**RIGA STRADINS UNIVERSITY**

**Natalija Vedmedovska**

# Fetal Growth Restriction

**For obtaining the degree of a Doctor of Medicine**

**Specialty: Obstetrics and Gynecology**

**Research supervisor: Assoc. Professor Dace Rezeberga, Riga Stradins University**

**Research Scientific Consultant: Prof. Gilbert GG Donders, Leuven University, Belgium**

Riga, 2011

Promotion work has been carried out at the Riga Maternity Hospital Histological examinations were performed at the University Children Hospital, Division of Pathology, Riga Stradins University

**Research supervisor: Assoc. Professor Dace Rezeberga, Riga Stradins University**

**Research Scientific Consultant: Prof. Gilbert GG Donders, Leuven University, Belgium**

## Reviewers:

**Professor Aigars Petersons, Riga Stradins University, Latvia**
**Assoc. Professor Povilas Sladkevicius, Malmo University Hospital, Sweden**
**Professor Ruta Nadisauskiene, Lithuanian University of Health Sciences, Lithuania**

## RELEVANCE OF THE SUBJECT MATTER OF THE STUDY

Intrauterine growth restriction (IUGR) is defined as the inability of a fetus to maintain its expected growth, with estimated fetal weight or actual birth weight below the $10^{th}$ percentile for gestational age (*American College of Obstetricians and Gynecologists-ACOG, 2001*). ACOG notes that the terms IUGR and small for gestational age (SGA) have been used interchangeably in the literature, creating confusion about the topic. For practical clinical purposes, the term SGA should be used to refer to the *infant* after birth, and the term "intrauterine growth restriction", or better, "fetal growth restriction" (FGR), should be used to refer to the *fetus* before birth (Chauhan, 2009). Fetal growth restriction occurs in 5 to 10% of all pregnancies. Up to 70% of FGR's are constitutionally small but healthy (*Alberi, 2007*). Failure of the fetus to achieve its genetically determined potential size may be the result of variable pathologic pathways, many of which are still largely unravelled (*Maulik, 2006b*).

Fetal growth restriction is associated with significant perinatal morbidity and mortality, including iatrogenic prematurity, fetal compromise in labor, need for induction of labor and cesarean delivery. Swedish research (*Cnattingius, 1998*) showed a 10-fold increase in late fetal death among very small fetuses. Similarly, Gardosi *et al*. (*1998*) noted that nearly 40% of stillborns without malformation were small for gestational age. Surviving fetuses with FGR are at increased risk for severe neonatal morbidity such as necrotizing enterocolitis, thrombocytopenia, temperature instability, and renal failure (*Hackett, 1987; Aucott, 2004*). Recently, Figueras *et al*. (*2009*) discovered that full-term SGA newborns performed worse in all of the neuro-behavioral tests than did normal weight full-term babies, and had significantly lower scores in tests for attention deficit, motoric development and social-interactive skills. Intrauterine deprivation is associated with delay in childhood motoric, cognitive and social development (*Zubrick, 2000; Dubois and Girard, 2006*). In prospective studies, FGR infants rarely developed major neurological impairment such as cerebral palsy and seizures, although Fitzhardinge and Stevens reported an incidence of 7% major neurological sequels at 4-6 years of age for children born with FGR (*Stevens, 1972*). At least one-third of FGR newborns never

achieve normal height (*Fitzhardinge, 1989*). Additionally, Leitner *et al.* (*2002*) have reported decreased sleeping time and poorer sleep efficiency in children having FGR. Furthermore FGR is related to cardiovascular and metabolic diseases in adulthood (*Kaiser, 2009*).

Different maternal risk factors are known to be involved, such as hypertensive, renal and autoimmune diseases, and the use of medication and illicit drugs. Furthermore, maternal life style factors, like smoking and awkward dietary habits, interfere with mechanisms regulating fetal growth (*Maulik, 2006b; Ramon, 2009*). Complications of previous pregnancies (such as very long and short interpregnancy interval, previous small for gestational age babies, previous stillbirth) may increase the risk of fetal growth restriction (*McCovan, 2009*). There is a correlation between maternal characteristics and abnormal placental growth, resulting in low placental weight and impaired fetal growth (*Baptiste-Roberts, 2008*). The advances described in this work regarding assessment of fetal circulation and examination of placentas from FGR pregnancies should help to improve antenatal care and significantly reduce perinatal mortality and morbidity in Latvia.

