

ULTRASONOGRAPHIC, PHARMACOLOGIC AND PATHOLOGIC EXAMINATION OF THE FETOPLACENTAL CIRCULATION

Ph.D. Thesis

Mária Jakó, M.D.
University of Szeged
Faculty of General Medicine
Department of Obstetrics and Gynecology

Supervisors:

György Bártfai, M.D., D.Sc.
University of Szeged
Faculty of General Medicine
Department of Obstetrics and Gynecology

Andrea Surányi, M.D., Ph.D.
University of Szeged
Faculty of General Medicine
Department of Obstetrics and Gynecology

Director of Doctoral School of Clinical Medicine:

Lajos Kemény, M.D., D.Sc.

Director of Reproductive Health Programme:

György Bártfai, M.D., D.Sc.

**University of Szeged
Faculty of General Medicine
Department of Obstetrics and Gynecology
Albert Szent-Györgyi Medical Centre
Szeged, 2018.**

1. List of Related Publications

- I. Jakó M, Surányi A, Kaizer L, Gáspár R, Domokos D, Bártfai G.
A köldökzsinór rendellenességei az intrauterin fejlődési visszamaradásban
Orv Hetil 2014, 155(50) 1989-95.
- II. Jakó M, Surányi A, Janáky M, Klivényi P, Kaizer L, Vécsei L, Bártfai G, Németh G.
Postnatal outcome and placental blood flow after plasmapheresis during pregnancy.
J Matern Fetal Neonatal Med. 2017, 30(22) 2755-2758. **IF₂₀₁₇:1.826**
- III. Molnár A, Surányi A, Jakó M, Németh G.
Intraoperative Surgical Treatment of Undiagnosed Placenta Percreta
J Clin Case Rep 2016 6:12 doi: 10.4172/2165-7920.1000910.
- IV. Jakó M, Surányi A, Kaizer L, Domokos D, Bártfai G.
The Correlation of Ultrasonographic and Pathophysiologic Measurements of Umbilical
Vessels in Gestational Diabetes
Southeastern European Medical Journal 2017, 1(1) 40-49.
- V. Molnár A, Surányi A, Jakó M, Nyári T, Németh G.
A 3-dimenziós power Doppler indexek és a perinatális kimenetel vizsgálata méhen
belüli növekedési restriktióval szövődött terhességekben
Orv Hetil 2017, 158(26):1008–1013. **IF₂₀₁₇:0.322**

Other publications

1. Vanya M, Jakó M, Szabó K, Nagy N, Farkas K, Janovák L, Bártfai Gy.
Új nanotechnológiai terápiás lehetőségek és genetikai predispozíció vizsgálata
recurrens vulvovaginális candidiasisban és bakteriális vaginózisban szenvedő
reproduktív korú nők körében
Magy Nőorv L 2014, 77(5) 20-25.
2. Deák J, Jakó M, Bártfai Gy.
Herpes simplex vírus 1 és 2 által okozott fertőzések és diagnosztikájuk
Focus Med 2015, 17(3), 3-9
3. Vanya M, Fejes I, Jakó M, Tula A, Terhes G, Janáky M, Bártfai Gy.
Lyme Disease Associated Neuroretinitis- Case Report
Acta Microbiol Immun Hung 2015, 62(4) 403-408. **IF₂₀₁₅:0.921**
4. Vanya M, Jakó M, Terhes G, Szakács L, Kaiser L, Deák J, Bártfai Gy.
Oropharyngealis humán papillomavírus ritka előfordulása cervicalis laesióval
rendelkező nőkben
Orv Hetil 2016, 157(2), 70–73. **IF₂₀₁₆:0.349**
5. Jakó M
Beszámoló a III. Polgári és Büntetőjogi Felelősség az Egészségügyi Gyakorlatban
című konferenciáról
Magy Nőorv L 2017, 80 133-134

