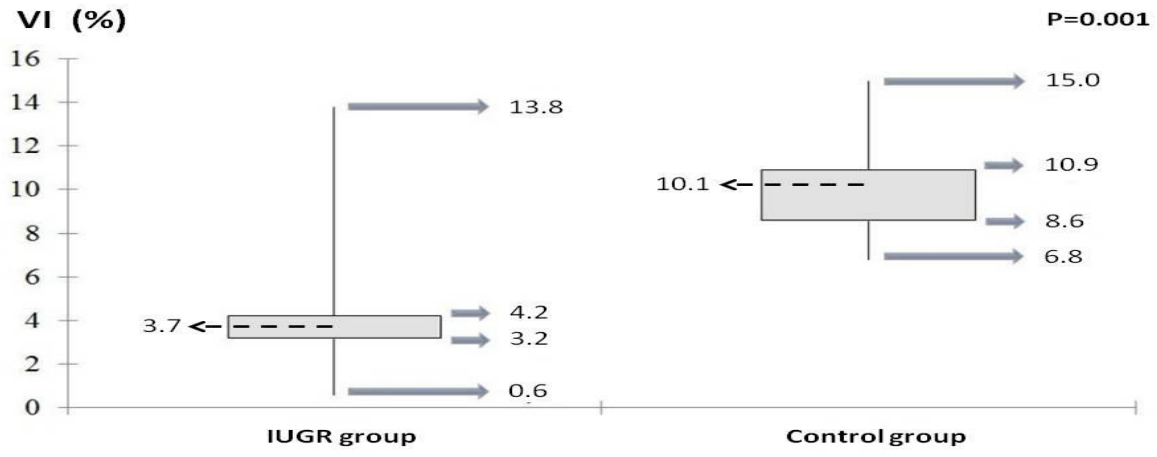
	Control group (n=171)	IUGR group (n=52)	*P* value
Age, y	31.0 (28–34)	30.0 (26–34)	0.1
Pregnancy duration at the time of ultrasonographic examination, wk	30.4 (19.0–37.0)	31.9 (24.1–37.6)	0.1
Median length of pregnancy, wk	39.1 (38.1–40.0)	38.4 (37.3–39.1)	0.2
Gravidity			
<3	123 (71.9%)	28 (53.8%)	0.01
≥3	48 (28.1%)	24 (46.1%)	0.01
Parity [b]			
<3	87 (83.6%)	26 (76.4%)	0.01
≥3	17 (16.4%)	8 (23.6%)	0.01
Pre-pregnancy BMI	22.1 ± 2.5	21.9 ± 1.5	0.01
Perinatal complications	12 (7.0)	12 (23.1)	0.01
Neonatal care	13 (7.6)	16 (30.8)	0.01
Cesarean delivery	45 (26.3)	31 (59.6)	0.01
Apgar score			
1 min	8.9 ± 2.3	9.0 ± 1.4	0.12
5 min	9.3 ± 1.3	9.7 ± 0.9	0.01
10 min	9.5 ± 0.8	9.8 ± 1.0	0.01
Birth length, cm	49.6 ± 2.45	46.5 ± 3.4	0.01
Birth weight, g	3351.9 ± 522.4	2674.4 ± 752.1	0.01
Umbilical cord arterial pH	7.2 ± 0.2	6.9 ± 1.6	0.01
Placental location			
Anterior wall	97 (56.7)	29 (55.8)	0.23
Posterior wall	74 (43.3)	23 (44.2)	0.22

[a] Values are given as median (interquartile range), number (percentage), or mean ± S.D. [b] Data available for 104 participants in the control group and 34 in the IUGR group. ***IUGR****: intrauterine growth restriction,* ***BMI****: body mass index,* ***wk****: week,* ***y****: year*

Placental location [(control: anterior wall: 97 (56.7%), p=0.23; posterior wall: 74 (43.3%), p=0.22) vs. (IUGR: anterior wall: 29 (55.8%), p=0.23; posterior wall: 23 (44.2%) p=0.22)] and 1-minute Apgar score (mean ± S.D.) [control (8.9 ± 2.3) vs. IUGR (9.0 ± 1.4) p=0.12] did not differ significantly between groups. Gravidity [control: <3: 123 (71.9%), p=0.01, ≥3: 48 (28.1%), p=0.01) vs. IUGR: (<3: 28 (53.8%), ≥3: 24 (46.2%), p=0.01)], parity [control: (<3: 87 (83.6%), ≥3: 17 (16.4%), p=0.01) vs. IUGR: (<3: 26 (76.4%), ≥3: 8 (23.6%), p=0.01)], pregestational BMI (mean ± S.D.) [control (22.1 ± 2.5) vs. (IUGR: (21.9 ± 1.5), p=0.01], umbilical cord artery pH (mean ± S.D.): [control (7.2 ± 0.2) vs. IUGR (6.9 ± 1.6), p=0.01.], and 5-minute and 10-minute Apgar scores (mean ± S.D.): [control (9.3 ± 1.3; 9.5 ± 0.8) vs. IUGR: (9.7 ± 0.9; 9.8 ± 1.0), p=0.01] did differ significantly.