FGR remains a challenging problem for clinicians. Most cases of FGR occur in pregnancies in which no risk factors are present; therefore, obstetricians must be alert to the possibility of a growth disturbance in all pregnancies. No single measurement can secure the diagnosis and therefore a more comprehensive assessment is necessary. The ability to diagnose the disorder and understand its pathophysiology still outpaces the ability to prevent or treat its complications. Ultrasonographic evaluation of fetal size and growth, as well as fetal hemodynamic changes is main issues in antenatal care.

The present study concentrates on exploring the impact of maternal and fetal factors on fetal growth in Latvia on the one hand, and the possibility to use the Doppler velocimetry as a sensitive predictive tool on the other hand. The study of fetal growth restriction was used as a model for achieving the set objective.

## STUDY OBJECTIVE

The **aim** of this study is to define risk groups and give evidence-based recommendations for development of clinical guidelines for management of FGR pregnancies in Latvia

The following **objectives** have been set for achieving this aim:

1) To determine the maternal and fetal risk factors for FGR

2) To study the arterial and venous redistribution (fetal and maternal hemodynamic changes) in cases of FGR and its effect on mode of delivery, pregnancy outcome and neonatal morbidity and mortality.

3) To analyze the macroscopic and microscopic changes of the placenta in detail, in order to test the hypothesis that vascular damage due to decreased maternal vascular perfusion may be responsible for intrauterine growth restricted fetuses.

## RESEARCH HYPOTHESIS STATEMENT

1) Maternal factors as well as fetal disorders interfere with normal fetal growth.

2) Placental pathology and umbilical cord abnormalities play an important role in the development of FGR.

3) Management of FGR cases can be improved in Latvia. This will require the adaption of national guidelines. Concrete proposals will be provided.

## SCIENTIFIC VALUE

**Novelty of this study**: FGR was studied during pregnancy, including assessment of maternal and fetal risk factors, evaluation of fetal circulatory redistribution, with special

emphasis on splenic artery and left portal vein system, and finally histological macroscopic and microscopic changes of the placenta. The results of the study propose to help compose or adapt specific clinical guidelines related to FGR management, which should improve the perinatal morbidity and mortality rates of affected children in Latvia. We also discovered a negative effect of STI and bacterial vaginosis and pre-pregnancy smoking on fetal growth. We drew attention to bleeding in early pregnancy as a prognostic factor for developing FGR and the relation of the former to more severe hemodynamic abnormalities. To our knowledge, we are among the first to study the adaptive redistributional changes of splenic artery and the left portal vein in intrauterine growth restriction. Finally our data provide consistent evidence by Doppler flow patterns as well as histological examination of the placenta, that abnormal blood flow in maternal circulation is an important factor in the causation and prognosis of FGR.

## THE STRUCTURE OF THE STUDY

The dissertation consists of 15 chapters: Introduction, Relevance of the subject matter of this study, Review of literature, Study objective, Research hypothesis statement, Scientific value, Materials and methods, Results, Discussion, Conclusions, Clinical implications and future aspects, References, Original publications, Supplement and Acknowledgements. The dissertation is written on 166 pages, including 23 tables, 33 figures and 1 supplement. The list of references consists of 302 titles.

## APPROBATION OF STUDY

A total of 14 presentations have been prepared in relation to the subject-matter of the study: twelve of them were presented at different international scientific congresses, two – at scientific conferences taking place at Riga Stradins University.

## PUBLICATIONS ON SUBJECT-MATTER OF STUDY

Six articles have been published in peer-reviewed scientific journals. The list of publications is included at the end of the summary.

## MATERIALS AND METHODS

A prospective case-control study was conducted in Riga Maternity Hospital from May 2007 until December 2009.

### 1. Patients

Ninty-nine unselected consecutive women with the antenatally suspected diagnoses of intrauterine growth restriction were recruited.