2. Abbreviations:

3DPD	3 dimensional power Doppler ultrasound
5-HT	5-hydroxy-triptamine, serotonin
AC	abdominal circumference
AED	absent end diastolic flow
BPD	biparietal diameter
FI	flow index
FOD	frontooccipital diameter
FL	femur length
GABA	gamma-amino-butyric-acid
GDM	gestational diabetes mellitus
HC	head circumference
HE	hematoxyllin/ eosine
IOD	intraorbital distance
IUGR	intrauterine growth restriction
NADPH	nicotinamide-dinucleotide-phosphate
ns	not significant
OT	oxytocin
OTR	oxytocin receptor
PED	positive end diastolic velocity
RED	reverse end diastolic flow
S/D	peak systolic velocity/end diastolic velocity
SGA	small for gestational age
SUA	single umbilical artery
ThAPD	thoracic anteroposterior diameter
ThTD	thoracic transversal diameter
UCI	umbilical coiling index
UtBF	uterine artery blood flow
relUtBF	relative uterine artery blood flow
UtPI	uterine artery pulsatility index (peak systolic velocity-end diastolic velocity)/ mean velocity
VFI	vascularization flow index
VI	vascularization index
VOCAL	virtual organ computer aided analysis
VP	vasopressin
V1aR	type 1a vasopressin receptor
WHO	World Health Organisation

3. Introduction

3.1. Intrauterine Growth Restriction

Birthweight is one of the most sensitive measures of the newborns' wellbeing. According to the World Health Organization (WHO), intrauterine growth restriction (IUGR) is diagnosed upon the estimated birthweight being below the 10th percentile of the recommended gender-specific birthweight for gestational age reference curves. Although early preterm IUGR is associated with the highest rates of mortality and morbidity, late-pregnancy IUGR remains a leading cause of unexpected perinatal death and morbidity after 34 weeks of gestation. Estimated fetal weight (EFW) is generally calculated through several steps: fetal biometric measurements are converted into a fetal weight estimate using one of several formulae. The most widely used cut-off is the 10th percentile of EFW, as suggested by the WHO and the American College of Obstetricians and Gynecologists.

The newborn's gestational age is determined by the first day of the mother's last menstrual period and during the early ultrasound examination. IUGR newborns are distinguished from SGA (small for gestational age) newborns by being thin, with reduced subcutaneous fat tissue. Their mortality rate is 3-4 times higher, their morbidity rate is also higher by 4-5% related to healthy infants. With the severity of IUGR, the risk for premature birth, fetal distress, neonatal hypoglycaemia, hypocalcaemia and polycythaemia increases. There is no conclusive evidence that this relationship would vary depending on the various etiologic factors. IUGR complicates about 8% of pregnancies and maternal, fetal or placental factors play a role in its pathogenesis.

3.2. Gestational Diabetes

The postprandial blood glucose value increases during pregnancy while the insulin sensitivity decreases. The glucose transport through the placenta is directly proportional to the maternal blood sugar level, and the elevation can increase the transport flow by up to five times. Infant respiratory distress syndrome (IRDS), cardiomyopathy, hypoglycaemia, hypocalcaemia, hypomagnesaemia, polycythaemia and hyperviscosity are more common in fetuses exposed to high blood sugar level. There are many, noncorresponding data regarding the prevalence of GDM in Hungary. In a populationbased screening program, 8.7% of pregnant women were diagnosed with GDM, and the risk increased linearly with maternal age. The first line of management of women with gestational diabetes is medical nutrition therapy and a given minimum of exercise. Patients who fail to maintain normal glycemic values via diet and

exercise therapy receive insulin. The mother's chance for type 2 diabetes ranges from 2.6 to 70.0% within 28 years postpartum. As umbilical cord vessels represent a suitable model for the study of vascular alterations brought about by GDM, the aim of the present work was to compare the ultrasonographic vascular flow measurements to pathological microvascular changes, and also to test the vasoreactivity of the vessels.

3.3. Placental circulation

The functional and structural integrity of the microcirculation of the placenta is essential for the satisfactory functioning of intrauterine transport of nutrients, gas and metabolites. The relationship between placental morphometry and unfavorable perinatal outcome is known. The smaller size, the decreased surface and the reduced volume correlate with the prevalence of IUGR. The placenta is gradually growing during pregnancy, but its mass and volume in IUGR pregnancies is below normal value. Via ultrasound, the volume of the placenta can be measured and the growth restriction of the placenta can precede fetal growth restriction by weeks. Pathological lesions affecting the placental vessels may further complicate the circulation (calcification, reduced capillarisation, decreased cytotrophoblastic proliferation, chronic vascular stenosis, infarction, fibrin deposition) that can prevent fetal development.