Women in the IUGR group had significantly lower 3-DPD index values (VI, FI, VFI) than did the control group [VI (median (interquartile range) (%): control: 10.1 (8.6-10.9) vs. IUGR: 3.7 (3.2-4.2), p=0.001; FI (median (interquartile range)): control: 45.1 (44.1-53.1) vs. IUGR: 40,0 (39.7-42.5), p=0.0012; VFI (median (interquartile range)): control: 4.8 (4.4-5.3) vs. IUGR: 2.2 (2.1-2.4), p=0.0001] *(Figure 15. a-c)*.

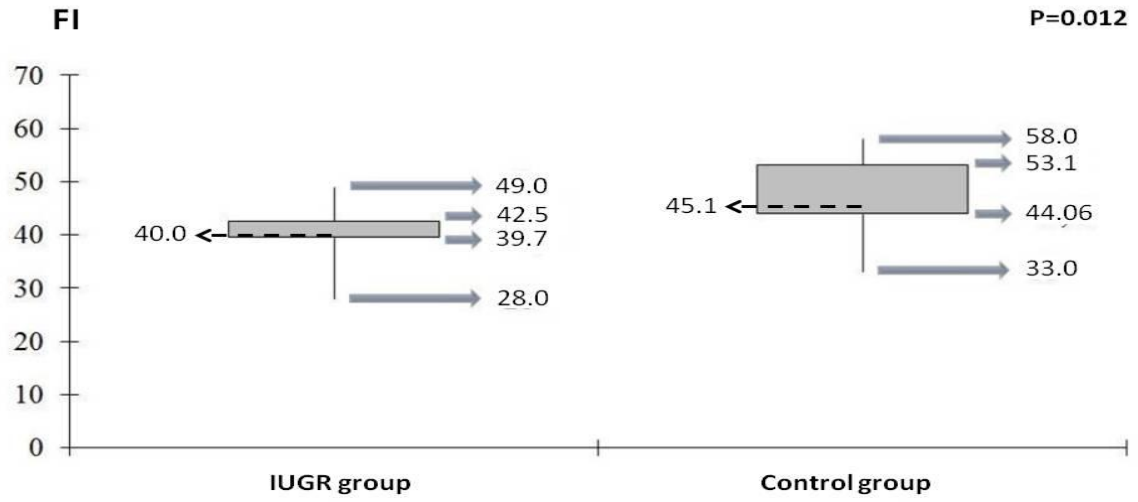
Figure 15. a. ***Vascularisation Index*** *in* ***IUGR*** *and* ***control*** *groups (median, interquartile range, p=0,001)*



IUGR*: intrauterine growth restriction*

VI: *vascularisation index*

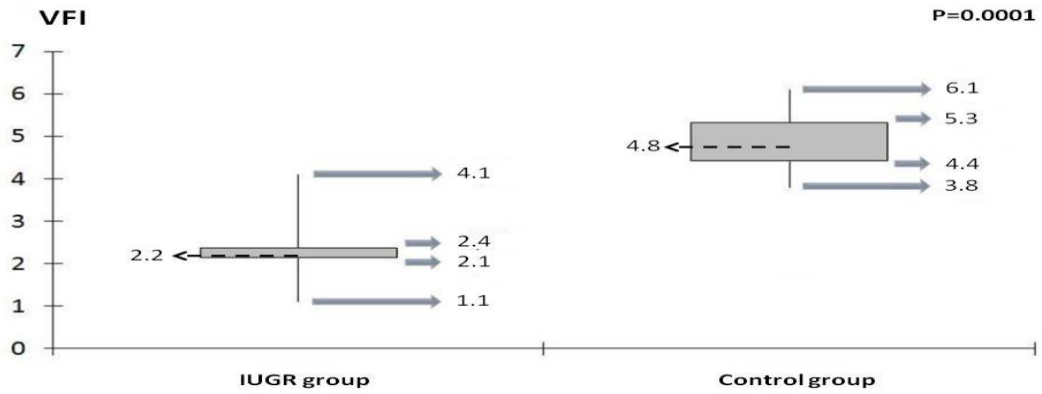
Figure 15.b. ***Flow Index*** *in* ***IUGR*** *and* ***control*** *groups (median, interquartile range, p=0,012)*



IUGR*: intrauterine growth restriction*

FI*: flow index*

Figure 15.c. ***Vascularisation Flow Index*** *in* ***IUGR*** *and* ***control*** *groups (median, interquartile range, p=0.0001)*



IUGR*: intrauterine growth restriction*

VFI*: vascularisation flow index*

Table 8 shows the comparison of the vascularisation indices among women with different gravidities, parities, and pregestational BMIs. Gravidity, parity, and pregestational BMI had no significant effect on VI, FI, VFI by quantile regression analyses ($p>0.01$), but there were significant differences in VI, FI, VFI between the control and IUGR group ($p<0.01$).

Perinatal complications occurred among 12 (23.1%) neonates in the IUGR group and 12 (7.0%) in the control group ($p=0.01$). Neonatal intensive care was needed for 16 (30.8%) infants in the IUGR group and 13 (7.6%) in the control group ($p=0.01$). Median length of pregnancy was 38.4^{th} weeks (interquartile range 37.3–39.1) in the study group and 39.1^{th} weeks (interquartile range 38.1–40.0; $p=0.2$) in the control group. The mean birth weight was 2674.4 ± 752.1 g in the study group and 3351.9 ± 522.4 g in the control group ($p=0.01$). The mean birth length was 46.5 ± 3.4 cm in the study group and 49.6 ± 2.45 cm in the control group ($p=0.01$). The mean z-score for the estimated fetal weight in IUGR pregnancies measured from 30^{th} to 38^{th} weeks of pregnancy was –2.9, indicating that the estimated fetal weights in the IUGR group were below the average measured in the control group by nearly three times the standard deviation. There were no associations between placental 3-DPD indices and 2-DCD indices (RI and PI of umbilical and uterine arteries) which were laid down by Spearman's rank correlations ($p<0.01$).