**Inclusion criteria were:**

- Sonographically estimated birth weight below the $10^{th}$ percentile for gestational age and gender
- Intention to deliver in Riga Maternity Hospital
- Singleton pregnancy

**Exclusion criteria were:**

- Multiple pregnancy
- Rh immunization
- Woman's refusal to participate in the study

Inclusion criteria for the controls matched for gestational age (Paper I-V) were the following:

- Sonographically estimated birth weight appropriate for gestational age and gender
- Intention to deliver in Riga Maternity hospital
- Singleton pregnancy

Exclusion criteria were:

- Multiple pregnancy
- Rh immunization
- Woman's refusal to participate in the study

Inclusion criterion for the control group for Paper VI was the following:

- The next two women who gave birth subsequently to FGR case, irrespective of birth weight

Exclusion criteria for Paper VI were:

- Multiple pregnancy
- Rh immunization

## 2. Study design

Women who attended Riga Maternity Hospital with the suspected diagnosis of intrauterine growth restriction of singleton fetus were invited to participate as cases. A control group was selected according to the protocol and the aims of the study. In the study of the influence of maternal factors (Paper I), the study about associated placental pathology (Papers II and III) and the studies about the possible interference on the growth of Doppler findings (Papers IV an V), controls were selected as each succeeding case with a normally developed, appropriately growing fetus, matched for gestational age, presenting after each confirmed FGR case**.** Both cases and controls filled out a specially designed questionnaire about possible risk factors for FGR.

For the study addressing the perinatal outcome (Paper VI), a different control group was chosen to provide a more appropriate assessment of comparative perinatal factors. These controls consisted of the next two women who gave birth subsequently to a FGR case, irrespective of their birth weight. The questionnaire included information obtained from standardized medical records.

All patients were informed of the goals and methods of the study before enrolment, and signed the Informed Consent form before being included in the study. The study conformed to the standards set by the *Declaration of Helsinki.* The Ethics Committee of Riga Stradins University has approved the study.

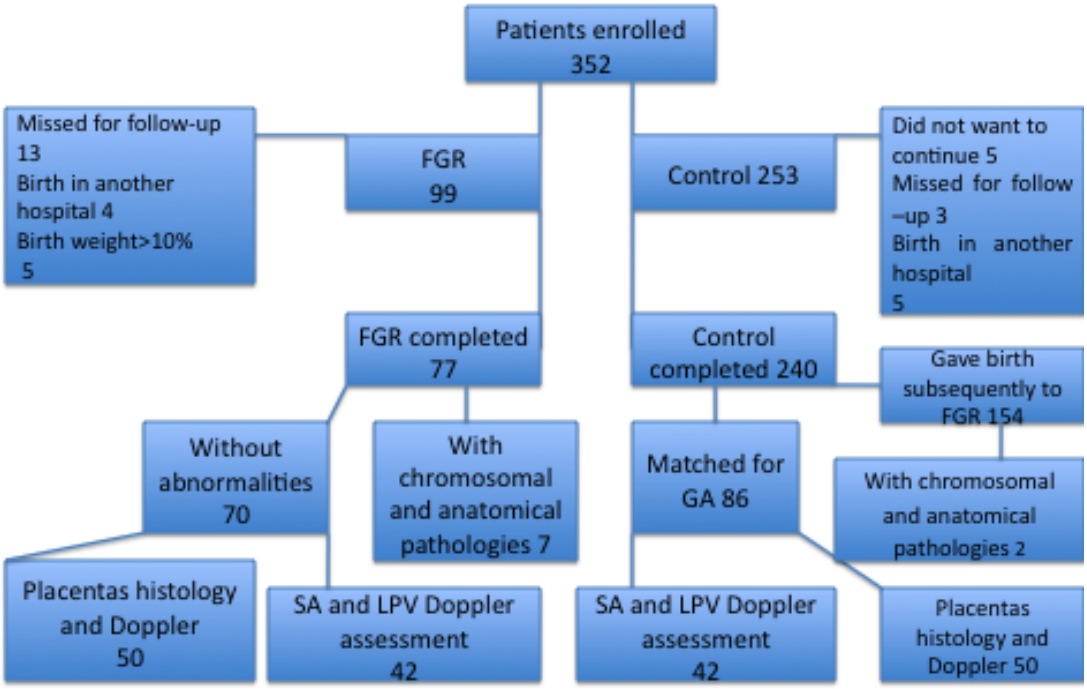


***Figure N 1***

A total of 317 patients from the sample of 352 have completed the study. Of the 99 fetuses with estimated fetal weight below the10th percentile included in the study after ultrasound examination, five had a birth weight above 10 percentile, four gave birth in another hospital and another 13 missed the follow up study at some stage, leaving 77 **Cases** eligible for analysis, seven of which had different kinds of chromosomal and anatomical abnormalities.