3.4. Ultrasonographic examination of the placenta

3.4.1. 2-dimensional ultrasonography

The functionality of the placenta is characterized by the Doppler flowmetry of the uterine arteries, umbilical arteries and arteria cerebri media, and the fetal biometry is used to monitor intrauterine fetal growth. The flow velocity in the umbilical arteries shows low resistance in the third trimester of healthy pregnancies. The increase in the resistance of the umbilical artery is a sign of circulatory insufficiency.

For IUGR fetuses, amount of blood flow through uterine artery at a time and its calculated value for fetal weight are significantly lower. However, abnormal uterine artery circulation does not necessarily mean abnormal intrauterine circulation and some of the fetuses are born with normal weight and are healthy. This implies placental compensatory factors with the fact that they have not shown a close correlation between blood flow through uterine arteries and umbilical arteries during a time unit. IUGR still remains a diagnostic challenge, since ultrasonographic biometry has only a 50% detection rate.

3.4.2. 3-dimensional ultrasonography

The VOCAL program calculates automatically the indices from gray-scale and color values of the acquired spherical placental sample. The vascularisation index (VI, the volume occupied by vessels in a particular tissue segment), the flow index (FI, the amount of blood flowing through the examined volume in a given time) and the vascularization flow index (VFI, derived from the combination of the previous two indices) can be assessed to examine the functional capacity of the placenta. The value of these indices does not change during normal pregnancy, the development of the vascular network is proportional to the placental growth.

3.5. Pharmacology

3.5.1. Oxytocin and Vasopressin

Since the placenta and umbilical cord does not have an autonomic innervation, the vascular tone is mediated by humoral factors. Mast cells along the blood vessels are potent sources of such regulative vasoactive substance like oxytocin (OT), which is physiologically present during pregnancy and passes through the placenta and arginine-vasopressin (AVP). Both are peptide hormones, they elicit constriction via transmembrane receptors. The expression of the OT gene and receptor (OTR) can be detected in the epithelial cells of chorionic, deciduous and amniotic cells after birth. Structural matching between the two peptides (approximately 80%) and the homology of the OT and AVP receptors may result in cross reaction. The expression of the vasopressin receptor 1a (V1aR) mRNA is known in sheep's placenta, but not proven in human, and the absence of vasopressin mRNA is also known. Pharmacological studies disclose controversial data regarding vasodilator function of these receptors and ligands in the vascular wall. According to our best knowledge and information, the presence of these receptors in the umbilical cord arteries has not been studied and the description of the fetal origin of the ligand exists only in hypothetical form.

3.5.2. Serotonin and Ketanserin

It has been established that serotonin stimulates both, contraction and relaxation of blood vessels. Due to the efforts made to identify the serotonin receptors involved in vasoregulation, thirteen different mammalian G-protein coupled 5-HT receptor types have been identified by molecular cloning, which have been grouped into seven families. 5-HT₁-like receptors were reported to trigger relaxation of blood vessels independent of the presence of an intact endothelium. Smooth muscle contraction is induced independently of the endothelium by activation of 5-HT_{2a} receptors. It is clear that serotonin regulates vasoconstriction and

vasorelaxation in a complex way which involves the interaction of several serotonin receptor subtypes with conflicting functional effects. Serotonin is a potent regulatory factor in fetoplacental circulation and it can also be found physiologically in measurable quantity in the umbilical cord blood. Its contractile effect was confirmed in both normal and preeclamptic pregnancies. Its dose-effect curve is associated with mild vasodilation, which disappears at a dose of 10^{-7} M and is dominated by contraction afterwards.