Table 8. Comparison of vascularization indices among women with different gravidity, parity, and pregestational BMI in the control group and IUGR group

	Mean vascularization index, %		Mean flow index		Mean vascularization flow index	
	Control group	IUGR group	Control group	IUGR group	Control group	IUGR group
Gravidity	[a]	[a]	[b]	[b]	[a]	[a]
1[c]	9.4	3.5	44.0	39.7	4.4	2.1
2[d]	10.9	3.5	45.5	39.7	5.3	2.4
3[e]	10.5	3.8	47.1	39.8	5.0	2.2
4[f]	10.0	3.3	42.5	42.5	4.6	2.3
5[g]	8.6	–	53.1	–	5.1	–
Parity	[h]	[h]	[i]	[i]	[j]	[j]
1[d]	9.4	5.3	44.2	39.5	4.4	2.0
2[e]	11.3	4.8	46.0	42.5	5.5	2.0
3[f]	11.1	6.4	47.1	39.0	5.5	2.7
4	4.6	–	43.0	–	2.0	–
Prepregnancy BMI	[k]	[k]	[l]	[l]	[j]	[j]
Normal	5.4	4.8	44.7	39.9	5.1	2.2
Obese	4.0	3.4	42.3	38.6	3.5	1.6
Normal but excessive weight gain during pregnancy [m]	5.3	4.4	46.3	39.8	4.5	2.1

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by the square of height in meters); IUGR, intrauterine growth restriction.

[a] Within-group comparison P=0.1.

[b] Within-group comparison P=0.3.

[c] Control group n=67; IUGR group n=18.

[d] Control group n=56; IUGR group n=10.

[e] Control group n=31; IUGR group n=16.

[f] Control group n=14; IUGR group n=8.

[g] Control group n=3; IUGR group n=0.

[h] Within-group comparison P=0.012.

[i] Within-group comparison P=0.068.

[j] Within-group comparison P=0.08.

[k] Within-group comparison P=0.02.

[l] Within-group comparison P=0.28.

[m] According to guidelines from the Mayo clinic [60].

7. Discussion

7.1. GDM/T1DM

We found a negative correlation between 3-DPD indices and GA in normal pregnancies. The declining tendency of 3-DPD indices was more remarkable in diabetic pregnancies. The unaffected cases had constant values of 3-DPD indices. The control group and the diabetic groups had overlaps of VI, FI, VFI values; hence 3-DPD indices are not diagnostic in diabetes. In a previous study by *de Paula et al*. [57] all placental vascular indices showed constant values, while increased volume expansion of placenta was observed in connection with GA. In diabetes the placental vascular alteration is a consequence of change in maternal glucose metabolism [17, 61].

The quantitative changes in the vascular tree in the placenta in pregnancies complicated by GDM/T1DM are not well defined. Decreased placental VI, VFI in diabetes could be associated with down-regulated angiogenesis, diminished number of arterioles and the hypertrophic wall of these vessels [17]. The depressed FI might represent narrower inner vascular diameter [17, 61, 62].

In contrast to our results, a preceding article by *Rizzo et al*. [55] reported an increased placental circulation in diabetic pregnancies from 11th week until the end of 13th week of gestation, and a poor glycemic control was associated with higher placental 3-DPD indices. The possible explanation for this paradox could be that the distinct placental changes associated with diabetes mellitus depend on the gestational period. In the first trimester the high glucose level triggers vasculogenesis, but vasculopathy is not present yet [17]. In later stage of pregnancy the vasculopathy destroys the blood flow in placenta, especially if poor glycemic control exists. Reasons for reduced placental blood flow (FI) in diabetes are: inadequate opening of spiral arteries, acute atherosis and reduction in intervillous space volume because of edema and higher number of bulbous villi [17, 61]. GDM correlates to diminished branching, attenuated coiling, mitigated vascular diameters. The alteration of placental vasculature leads to diminished function of placenta [17, 61]. Since in GDM/T1DM the volume and weight of placenta increases (the placental parenchymal and villous tissue content is higher than in non-diabetic normal pregnancies), VI is proportionally reduced scaled to volume unit.

The uteroplacental circulation can be detected by flowmetry of the umbilical and uterine arteries. Our study confirms the results of other research groups [63, 64] referring to their statements that GDM does not induce changes in umbilical and uterine circulation. In

diabetes, increased blood flow resistance of the umbilical artery is just a late pathognomonic sign of ischemic vascular changes in placenta [62, 65], therefore we added the analysis of the placental microcirculation to the conventional indirect uteroplacental sonographic examination.

There are some available methods reported in the literature on measuring placental vascular flow [52-55, 59]. Our study was designed based on a literature search for this issue and our aim was to find a technique, which is applicable even if the whole placenta can not be visualized and irrespectively of the placental volume it can be used throughout the whole pregnancy. The *"Mercé-type sonobiopsy"* [55] was applied in our study as a well-defined, validated method [66], in which both the intraobserver and interobserver errors are low [52]. This method can be used not only in the first trimester, when the overwhelming majority of the abnormalities in high-risk pregnancies has not been developed yet, but also in the second and third trimester.