The control group for the five sub-studies mentioned above consisted of 99 women matched for gestational age. Thirteen of 99 of them were withdrawn from the study due to the following reasons: five patients decided to deliver in another maternity hospital and could not be traced, five refused to undergo serial ultrasound examinations and three were invited for examinations but did not attend the follow-up procedure due to logistical reasons (see *Figure N 1)*. According to the financial plan, 100 placentas (50 FGR and 50 prospective controls) were examined histologically, restricting the study of histology with Doppler measurements to that number. The techniques of investigation and measurement

of the left portal vein and splenic artery Doppler velocimetries were trained during the author's month-long visit to the Department of Clinical Medicine, University of Bergen, Norway. In total 84 patients (42 FGR and 42 prospective controls) LPV and SA Doppler velocities were adequately assessed by this new and largely unexplored technique.
The control group for paper VI included 154 patients, of whom two had chromosomal aberrations.

## 3. Ultrasound measurements

Studies of fetal growth included serial ultrasonographical and Doppler velocimetry examinations by. We used a 2-5, 2-7 or 4-8 MHz abdominal transducer (Philips, AU 22, USA) with color Doppler and pulsed Doppler facilities. The high-pass filter was set as low as possible, at 70 Hz. The mechanical and thermal indices were below 1.1 and 0.9, respectively, in most of the session, and were always kept below 1.9 and 1.5.

Standardized measurements are important when using reference charts. We measured four biometric parameters three times, noted a mean for each and used these data to calculate the estimated fetal weight according the formula of Combs et al. (1993).

### 3.1. Head size

More than 30 years ago, when the first charts based on PBD were introduced (*Campbell, 1969*), the advantage of a well -defined leading edge made the outer-inner measurement a commonly used method (*Persson, 1978, Altman, 1997*). However, with improved ultrasound technology the general principle otherwise used in morphometric techniques could also be applied for the head biometry, and the outer-outer measurements of BPD was introduced in many countries (*Hadlock et a. 1982, Hansman 1985*). In the present study we used BPD measured outer-outer.

### 3.2 Abdiominal size

The abdominal measurements are obtained in a transverse section of abdomen at the level where the umbilical veins enters the liver.

### 3.3. Femur length

The fetal FL is obtained in a longitudinal section by placing the calipers at the end of the diaphysis (*Goldstein, 1987*).

### 3.4. Doppler studies

At each session we aimed at measuring blood flow velocity in the 1) uterine artery (UtA*),* 2) umbilical artery (*AU*), 3) middle cerebral artery (*ACM*), 4) left portal vein (LPV), 5) splenic artery (SA), 6) *ductus venosus* (*DV*). We applied standardized techniques for assessing vessels (*Kiserud et al. 1991; Mari et al. 2005; Kessler et al., 2007a*). All recordings have been obtained in the absence of fetal breathing and fetal movement with the insonation along the vessel axis. The angle of insonation was kept as low as possible and always lower than 30°. Each examination time did not last more than 1 hour, and we placed women in a semirecumbent position.

Color Doppler imaging was used to identify the UtA (Gomez, 2008). The probe was placed on the lower quadrant of the abdomen, angled medially, and again color Doppler imaging was used to identify the UtA at the apparent crossover with the external iliac artery. Measurements were taken approximately 1 cm distal to the crossover point. In all cases the pulsed Doppler gate was placed over the whole width of the vessel. Angle correction was then applied and the signal updated until three similar consecutive waveforms had been obtained. The PI of the left and right arteries was measured, and the mean PI was calculated. The presence or absence of a bilateral early protodiastolic "notch" was noted. A "notch" was defined as a persistent decrease in blood flow velocity in early diastole, below the diastolic peak velocity.

The *AU* recordings performed in a free-floating section of the umbilical cord. Angle correction was then applied and the signal updated until three similar consecutive waveforms had been obtained.

The *ACM* was visualized using color flow mapping in an axial section of brain. The Doppler beam has been directed along the *ACM*, and the sample volume was placed over

the proximal section where *ACM* emerges from circle Willis. When the *ACM* near the field was not able to interrogate, the ACM of the opposite site has been used.

Using color Doppler, the *ductus venosus* was identified in mid-sagittal or oblique transaction as a vessel connecting the umbilical vein with inferior *vena cava* and exhibiting the typical aliasing of high velocities compared with the umbilical vein.