Ketanserin is a selective 5-HT_{2a} antagonist but shows measurable affinity for the 5-HT_{2c} receptor, α ₁ and α ₂-adrenergic receptors, and 5-HT_{1d}, 5-HT_{2b}, 5-HT₆, and 5-HT₇ receptors. It is used in pre-eclampsia for the treatment of maternal hypertension, as it has only peripheral vascular effect, it has no chrono-, dromo- or bathmotrop effect. The optimal therapeutic dose has not yet been determined. It is transported through the placenta fairly enough to develop its effect on the fetal circulation.

4. Aims and hypotheses:

Our objectives were to investigate the fetoplacental circulation and the vasoregulation of the umbilical and placental vessels in healthy pregnancies and pregnancies complicated by IUGR or GDM. Our hypothesis is that in case of placental insufficiency and compromised fetal growth a misregulation of vascular resistance and vascularization leads to the unfavourable neonatal outcomes. In order to prove our hypothesis we assessed the following data and investigated their relationship:

- 1) We assessed ultrasonographic biometry, flowmetry and 3-dimensional ultrasonographic indices of the afore mentioned pregnancy groups. Ultrasonographic data was compared to blood vessel response to vasoactive agents.
- 2) We investigated whether the vasoreactivity to the investigated agents is altered in the fetoplacental circulation in pregnancies complicated by IUGR or GDM.
- 3) We investigated the pathological and histological alterations in the placenta and umbilical cord and whether these findings can affect or be a result of compromised vasoregulation and may lead to restricted fetal growth.

5. Materials and Methods

5.1. Patient recruitment and ultrasound examination

We recruited pregnant patients at the Department of Obstetrics and Gynecology, University of Szeged, between January 2014 and May 2017. Patients have read and signed the Informed

Consent (Ethics Registry Number: 49870-3773 / 2014 / ECU 586) after which estimated fetal weight was determined by Hadlock 'B' formula:

$$\text{Log}_{10} \text{ EFW} = 1,335 - 0,0034 \text{ ACxFL} + 0.0316\text{BPD} + 0.0457\text{AC} + 0.1623\text{FL}$$

IUGR and control group were set up based on the EFW, in the IUGR group the estimated fetal weight in the 2nd and 3rd trimester of pregnancy was below the 10th percentile. Additionally, premature deliveries, newborns with genetic malformation, chromosome or developmental disorders, pregnancies complicated by hypertension, diabetes were excluded from the control and IUGR group.

The GDM group consisted of pregnant patients diagnosed at the 24-28th week 75 g oral glucose tolerance test based on the WHO diagnostic criteria, all of whose blood glucose levels were controlled by diet, those who needed insulin therapy were excluded from this study. None of the recruited patients had immunologic, cardiovascular, gastrointestinal or pulmonological disease. Patients with twin pregnancy, history of habitual abortion or assisted reproduction, fetal developmental malformations were excluded.

All pregnancies were examined in a semirecumbent position with "Obstetrics / 2-3 trimester" in 2D mode. Conventional color Doppler assay was used to determine the flow values of the umbilical artery. 3D power Doppler Ultrasound volume image was made at the placental insertion of the umbilical cord. We performed the Mercé type sonobiopsy to calculate the 3DPD indices by VOCAL program.

5.2. Pharmacologic studies:

The umbilical cord and placenta were immediately placed into Krebs-Henseleit buffer solution at pH 7.4 and 4°C. The cord was removed from the placenta and perfused with the solution. For the tissue bath experiments fresh buffer was prepared and heated up to 37°C and the examination took place within 24 hours after delivery. The vessels were cut into 3-5 mm long segments and mounted on stainless steel hooks. During incubation the vessels were marched to 2g initial tension, bubbled with carbogen gas. During the incubation, tissues were washed through with fresh solution in every fifteen minutes and reached equilibrium within 60 minutes, and at logarithmic single dose levels (10^{-10}M - 10^{-7}M) of oxytocin (Sigma-Aldrich O3251) or desmopressin (vasopressin analogue, Sigma-Aldrich D0650000) was added to the system in noncumulative fashion.