The studies about reproducibility demonstrated that the measurement of placental microcirculation by sonobiopsy can be appropriate [55, 66] for opting for measuring placental indices for a long time-period (between 10 and 41 weeks of gestation). The umbilical cord insertion can be visualized until the end of pregnancy. From the early second trimester on it is impossible to visualize the placenta completely on the posterior wall that is why we excluded the cases with posterior placenta. In addition, the growth of the fetal skeleton reduces the effect of ultrasound examination.

Pregnant women with DM in our study did not have any other microvascular diseases such as retinopathy or nephropathy, though fetal vessels of placenta showed dramatic changes in VI, FI and VFI. This assumes, that placental microvascularisation in diabetes is under control of fetal circulation factors as well [67].

In our study, only the most vascularised placental region (the site of umbilical cord insertion) was measured correlated to a standard volume unit. We applied sonobiopsy as the most accurate measurement method concerning vascularisation [53, 59]. Placental 3-DPD indices, measured by sonobiopsy clearly point to a decreasing tendency in regard to increasing GA in diabetic mothers. In addition, our measurements demonstrate that the placental region close to the umbilical cord insertion has constant vascularisation during normal pregnancy. However, significant difference can not be observed between cases when diabetes has been developed prior to and during mid as well as late pregnancy.

7.2. IUGR

We found that 3-DPD indices were significantly decreased in the IUGR pregnancies compared to the normal pregnancies. There are some examples in the international literature that the placenta was examined by 3-DPD technique; however, the vast majority of the authors have only analyzed the changes in placental vascularisation [54, 56, 58] in IUGR, while we investigated the influence of 3-DPD indices on perinatal outcome as well. In addition, we applied a technique which is adequately reproducible until the end of the gestation period.

Ultrasound plays an important role in diagnosis of IUGR. Parameters most commonly used for estimating fetal weight by ultrasonography include BPD, HC, AC and FL [42]. A reduced AC established by ultrasonographic evaluation is reported to be the most sensitive biometric measurement in predicting IUGR. An AC within the normal range reliably excludes IUGR with a false-negative rate of less than 10% [69]. In the most cases of IUGR we can find decreased amniotic fluid volume [70]. Oligohydramnios is considered to be present when the largest vertical pocket of amniotic fluid is less than 2 cm or the amniotic fluid index (AFI) is less than 5 cm. This index is the sum of the vertical amniotic fluid pocket in the 4 abdominal quadrants [71, 72]. Doppler impedance measurement of umbilical and uterine artery is well suited to be a pivotal test in the management of IUGR fetus. If placental failure is the cause of intrauterine restriction, we can measure high impedance in umbilical and uterine arteries and notch in uterine artery [30]. Doppler examinations of the fetal circulation have the potential to reduce perinatal death and overtreatment such as unnecessary preterm labour induction [73-75]. The use of 3-D ultrasonography may increase diagnostic precision in the estimation and detection of fetal malformations [76].

Guiot et al [58] determined indices at 5 different places on the placenta. *Noguchi et al* [54] examined the entire placenta attached only to the anterior wall. Their testing method was to average the results of 9-12 sampling spheres by sonobiopsy. They investigated the vascularisation of the entire placenta, and neither the sampling volume nor the number of sampling spheres was standardized. Samples from barely vascularised edges and better vascularised centre parts were averaged, which may have led to significantly modified statistics. In 2009 *de Paula et al* [57] measured the vascularisation of the placenta as a whole, while *Mercé et al* [59] examined a 3x3x3mm volume part of the placenta directly at the adhesion of the umbilical cord, which is the most vascularised portion of the placenta *(Mercé-type sonobiopsy)*. We applied this technique for our examinations, which was necessary

because the entire placenta is difficult to visualize in the third trimester, especially when it adheres to the back wall of the uterus. The method is well standardized and reproducible, and intra- and inter-observer errors are also infrequent [68]. There is a debate over the normal reference range of placental indices established by various authors. Our evaluation verifies the results of the following authors: *Guiot* [58] and *de Paula* [57]. They found placental indices constant throughout the pregnancy. A possible explanation for the discrepancy noted above between the results of different authors is the diverse measuring technique, low case number, and the unpublished volumes and standardized settings of the ultrasound equipment.

In our prospective study focusing on the determination of placental vascularisation indices (VI, FI, VFI) using *Mercé-type sonobiopsy*, we experienced that placental vascularisation is lower in the case of IUGR pregnancies compared to normal pregnancies, which supports the results of *Pomorski et al, Noguchi et al and Guiot et al* [54, 56, 58]. Placental PD vascularisation indices significantly decline compared to the control group.