The Splenic Artery was visualized in the horizontal insonation that identified its origin at the CA in front of the aorta and its course behind the stomach to the spleen. The sample volume placed over the proximal part of the vessel.

The left portal vein was identified with color Doppler in a transverse insonation as an extension of the umbilical vein after the branching site of the *ductus venosus*.

### 3.5. Surveillance assessment

For the evaluation of fetal wellbeing the biophysical profile was used. The biophysical profile (Manning, 1999) has 5 components: 4 ultrasound assessments and a nonstress test. The nonstress test evaluates fetal heart rate and response to fetal movement. Amniotic fluid was considered abnormal in presence of amniotic fluid index less than 5 cm.

Each ultrasound assessment is graded either 0 or 2 points, and then added up to yield a number between 0 and 8. Each variable receives 2 points for a normal response or 0 points for an abnormal or absent response. A BPP of 6 to 8 is generally considered reassuring.

Delivery was indicating in presence of either an abnormal biophysical profile or by the presence of variable deceleration characterized by a decrease in heart rate from the baseline of at least 30 beats per minute (at least six in 60 minutes) and/ or in a case of abnormal Doppler studies depending on degree of fetal compromise. The decision on the best mode of delivery was based on the gestation, fetal condition and cervical status. In cases with evidence of fetal acidemia, cesarean section was performed

## 4. Outcome assessments

*Data collection:* A questionnaire was designed to inquire about the possible risk factors for FGR (*see Supplements, appendix 1*). The standard antenatal files were used for

collecting all data concerning medical history, STI screening, medication and recreational drug use, including alcohol and smoking, during pregnancy.

*Infectious laboratory techniques.* Upon inclusion in the study, a screening for STI was performed for all who did not receive one during pregnancy. The screening was done according to Latvian antenatal program and existing guidelines, using standardized procedures, including serology for antibodies (lues, HIV), Gram smear (*Trichomonas vaginalis, Neisseria gonorrhoeae*, dominant vaginal flora, presence of leucocytes), Amsel criteria for BV, and additionally ELISA for *Chlamydia trachomatis* (The regulations of Latvian Cabinet of Ministers N 611; LAGO clinical guidelines, 1999; *Krowchuk, 1988)*.

*Doppler studies*. Doppler waveforms were recorded before delivery. When more than one Doppler study was performed in the same fetus, the last Doppler study preceding delivery was used for analysis.

•Abnormal uterine artery velocimetry was considered as a mean (left and right) PI value above the 95$^{th}$ percentile for gestational age (GA) based reference ranges (*O.Gomez, 2008*) and/or presence of early diastolic "notch".

•Abnormal umbilical artery velocimetry was defined as PI above the 95$^{th}$ percentile for GA based reference ranges (*G.Acharya, 2005*) and/or absent or reversed end diastolic flow.

•Abnormal middle cerebral artery velocimetry was defined as PI below the 2.5$^{th}$ percentile for GA based reference ranges (*C.Ebbing, 2007*).

•Abnormal *ductus venosus* was defined as PIV above the 95$^{th}$ percentile for GA based reference ranges and/or absent or reversed a-wave flow (*J.Kesler, 2006*).

•Abnormal left portal vein was defined as Time averaged maximum velocity (TAMXV, cm/s) below the 5$^{th}$ percentile for GA based reference ranges (*J.Kesler, 2007a*).

•Abnormal splenic artery velocity waveforms were defined as PI below the 5$^{th}$ percentile for GA based reference ranges (*C.Ebbing, 2007*).

Intrauterine growth restricted newborns were grouped as follows:

- Group I - neonates with an estimated weight below the 10$^{th}$ percentile and normal blood velocity waveforms;

- Group II - an abnormal uterine artery velocimetry PI and/or presence of early diastolic “notch”;
- Group III - an abnormal umbilical artery PI;
- Group IV - an abnormal *AU* and middle cerebral artery PI;
- Group V - *AU* absent or reversed end diastolic flow and/or an abnormal *ductus venosus* PIV.

*Delivery.* Information about birth weight, duration of gestation at delivery, mode of delivery and length of hospital stay were obtained from standardized medical records.
*Neonates.* Gender, Apgar score below 7 at five minutes, neonatal health condition, admission to neonatal intensive care unit (NICU), transfer to the pediatric hospital for the further treatment and neonatal death were assessed. In cases of severe FGR, neonates were screened for serological (cytomegalovirus, toxoplasmosis, herpes simplex) infections. Standardized medical files were used. According to the clinical guidelines for all transferred to NICU infants the culture was made from blood to detect the presence of pathogenic microorganisms. Perinatal outcome end points included perinatal mortality. Perinatal mortality was defined as mortality occurring between 21 weeks of gestation and 28 days after birth.