In the second study under the same conditions, serotonin (Sigma-Aldrich H9523) was added to the vessels in the similar pattern as oxytocin. We administered serotonin in logarithmic

concentration (10^{-9}M - 10^{-5}M) in every 6 minutes cumulatively. To isolate receptor subtypes, a new study was performed by incubating the blood vessels with 10^{-8}M ketanserin (Sigma-Aldrich S006) for 6 minutes and testing for the cumulative dose-response curve of serotonin. In the 10^{-9}M - 10^{-5}M range of ketanserin, we found that 10^{-8}M concentration alone did not affect the tone.

5.3. Pathological examination:

The volume of placenta was determined by water displacement method. The tissues were assigned a numeric code after delivery and restored for further examinations.

The pathological study was performed after 3 to 7 days of formalin fixation, measuring the placental weight and volume according to the Royal College of Pathologists Guideline. After measuring the diameters, the placenta was cut into 1 cm thick stripes along the longest diameter and its thickness was determined at the umbilical cord insertion. We prepared histological samples from the placental end of the umbilical cord. We digitally recorded the longest cross-sectional diameter of the cord, the cross-sectional area of blood vessels and the thickness of the vessel wall. Due to the coiling of the umbilical cord, the vascular image was never a perfect mathematical cross section, therefore the arterial wall was measured at the following locations: the most and the least distorted location and a third measurement was performed at 3/6/9 o'clock depending on the previous two data. The measurements were done blindly, the samples were identified only by their numeric code. Histological samples of the placenta were taken from the umbilical cord insertion, the peripheral area, the maternal and the fetal side, and from the pathological alteration, if there were any.

5.4. Statistical analysis:

When comparing maternal and fetal parameters with normal distribution, Student's t-test was used. The total thickness of the umbilical cord, the vessel wall thickness and the various lumen shapes and the dose-response curve of the active compounds were analyzed by ANOVA. The area under the curve (AUC) was determined by the ISOSYS Program software (SOFT-02-ISO S.P.E.L. ADVANCE ISOSYS, MDE Kft. 1062 Budapest) at each dose and each vessel.

Data showing non-normal distribution were analyzed with Kruskal-Wallis probe with Bonferroni correction. Differences were considered significant when $p < 0.05$. Linear regression was studied between placental morphometry and newborn parameters. The

Pearson's correlation was judged if two variables co-varied. For statistical analysis, Prism 6 (2016, Graph Pad Software Inc. La Jolla, California, USA) software was used.

6. Results

6.1. Results of IUGR/GDM/control study with oxytocin and desmopressin

There were no significant differences among the groups except for birthweight (mean \pm SD) that was $3221\text{g} \pm 266.1$ in controls, $2350\text{g} \pm 194.0$ in IUGR ($p=0.0023$) and $3627\text{g} \pm 363.5$ in the GDM group ($p=0.0411$), the IUGR newborns were also shorter, their head circumference and abdominal circumference were smaller than in the other two groups that did not differ from each other significantly, the birthweight/placental weight ratio was also smaller ($p=0.0112$). The 1 minute Apgar was 9.46 ± 0.52 , 8.35 ± 1.63 ($p=0.0360$) and 8.20 ± 1.10 ($p=0.0213$) respectively. There were no significant differences between medications and drugs taken during pregnancy, and proportion of those who did not take any medication. The caesarean section frequency was 17% in the control group, 75% in the IUGR group and 40% in the GDM, there was no statistical difference in pregnancy history among the groups. In all pathological histological studies, 3 vessels were found throughout the length of the cords. Typically, the cords are twisted counterclockwise. The umbilical coiling index (UCI) was smaller in the IUGR group ($p=0.0418$). The coiling pattern was determined on the basis of a study published by LM Ernst in 2013, in umbilical cords from IUGR pregnancies, the undulating pattern was uniformly found, while in the other groups the rope pattern was dominant. The cord cross-sectional area was smallest in case of GDM pregnancies ($p=0.0027$) but the vascular cross-sectional area was smallest in the IUGR group ($p=0.0058$). In the umbilical cord of IUGR fetuses, we could see perivascular haematoma and once haemangioma cavernosum, several monocytes, and mild funitis and an edemic cord in GDM. There was no significant difference between control and IUGR samples.