We did not find a significant discrepancy with regard to maternal age or gestational age at the time of examination between the IUGR and the control group; thus maternal age had no influence on the condition of arteries or on angiogenesis during pregnancy. Placental vascularisation indices are more or less constant during pregnancy [57, 58, 77]. The frequency of Cesarean section was significantly higher in the case group compared to the control group. Growth-restricted infants respond sensitively to stress during labour and tolerate vaginal delivery badly; thus Cesarean section is performed more frequently. In our study gestational and maternal age in the two groups had no influence on placental vascularisation indices. 5-minute and 10-minute Apgar scores showed significant differences with regard to VI, FI and VFI, which can be explained by the poor postnatal adaptation of the infant restricted in the uterus. Complications, such as hypothermia, hypoglycemia, polycythemia, hyperviscosity and breathing problems emerge, and neonatal intensive care is more often needed compared to infants in the control group. The decrease in vascularisation indices is significant; the value of VFI, which is the most sensitive factor in indicating complications, is particularly predictive. However, we recommend the use of FI screening, since this parameter is minimally influenced by other factors, such as equipment settings. FI has the lowest coefficient of variation and intra-/inter-observer errors in measurements.

Our study confirms statements made in a previous article [78] that there is no significant discrepancy between the location of the placenta and placental 3-DPD indices. *Guimarães Filho HA et al* [78] examined 283 women between 26-35th weeks of pregnancy and monitored the effect of the position of the placenta on the change of placental

vascularisation indices. The results of their research revealed that there is no significant coherence between FI, VI, VFI and the position of the placenta. We excluded placental adhesion anomalies and morphological disorders, because insufficient circulation may occur from the beginning of this type of pregnancy which could have falsely influenced our results, as indicated in a review article by *Srividhya S et al*. [29].

During our examinations we aimed at using an appropriately validated and reproducible method, which could be adopted in maternal care as a routine. Previously published methods cannot be applied comprehensively in general maternal care, partly because sampling volumes [57, 58] as well as the place and number of sampling [56] were not standardized; moreover, they are connected to the visualization of the entire placenta [54, 56, 58], which can only be realized in selected cases, since it is not possible to visualize the whole placenta in the third trimester.

We think that the machine settings could significantly influence comparisons of study findings, though they do not affect the observation of changes of 3-D vascular indices during pregnancy and the differences in case-control studies [54, 56, 58]. In conclusion, the main message of our study is that differences in placental vascular flow indices between normal and IUGR pregnancies can easily be established and not biased by our results. Therefore, we used standard machine settings. Volume acquisition was done during a time interval varying from 5 to 15 seconds lacking fetal motion and with the mother remaining as motionless as possible to prevent the display of any sort of artefacts.

Our results confirm the hypothesis that there is a strong correlation between the deterioration of placental vascularisation and perinatal outcomes in pregnancies complicated by IUGR. We strongly consider the method of vascular sonobiopsy of the placenta beneficiary and applicable in monitoring patients so as to reduce the rate and range of perinatal complications. This may become a relevant method in ultrasound examinations; however, it is important to emphasize the recommendation that a therapeutic conclusion may only be drawn when it is based on a complete clinical background.

8. Conclusion

A significant reduction in placental 3-DPD indices could be measured in pathological pregnancies (diabetic and growth restricted) applying 3-D sonobiopsy, which is a valid alternative for evaluation of the placental vascular tree when visualization of the entire placenta is not feasible. The evaluation of altered placental vascularisation by measurement of 3-DPD indices (VI, FI, VFI) could be useful in checking the pathological pregnancies, however further studies on association between other pregnancy characteristics and 3-DPD indices are necessary in order to evaluate the usefulness of this method in screening complications of pathological pregnancies.

In the future we would like to continue our research on this method, to develop and promote the accessibility of this method in clinical centres, but later in county hospitals as future screening centres as well and to make the possibilities of conventional identification of risk-pregnancies complete. Therefore we can affirm with certainty that after the evaluation of the findings and the adaptation of this method to the routine screening practice, we would improve the perinatal outcomes regarding morbidity and mortality. Our study group wishes to contribute to the creation of these potential facilities and I hope that based upon our research results this screening method will once become part of the pregnancy-care protocol. If we had the opportunity to accomplish this method in Hungary, we would be pioneers in the methodology and quality level of pregnancy care in Central and Eastern Europe.

9. The new results of the thesis

1. The placental 3-DPD indices are nearly constant in normal pregnancies.
2. The placental 3-DPD indices are significantly decreased in GDM/T1DM.
3. None of placental indices showed significant difference between T1DM and GDM pregnancies.
4. The placental 3-DPD indices are influenced by GA in GDM/T1DM pregnancies.
5. The placental 3-DPD indices are significantly reduced in IUGR pregnancies.
6. The placental 3-DPD indices are not influenced by maternal age, gravidity, parity, pregestational BMI and placental localization in IUGR pregnancies.
7. There is a connection between 3-DPD indices and perinatal outcome.

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11. References

1. Campbell S. Placental vasculature as visualized by 3D power Doppler angiography and 3D color Doppler imaging. Ultrasound Obstet Gynecol. 2007; 30(6):917-20.

2. Hata T, Tanaka H, Noguchi J, Hata K. Three-dimensional ultrasound evaluation of the placenta. Placenta. 2011; 32(2):105-15.

3. Regnault TRH, Galan HL, Parker TA, Anthony RV. Placental development in normal and compromised pregnancies: a review. Placenta. 2002;23 (Suppl A):S119-29.

4. Sibley CP, Turner MA, Cetin I, Ayuk P, Boyd CAR, D'Souza SW, et al. Placental phenotypes of intrauterine growth. Pediatr Res. 2005; 58(5):827-32.

5. Desoye G, Hauguel de Mouzon S, Shafrir E. The placenta in diabetic pregnancy. In: Textbook of Diabetes and Pregnancy. Hod M, Jovanovic L, DiRenzo G-C, deLeiva A, Langer O editors. London, Dunitz M; 2003. p. 126–47.