*Macroscopic and microscopic examination of placenta.* Placenta and membranes were trimmed, dried and clots were removed before weighing. Length, insertion type and particularities of the umbilical cord were recorded on the special standardized form. Implantation site of the umbilical cord was registered as follows: central (at the centre), eccentric (between the centre and the margin of the chorionic disc), marginal (at the margin of the chorionic disc) or velamentous (to the membranes). All placentas were examined by the same histopathologist (IM) in the Division of Pathology of the University Children Hospital, Riga Stradins University.
The thickness of placental tissues was obtained from the area of cord insertion, intermediate and marginal portions of placental disc. The specimens were fixed in buffered formalin, dehydrated and embedded in paraffin wax. Three μm serial sections

were cut and stained with heamatoxylin and eosin (Benirschke, 1961). The specimens were viewed in the light microscope *Leica DM 3000* at 10 magnifications.

### 5. Statistical analysis

Both parametric and non-parametric statistics were used. The relationships between variables were assessed using chi-square, t-test or Fisher's exact test. A two-tailed p value <0.05 was considered significant. Relationships among variables were evaluated using either Pearson's correlation or Spearman's rank-order correlation with modeling performed using simple linear regression (*Altman*, 1999, 2000; *Rosner*, 2000; *Teibe*, 2007). All statistical analyses were performed using SPSS version 18.0.

## RESULTS

### 1. Demographic data and socioeconomic determinants of patients

The demographic characteristics of women participated in the present study are shown in *Table N 1***.** The mean age and SD of patients with FGR being 28.3 ± 5.4 y compared with 27.5 ± 4.5 y for the control group. A *t-test,* showed no significant difference in age between the two groups (p=0.17).

**Table N 1.** The demographic characteristics of patients from FGR and control groups

| | **FGR (N = 70)** | **Control (N= 86)** | **p value** |
|---|---|---|---|
| **Age** (years, mean ± SD) | 28.3 ± 5.4 | 27.5 ± 4.5 | 0.17 |
| **Type of residence** | | | |
| Urban | 54 (77.1) | 66 (76.7) | 0.98 |
| Rural | 16 (22.9) | 20 (23.3) | 0.96 |

| | | | |
|---|---|---|---|
| **Level of education** | | | |
| Basic | 6 (8.6) | 7 (8.1) | 0.92 |
| Secondary | 19 (27.1) | 26 (30.2) | 0.75 |
| Secondary/professional | 11 (15.7) | 10 (11.7) | 0.51 |
| High/university | 34 (48.6) | 43 (50) | 0.91 |
| **Marital status** | | | |
| Unmarried | 31 (44.3) | 34 (39.5) | 0.7 |
| Married | 39 (55.7) | 52 (60.5) | 0.75 |
| **Employment status** | | | |
| Employed | 59 (84) | 71 (82.5) | 0.93 |
| Unemployed | 11 (16) | 15 (17.5) | 0.8 |

Data are given as numbers, percentages in parentheses

In like manner, checking the hypothesis on the patients' place of residence, no statistical differences were found between groups (*Table N 1*). According to the statistical analyses 77.1% of seventy FGR pregnant women lived in urban areas compared with 76.7% of the control group (p =0.98).

Both groups were similar in respect to level of education. Basic level had 8.6% of study group vs. 8.1% in controls (p=0.92). At the same time 48.6% of the FGR patients had the high or university level of education comparing to 50% of the control group, not significant (p=0.91).

Among study patients 44% were unmaried compared with 39.5% for the controls, a non significant difference (p =0.7).

It turned out that employment status in both groups were not statistically different with 16% unemployed in FGR group vs. 17.5% for the controls (p=0.8).

**2. Obsterical characteristics**

The clinical characteristics of women are shown in *Table N 2.* Both groups were similar in respect to parity. Sixty-seven percent of FGR patients gave birth for the first time compared with 57% of controls (p=0.52). The rate of multiparous patient was not significantly different between the groups: 32.8% in FGR group versus 43% for the controls (p=0.38).