The 3DPD indices were (mean \pm SD) FI: 49.16 ± 1.76 , VI: 10.08 ± 0.49 and VFI: 4.9 ± 0.16 in controls while 38.48 ± 1.74 ($p=0.015$), 4.53 ± 0.61 ($p=0.0004$), 2.70 ± 0.74 ($p=0.0194$) in IUGR and 35.50 ± 5.89 ($p=0.0318$), 4.90 ± 1.85 ($p=0.0136$), 1.83 ± 1.01 ($p=0.0069$) in GDM pregnancies respectively.

The change in vascular tone elicited by logarithmic non-cumulative oxytocin dose was not significant in the arteries or veins in any of the groups, and the measurement results reflect the dynamic variability of baseline equilibrium. Since oxytocin elicits vascular effect via type 1 vasopressin receptor, a more powerful agonist, desmopressin, was administered during the

experiment, but it was again found that there was no significant alteration in vascular tone. The viability of the vessels were tested with contractions elicited by 10^{-5} M serotonin.

6.2. Results of IUGR/control study with serotonin and ketanserin

There were no significant difference in blood parameters that could alter the blood viscosity meaning that the difference in oxygen and nutrient supply is rather due to the regulation of vascular resistance. The systolic/diastolic flow velocity in the umbilical arteries of IUGR fetuses were 3.39 ± 0.38 ($p=0.0006$) and 2.22 ± 0.11 in controls, the birth weight ($p=0.0023$) and Apgar scores ($p=0.036$) were lower in IUGR newborns related to the control group. There were no significant differences in patient histories.

From the 3DPD data, we can conclude that there is a more prominent difference in the number of vessels in a unit volume, than the blood flowing through a corresponding volume of the placenta in a certain time. Reducing the vascular resistance increases the blood flow through a given volume of the placenta. By reducing resistance, the placenta can compensate hypovascularization to a certain level, a significant reduction in the blood flow still can be observed but not as pronounced as in vascularization. The umbilical artery S/D ratio did correlate well with the maximum response to serotonin ($r=0.5328$).

Serotonin is stored in the umbilical cord in perivascular mastocytes. When released into the vessels, it has a vasoconstricting effect on the 5-HT₁₋₂ and 7 receptors, which was evaluated on a total of 240 vessel segments. It elicited significant contraction in control and IUGR umbilical arteries at the concentrations of 10^{-6} M. In case of umbilical veins significant contraction occurred at 10^{-7} M concentration in controls, while in IUGR at 10^{-6} M. The placental arteries contracted at 10^{-6} M concentrations in controls and only at 10^{-5} M in IUGR. In the placental veins the effect reached significance at 10^{-7} M in controls, but no significant reaction could be observed in IUGR. However, ketanserin inhibition results in a significant reduction in contraction and this effect is more relevant in IUGR fetuses ($n = 30$) than in healthy pregnancies ($n = 48$). While in the umbilical arteries the reaction to serotonin without ketanserin incubation does not show significant difference, after ketanserin incubation, the reduction in IUGR vessel reactivity is more prominent and in controls the difference reaches significance only at 10^{-7} M concentration. In the case of umbilical veins the control group shows more contractility over 10^{-7} M concentration, but after ketanserin incubation the reactivity of the IUGR veins is nearly eliminated yet the control vessels show as much reactivity as the IUGR without the antagonist. Both the placental arteries and veins are more

contractile to serotonin in the control group but the IUGR vessels differ at only 10^{-7} M and 10^{-6} M concentrations respectively. After ketanserin incubation the responsiveness to the agonist decrease in both groups, in controls over 10^{-6} M and in IUGR over 10^{-7} M. In case of IUGR placental veins neither the contraction to serotonin nor the effect of ketanserin reaches significance. This difference between the case and the control group may be due to the higher relative density of the 5-HT₂ receptor in the umbilical veins of IUGR fetuses, resulting a smaller contraction on the fewer 5-HT₁ receptor and reduced effectiveness of ketanserin on the elicited contraction.