6. Desoye G, Myatt L: The placenta. In Diabetes in Women: Adolescence, Pregnancy, and Menopause. 3rd ed. Reece EA, Coustan DR, Gabbe SG editors. Philadelphia, Lippincott Williams & Wilkins; 2004. p. 147–57.

7. Desoye G, Shafrir E. The human placenta in diabetic pregnancy. Diabetes Reviews 4. 1996. p. 70–89.

8. Pedersen J. Diabetes and pregnancy; blood sugar of newborn infants during fasting and glucose administration. Nord Med. 1952;47(30): 1049.

9. Hiden U, Glitzner E, Hartmann M, Desoye G. Insulin and the IGF system in the human placenta of normal and diabetic pregnancies. J Anat. 2009;215(1):60–8.

10. Beckman JA, Creager MA, Libby P. Diabetes and atherosclerosis: epidemiology, pathophysiology and management. J Am Med Assoc. 2002;287(19):2570–81.

11. Tabit CE, Chung WB, Hamburg NM, Vita JA. Endothelial dysfunction in diabetes mellitus: molecular mechanisms and clinical implications. Rev Endocr Metab Disord. 2010;11(1):61–74.

12. Wang K, Jiang YZ, Chen DB, Zheng J. Hypoxia enhances FGF2- and VEGF-stimulated human placental artery endothelial cell proliferation: roles of MEK1/2/ERK1/2 and PI3K/AKT1 pathways. Placenta. 2009;30(12):1045–51.

13. Myatt L. Placental adaptive responses and fetal programming. J Physiol. 2006;(1)572:25–30.

14. Radaelli T, Varastehpour A, Catalano P, Hauguel-de MS. Gestational diabetes induces placental genes for chronic stress and inflammatory pathways. Diabetes. 2003;52(12):2951–8.

15. Evers IM, Nikkels PG, Sikkema JM, Visser GH. Placental pathology in women with type 1 diabetes and in a control group with normal and large-for-gestational-age infants. Placenta. 2003;24(8-9):819–25.

16. Laurini RN, Visser GH, van Ballegooie E, Schoots CJ. Morphological findings in placentae of insulin-dependent diabetic patients treated with continuous subcutaneous insulin infusion (CSII). Placenta. 1987;8(2):153–65.

17. Hiden U, Froehlich J, Desoye G. Diabetes and the Placenta. In: Kay HH, Nelson DM, Wang Y editors. The Placenta: From Development to Disease. 1st ed. UK: Oxford. Wiley-Blackwell; 2011. p. 228-30.

18. Kun A, Tornoczky J, Tabak AG. Prevalence and predictors of GDM in Hungary. Horm Metab Res. 2011;43(11):788–93.

19. Expert Committee on the diagnosis and classification of diabetes mellitus: Report of the Expert Committee in the diagnosis and classification of diabetes mellitus. Diabetes Care. 2011;34(Suppl.1):S66–8.

20. Vambergue A, Valat A, Dufour P et al. Pathophysiology of gestational diabetes. J Gynecol Obstet Biol Reprod (Paris). 2002; 31(6):4S3-10.

21. Brian AM, Philip O, James D, editors. Clinical obstetrics and gynecology. 2nd ed. Saunders Elsevier; 2009.

22. Alcazar JL. Three-dimensional power Doppler derived vascular indices: what are we measuring and how are we doing it? Ultrasound Obstet Gynecol. 2008;32(4):485–7.

23. Pairleitner H, Steiner H, Hasenoehrl G, Staudach A. Three-dimensional power Doppler sonography: imaging and quantifying blood flow and vascularization. Ultrasound Obstet Gynecol. 1999;14(2):139–43.

24. de Onis M, Garza C, Onyango AW, Rolland-Cachera MF. WHO growth standards for infants and young children. Arc Pedistr. 2009;16(1):47-53.

25. Lausman A, McCarthy FP, Walker M, Kingdom J. Screening, diagnosis and management of IUGR. J Obstet Gynecol Can. 2012; 34(1):17-28.

26. Romo A, Carceller R, Tobajas J. Intrauterine growth retardation (IUGR): epidemiology and etiology Pediatr Endocrinol Rev. 2009;6(3):332-6.

27. Gaudineau A. Prevalence, risk factors, maternal and fetal morbidity and mortality of intrauterine growth restriction and small-for-gestational age. J Gynecol Obstet Biol Reprod. 2013;42(8):895-910.

28. Surányi A. Prenatal and postnatal evaluation of foetal renal hyperechogenicity in pregnancies complicated with pre-eclampsia and intrauterine growth retardation (Ph.D. thesis). University of Szeged. 2000. p.48.

29. Srividhya S, Phillipa MK. Aetiology and Pathogenesis of IUGR. Best Practice and Research Clinical Obstetrics and Gynaecology. 2009;23(6):765-77.

30. Charnock-Jones DS, Kaufmann P, Mayhew TM. Aspects of human fetoplacental vasculogenesis and angiogenesis. I. Molecular regulation. Placenta. 2004;25(2-3):103-13.

31. Trudinger BJ, Giles WB. Elaboration of stem villous vesels in growth restricted pregnancies with abnormal umbilical artery Doppler waveforms. Br J Obstet Gynecol. 1996;103(5):487-9.