Obstetrical characteristics show no differences between the groups regarding most of the complications in current or previous pregnancies (*see Table N 2*). Bleeding in early pregnancy among FGR patients was reported significantly more often than in the control group, (13/18.5% vs. 6/6.9%, p=0.02). There were no significant differences regarding pregnancy anemia in current pregnancy (15.7% in FGR group vs. 16.2% for controls, p=0.3), progesterone use during pregnancy (8.5% vs. 4.6%, p=0.51) and threatened premature delivery (7.1% in FGR group vs. 3.4% in controls, p=0.47). Five out of 70 FGR patients (7.1%) had viral upper respiratory tract infection comparing to seven (8.1%) in controls (not significant, p=0.82). Two study pregnancies (2.8%) comparing to one (1.1%) in control group had complications involving urinary tract infections with AB use (p=0.59). Two FGR patients received antenatal care starting only from the second trimester of pregnancy. There were no late first antenatal visits for the control group (p=0.21, not significant). FGR women had more pregnancy-related increase in blood pressure (pre-eclampsia (13 /18.5% vs. 3/ 3.4%, p=0.005) and gestational hypertension (6 /8.5% vs. 1 /1.1%, p=0.05, respectively).

Regarding past history, we did not find any statistical differences between groups in respect of termination of pregnancy (TOP) (7.1% vs. 1.1%, p=0.09), recurrent miscarriage or stillborn (2.8% vs. 0, p=0.26) or premature deliveries in past history (3/ 4.2% vs. 2/ 2.3%, p=0.65). Gynecological anomalies (8 /11.4% vs. 3/ 3.4%, p=0.05) and interval between pregnancies more than 60 months significantly correlated with FGR (11/15.7% vs. 5/5.8%, p=0.043).

**Table N 2.** Obstetrical characteristics of patients from FGR and control groups

| | **FGR** | **Control** | **p value** |
|---|---|---|---|

| | **(N = 70)** | **(N = 86)** | |
|---|---|---|---|
| **Parity** | | | |
| Nulliparous | 47 (67.2) | 49 (57) | 0.52 |
| Multiparous | 23 (32.8) | 37 (43) | 0.38 |
| **Pregnancy complications** | | | |
| ***Current pregnancy*** | | | |
| Pregnancy anemia (n=25) | 11 (15.7) | 14 (16.2) | 0.3 |
| Bleeding in early pregnancy (n=19) | 13 (18.5) | 6 (6.9) | 0.02 |
| Threatened premature delivery (n=8) | 5 (7.1) | 3 (3.4) | 0.47 |
| Progesterone use (n=10) | 6 (8.5) | 4 (4.6) | 0.51 |
| Weight gain during pregnancy (kg, mean ± SD) | 10.1±5.2 | 14.3±5.6 | 0.001 |
| Gestational hypertension (n=7) | 6 (8.5) | 1 (1.1) | 0.05 |
| Pre-eclampsia (n=16) | 13 (18.5) | 3 (3.4) | 0.005 |
| Viral upper respiratory tract infection (n=12) | 5 (7.1) | 7 (8.1) | 0.82 |
| Urine tract infections/AB use (n=3) | 2 (2.8) | 1 (1.1) | 0.59 |
| Late antenatal care (n=2) | 2 (2,8%) | 0 (0) | 0.21 |
| ***In past history*** | | | |
| ≥3 or more miscarriages or TOP (n=6) | 5 (7.1) | 1 (1.1) | 0.09 |
| Complications after previous deliveries (n=2) | 2 (2.8) | 0 (0) | 0.2 |
| SC in previous history/uterine scar (n=4) | 1 (1.4) | 3 (3.4) | 0.62 |
| Intrauterine fetal death in previous history (n=2) | 2 (2.8) | 0 (0) | 0.26 |
| Premature deliveries in previous history (n=5) | 3 (4.2) | 2 (2.3) | 0.65 |
| Interval between pregnancies less than17 month (n=12) | 8 (11.4) | 4 (4.6) | 0.2 |
| Interval between pregnancies more than 60 month (n=16) | 11 (15.7) | 5 (5.8) | 0.04 |
| Gynecological anomalies (congenital uterine abnormalities, myoma) (n=11) | 8 (11.4) | 3 (3.4) | 0.05 |
| Extrauterine pregnancy in history (n=3) | 1 (1.4) | 2 (2.3) | 0.57 |

Data are given as numbers, percentages and the total of that group in parentheses

### 3. Reproductive tract infections and extragenital diseases

Reproductive tract infections (RTI) in anamnesis (no significant difference) or diagnosed during current pregnancy (P=0.02) were more frequent in the FGR group than in the control group. In FGR group's past history *C.trachomatis* was diagnosed in three cases; syphilis in two cases but during current pregnancy, *C. trachomatis* was diagnosed in four cases, and BV in six cases. In the control group' past history there were one case of syphilis and two cases of *C.trachomatis* infection but no STI was diagnosed during current pregnancy. BV was present in three control patients.