The morphometric examination of the placenta, showed that the volume measured after birth is more strictly correlated with the weight of the newborn than the mass or volume of the placenta after formalin fixation. Umbilical arterial cross-sectional area shows significant difference between the two groups and has a slight correlation with birth weight ($r = 0.336$). Two or more histopathological changes in the placenta is characteristic for IUGR pregnancies.

7. Discussion

Perinatal complications, such as necrotizing enterocolitis, low Apgar score, hypoxic brain damage, need for respiratory support, chronic lung disease, retinopathy, and prolonged perinatal intensive therapy are more likely in case of IUGR newborns. 2D ultrasonographic examination of the umbilical arteries showed that the flow velocity curve may be normal in growth restricted fetuses as compared to their gestational age, but its abnormal appearance can be considered as a diagnostic signal for intrauterine growth restriction. Compared to normal pregnancies, the arteria umbilicalis S/D ratio remains permanently high in IUGR fetuses and correlates with later complications. The flow rates were similar in our measurements.

The umbilical artery blood flow of GDM pregnancies showed a normal S/D ratio. Of the 3DPD indices, the VI has been low, so the placenta is hypovascularized related to normal and the blood vessels are damaged by high blood sugar levels. Increased glucose transport leads to the activation of pentose phosphate and NADPH oxidase resulting in excessive free radical formation. The free radical's proinflammatory effect leads to atherosclerosis. Initial ischemic signs include syncytial knot, hypovascularized villus, interstitial calcification, and extravascular fibrin deposition. Their combined, long-term consequence is the increase of their arterial resistance. In case of GDM pregnancies, the umbilical coiling index exceeded the

control, while the IUGR umbilical cord showed significantly lower number of turns. The thin, hypocoiled cord with less Wharton's jelly is unprotected against mechanical impacts, that makes the fetus and the newborn more vulnerable.

Since direct contraction was observed neither for oxytocin nor for desmopressin, it can be assumed that there is no type 1 vasopressin receptor in the umbilical cord vessels. The vascular rings were contracted with serotonin to confirm their viability. In a placenta perfusion model, vasopressin (30pg / ml to 60,000pg / ml) administered on the maternal side was measured at the fetal side with a maximum of 3110 pg / ml, which was $3.11 \times 10^{-8} \text{M}$. The highest concentration we have studied is 10^{-7}M , which is 30 times higher. If we could not contract the umbilical cord in vitro due to decreased receptor number, it can not be contracted with in vivo vasopressin dose because the amount of active substance does not reach the umbilical vessels.

Serotonin can be found in detectable amount in umbilical cord blood after birth and may also play a role in occlusion of the umbilical vessels. In vascular smooth muscle cells, serotonin exerts a contractile effect on its own receptor. Serotonin-induced vasoconstriction may be similar to or exceeds that of potassium-chloride and is reduced in calcium-free environment. In the case of umbilical arteries, the initial small vasodilation is followed by a dominant contraction. Endothelial receptors and release of nitric-oxide are responsible for the short dilatation, after removal of the endothel this transient reaction disappears. We studied the reactivity to serotonin in umbilical and placental vessels in normal and IUGR term deliveries. Regarding the umbilical cord arteries, the contraction of the IUGR vessel rings was stronger but with ketanserin inhibition, its reactivity decreased significantly ($p < 0.05$) and remained below the controls. In the case of umbilical veins, the control group's veins reacted with higher intensity but the difference persisted with ketanserin inhibition. In IUGR pregnancies the vascular tone in umbilical arteries is more elevated by serotonin than controls, resulting increased resistance and abnormal blood flow velocity. The physiologic effect of serotonin can be seen in the correlation between its effect and S/D ratio and their relationship with perinatal outcome. Ketanserin in the umbilical circulation would decrease vascular resistance thus providing beneficial circulatory environment for the fetus. This effect is more prominent in IUGR fetuses. In the placenta IUGR vessels were less contractile and this difference persisted with the presence of ketanserin. The difference in the vascular tone regulatory capacity can be seen in the 3DPD indices too; the difference in VI is more prominent than in

the FI. Although there are fewer vessels in a certain volume of the placenta in IUGR, these vessels are less contractile to serotonin, a humoral regulator, providing smaller vascular resistance and more blood flowing to the fetus. These results suggest that serotonin has a major role in regulating fetoplacental blood flow and might play a role in the circulatory environment in intrauterine growth restriction. Fetoplacental circulation might be improved by ketanserin. Regarding that the umbilical and placental circulation is balanced by several other humoral factors that can distort our data, additional studies are required to map the receptor spectrum of the foetoplacental unit to improve fetal circulation.