32. Jackson MR,Walsh AJ, Morrow RJ et al. Reduced placental villous tree elaboration in small-for-gestational age pregnancies: relationship with umbilical artery Doppler waveforms. Am J Obstet Gynecol. 1995;172:518-25.

33. Gilmartin AH, Ural SH, Repke JT. Gestational diabetes mellitus. Rev Obstet Gynecol. 2008;1(3):129-34.

34. American Diabetes Association: Diagnosis and classification of diabetes mellitus. Diabetes Care. 2011;34(1):S62–9.

35. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med. 1998;15(7):539-53.

36. HAPO Study Cooperative Research Group: Metzger BE, Lowe LP, Dyer AR et al. Hyperglycemia and adverse pregnancy outcomes. New England J Med. 2008;358(19):1991–2002.

37. Kerenyi Z, Tamas G. Diabetes in pregnancy: Epidemiology, classification, treatment and outcome. In: Williams DRR, Papoz L, Fuller JH, editors. Diabetes in Europe. London: John Libbey, London; 1994. p. 95–108.

38. Wollmann HA. Intrauterine growth restriction: definition and etiology. Horm Res. 1998;49(2):1-6.

39. K. Haram, E. Softland, R. Bukowski. Intrauterine growth restriction. Int. J of Gynecol and Obstet. 2006;93(1):5-12.

40. Loeffler FE. Clinical foetal weight prediction. J Obstet Gynecol Br. Commonw. 1967;74(5):675-7.

41. Basis JM, Eskes M, Pel M, Bonsel GJ, Bleker OP. Effectiveness of detection of intrauterine growth retardation by abdominal palpation as screening test in a low risk population: an observational study. Eur J Obstet and Gynecol eprod Biol. 2004;116(2):164-9.

42. Hadlock FP, Harrist RB, Sharman RS, Deter RL, Park SK. Estimation of fetal weight with the use of head, body and femur measurements: a prospective study. Am J Obstet Gynecol. 1985;151(3):333-7.

43. Nicolaides K, Rizzo G, Hecker K, Ximenes R: Methodology of Doppler assessment of the placental and fetal circulations: Uteroplacental circulation in Doppler in obstetrics. ISUOG. Fetal Medicine Foundation. 137 Harley Street, London, W1G 6BG United Kingdom [cited 20 Jan 2015] Available: http://www.fetalmedicine.com. Ch 3. pp.32-61.

44. Guimarães Filho HA, da Costa LL, Araújo Júnior E, Nardozza LM, Nowak PM, Moron AF et al. Placenta: angiogenesis and vascular assessment through three-dimensional power Doppler ultrasonography. Arch Gynecol Obstet. 2008;277(3):195-200.

45. Welsh AW, Humphries K, Cosgrove DO, Taylor MJO, Fisk NM. Development of three-dimensional power Doppler ultrasound imaging of fetoplacental vasculature. Ultrasound Med Biol. 2001;27(9):1161-70.

46. Matijevic R, Kurjak A. The assessment of placental blood vessels by threedimensional power Doppler ultrasound. J Perinat Med. 2002;30(1):26-32.

47. Konje JC, Huppertz B, Bell SC, Taylor DJ, Kaufmann P. 3-dimensional colour angiography for staging human placental development. Lancet. 2003;362(9391):1199-201.

48. Raio L, Ghezzi F, Cromi A, Nelle M, Dürig P, Schneider H. The thick heterogeneous (jellylike) placenta: a strong predictor of adverse pregnancy outcome. Prenat Diagn. 2004;24(3):182-8.

49. Welsh AW, Taylor MJO, Cosgrove D, Fisk NM. Freehand three-dimensional Doppler demonstration of monochorionic vascular anastomoses in vivo: a preliminary report. Ultrasound Obstet Gynecol. 2001;18(4):317-24.

50. Kurjak A, Mercé LT, Ahmed B. 3D power Doppler in the study of fetal and maternal angiogenesis and vasculogenesis. In: Kurjak A, Chervenak FA, editors. Donald School Textbook of Ultrasound and Gynecology. 2nd ed. New Delhi: Jaypee; 2008. p. 433-51.

51. Mercé LT, Barco MJ. Placental volume and circulation: the new concept of 'Virtual Placental Biopsy'. In: Hata T, Kurjak A, Kozuma S, editors. Current Topics on Fetal 3D/4D Ultrasound. Bentham Science Publishers; 2009. p. 187-208.

52. Mercé LT, Barco MJ, Bau S. Reproducibility of the study of placental vascularization by three-dimensional Power Doppler. J Perinat Med. 2004;32(3):228–33.

53. Pretorius DH, Nelson TR, Baergen RN, Pai E, Cantrell C. Imaging of placental vasculature using three-dimensional ultrasound and color power Doppler: a preliminary study. Ultrasound Obstet Gynecol. 1998;12(1):45-9.

54. Noguchi J, Hata K, Tanaka H, Hata T. Placental vascular sonobiopsy using three-dimensional power Doppler ultrasound in normal and growth restricted fetuses. Placenta. 2009;30(5):391-7.

55. Rizzo G, Capponi A, Pietrolucci ME, Aiello E, Arduini D. First trimester placental volume and three dimensional power doppler ultrasonography in type I diabetic pregnancies. Prenat Diagn. 2012;32(5):480-4.