Based on the pathological data it can be concluded that the volume of placenta shows a stronger correlation with the weight of the newborn than the weight of the placenta. Although morphometric results show differences between IUGR and control cases, they do not reflect the functional capacity of the placenta. A large placenta with continuous infarcts and calcifications is found among the upper region of the weight percentile chart, but the fetus can be IUGR due to insufficient transport function. By measuring the volume of the placenta and the 3DPD indices, we can obtain information that correlates with birth weight. For the purpose of improving IUGR diagnostics and pregnancy care, a percentile curve or diagnostic flowchart can be constructed from the aforementioned data. Our results also show that histological and ultrastructural differences may be present in milder forms in normal pregnancies, but two or more of these differences already pose a significant reduction in the compensative capacity of the placenta and can be diagnostic sign for IUGR. Based on our results, only the cross-section area of the arteries showed deviation, which corresponds to the data reported in the literature. This difference also plays a role in the differences in flow in the arteria umbilicalis, but the causative relationship is not clear yet.

Based on the results of this study, we can make the following statements:

The volume of the placenta is more closely related to the perinatal outcome than the placental weight. The volume of the placenta can be measured ultrasonographically in utero and therefore may be an informative part of IUGR diagnostics. However the functional volume of the placenta is more important than the absolute volume, to determine this, the 3DPD ultrasonographic indices allow the measurement of VI, FI and VFI values that correlate with clinical data of the newborn. With the establishment of percentile or cut off values it is possible to improve the monitoring of IUGR pregnancies.

In regulating the circulation of umbilical cord and placenta, the effect of oxytocin and desmopressin was investigated. Neither oxytocin nor desmopressin elicited a significant increase in vascular tone in healthy, IUGR or GDM pregnancies. Serotonin-induced contraction in umbilical cord arteries correlates with clinical data. The response of the placenta to serotonin reflects the value of the 3-dimensional ultrasonographic flow index.

The physiological role of serotonin in vascular tone regulation can be assumed but needs further research. The different contractivity of the vessels in IUGR and their altered response in ketanserin inhibition can be explained by difference in receptor-density and/ or difference in receptor subtypes. The altered density of 5-HT1 receptor itself can play a role in the pathogenesis of IUGR and the relatively higher density of 5-HT2 subtype can be another predisposing factor. As ketanserin passes from maternal blood to the fetoplacental circulation, its therapeutic use in IUGR should be investigated.

Further studies are needed to map the receptor spectrum of placenta and umbilical cord vessels and to understand the regulation of vascular tone. Based on our results, inadequate fetal development can be studied from a new etiologic perspective and a new therapeutic approach can be possible.

8. Conclusion

Among the placental 3-DPD indices the vascularisation index shows a more prominent decrease than the flow index suggesting that the vasoregulation of the fetoplacental circulation can compensate the hypovascularization of the placenta. Oxytocin and desmopressin are not likely potent vasoregulators of the fetoplacental circulation. Their effect was only observed on meconium-stained tissues. Based on our results, there is probably no type 1 vasopressin receptor in the veins of the umbilical cord or is present only in the inactive form. Serotonin elicits a dose-dependent vasoconstriction on both arteries and veins in both the placenta and the umbilical cord. The reactivity to serotonin is altered in IUGR pregnancies. This difference is magnified if the tissues are incubated with ketanserin, a selective 5-HT2 receptor antagonist. Serotonin seems to be one of the major humoral factors that determine the amount of blood flowing through the fetoplacental circulation. The difference in the receptor density of the IUGR and control placentas suggest that serotonin /serotonin receptors might play a role in the pathogenesis of IUGR.