56. Pomorski M, Zimmer M, Florjanski J, Michniewicz J, Wiatrowski A, Fuchs T, et al. Comperative analysis of placental vasculature and placental volume in normal and IUGR pregnancies with the use of three-dimensional Power Doppler. Arch Gynecol Obstet. 2012;285(2):331-7.

57. de Paula CF, Ruano R, Campos JA, Zugaib M. Quantitative analysis of placental vasculature by three-dimensional power doppler ultrasonography in normal pregnancies from 12 to 40 weeks of gestation. Placenta. 2009;30(2):142-8.

58. Guiot C, Gaglioti R, Oberto M, Piccoli E, Rosato R, Todros T. Is three-dimensional power Doppler ultrasound useful in the assessment of placental perfusion in normal and growth-restricted pregnancies? Ultrasound Obstet Gynecol. 2008;31(2):171-6.

59. Mercé LT, Barco MJ, Bau S, Kupesic S, Kurjak A. Assessment of placental vascularisation by three-dimesnional power Doppler "Vascular Biopsy" in normal pregnancies. Croat Med J. 2005;46(5):765-71.

60. Mayo Clinic. Pregnancy weight gain: What's healthy? http://www.mayoclinic.org/healthy-living/pregnancy-week-by-week/in-depth/pregnancy-weight-gain/art-20044360. Published March 4, 2014. Accessed Jan 11, 2014.

61. Leach L. Placental vascular dysfunction in diabetic pregnancies: intimations of fetal cardiovascular disease? Microcirculation. 2011;18(4):263–9.

62. Saldeen P, Olofsson P, Laurini RN. Structural, functional and circulatory placental changes associated with impaired glucose metabolism. Eur J Obstet Gynecol Reprod Biol. 2002;105(2):136-42.

63. Tan AE, Norizah WM, Rahman HA, Aziz BA, Cheah FC. Umbilical artery resistance index in diabetic pregnancies: the associations with fetal outcome and neonatal septal hypertrophic cardiomyopathy. J Obstet Gynaecol Res. 2005;31(4):296-301.

64. Boito SM, Struijk PC, Ursem NT, Stijnen T, Wladimiroff JW. Assessment of fetal liver volume and umbilical venous volume flow in pregnancies complicated by insulin-dependent diabetes mellitus. BJOG. 2003;110(11):1007-13.

65. Fadda GM, D'Antona D, Ambrosini G, Cherchi PL, Nardelli GB, Capobianco G et al. Placental and fetal pulsatility indices in gestational diabetes mellitus. J Reprod Med. 2001;46(4):365-70.

66. Tuuli MG, Houser M, Odibo L, Huster K, Macones GA, Obido AO. Validation of placental vascular sonobiopsy for obtaining representative placental vascular indices by three-dimensional power Doppler ultrasonography. Placenta. 2010;31(3):192–6.

67. Barth WH Jr, Genest DR, Riley LE, Frigoletto FD Jr, Benacerraf BR, Greene MF. Uterine arcuate artery doppler and decidual microvascular pathology in pregnancies complicated by type I diabetes mellitus. Ultrasound Obstet Gynecol. 1996;8(2):98-103.

68. Sankaran S, Kyle PM. Aetiology and pathogenesis of IUGR. Best Pract Res Clin Obstet Gynaecol. 2009;23(6):765–77.

69. Warsof SL, Cooper DJ. Little D, Cambell S. Routine ultrasound screening for antenatal detection of intrauterine growth retardation. Obstet Gynecol.1986;67(1):33-9.

70. Vandenbosche RC, Kirchner JT. Intrauterine growth retardation. Am Fam Physician. 1998;58(6):1384-90.

71. Chauhan SP, Doherty DD, Magann EF, Cahanding F, Moreno F, Klausen JH. Amniotic fluid index vs single deepest pocket technique during modified biophisical profile:a randomized clinical trial. Am J Obstet Gynecol. 2004;191(2):661-7.

72. Ott WJ. Reevaluation of the relationship between amniotic fluid volume and perinatal outcome. Am J Obstet Gynecol. 2005;192(6):1803-9.

73. Alfirevic Z, Neilson JP. Doppler ultrasonography in high risk pregnancies: systematic review with meta-analysis. Am J Obstet Gynecol. 1995;172(5):1379-87.

74. Davies JA, Gallivan S, Spencer JA. Randomised controlled trial of Doppler ultrasound screening of placental perfusion during pregnancy. Lancet. 1992;340(8831):1299-303.

75. Low JA. The current status of maternal and fetal blood flow velocimetry. Am J Obstet Gynecol. 1991;164(4):1049-63.

76. Dudley NJ. A systematic review of the ultrasound estimation of fetal weight. Ultrasound Obstet Gynecol. 2005;25(1):80-9.

77. Surányi A, Kozinszky Z, Molnár A, Nyári T, Bitó T, Pál A. Placental three-dimensional power Doppler indices in mid-pregnancy and late pregnancy complicated by gestational diabetes mellitus. Prenat Diagn. 2013;33(10):952–8.

78. Guimarães Filho HA, Araujo Júnior E, Mattar R, Da Costa LL, de Mello Júnior CF, Nardozza LM, et al. Placental blood flow measured by three-dimensional power Doppler ultrasound at 26 to 35 weeks gestation in normal pregnancies. J Matern Fetal Neonatal Med. 2010;23(1):69–73.

Appendix

I.

II.

III.