Out of four FGR women with STI two delivered at term, two preterm: one had spontaneus delivery at 35 weeks of gestation, another was induced at 25 weeks of gestation due to progressive fetal dystress. All FGR patients except one in whom BV was diagnosed delivered prematurely: two had spontaneous preterm delivery, three were terminated by cesarean section for medical reasons.

All control women having BV diagnosed during pregnancy delivered at term.

HIV infection among participants was presented in both groups with similar rates -one case in FGR group and one in the control group. Both HIV-infected patients used antiretroviral medications in order to reduce the risk of mother-to-child transmission.

Extragenital morbidities were present more often in FGR than in controls (p=0.03, see *Table N 3),* and the FGR patients used medication during pregnancy significantly more often (p=0.009). Among extragenital diseases, thyroid gland abnormalities (four cases), chronic arterial hypertension (two cases), bronchial asthma (one case) and epilepsy (one case), pituitary gland adenoma (one case) and renal pathology (one case) were reported. Most medications were taken for thyroid gland disease therapy (n=4), pituitary gland adenoma (n=1), Azithromycin for *C. trachomatis* (n=3), anticonvulsant (n=1) and antihypertensive drugs (n=8). According to antenatal files the therapy of thyroid gland abnormalities was effective and the serum TSH and FT4 (free T4) concentration were within normal ranges during pregnancy.

**Table N 3.** Concurrent medical problems of patients from FGR and control groups

| | **FGR N=70** | **Control N=86** | **p value** |
|---|---|---|---|
| Extragenital pathology (n=14) | 10 (14.2) | 4 (4.6) | 0.03 |
| STI/RTI in current pregnancy (n=13) | 10 (14.2) | 3 (3.4) | 0.02 |
| STI in history (n=8) | 5 (7.1) | 3 (3.4) | 0.3 |
| Use of medication for therapeutic reasons (n=23) | 17 (24.2) | 6 (6.9) | 0.009 |

*Data are given as numbers, percentages and the total of that group in parentheses*

**4. Life style determinants**

Current smoking (p=0.02), as well as pre-pregnancy smoking (p=0.01) was associated with FGR, but there was no difference in exposure to smoke (passive smoking) between both groups (p=0.1, *see Table N 4*).

In our series, we observed significantly less weight gain during pregnancy among pateints with FGR than among control women. The mean weight gain during pregnancy in the study group was 10.1±5.2 kg comparing to 14.3±5.6 kg in the control group (p=0.001).

**Table N 4.** Lifestyle determinants in FGR and control groups

| **Life style determinants** | **FGR N=70** | **Control N=86** | **p value** |
|---|---|---|---|
| Illicit drug use (n=1) | 1 (1.4) | 0 | 0.44 |
| Alcohol (n=1) | 1 (1.4) | 0 | 0.44 |
| Smoking in current pregnancy (18) | 13 (18.5) | 5 (5.8) | 0.02 |
| Smoking until current pregnancy (n=14) | 10 (14.3) | 4 (4.6) | 0.01 |
| Passive smoking (n=30) | 9 (12.8) | 21 (24.4) | 0.1 |

Data are given as numbers, percentages and the total of that group in parentheses

Factors associated with FGR in the multivariate analyses included (*see Table N* 5 and *N 6*) pre-pregnancy smoking (OR5.8; 95% CI 1.4-23.5) and smoking in current pregnancy (OR 5.7; 95% CI 1.4-22.8**),** STI/RTI in current pregnancy ( OR 4.9; 95% CI 1.1-21.6), interval between pregnancies more than 60 month (OR 5.1; 95% CI 1.4-17.9), bleeding in early bleeding in early pregnancy (OR 4.1; 95% CI 1.2-13.9) and weight gain during pregnancy equal or less than 10 kg (OR 29.8; 95% CI 9.0-98.7). Extragenital pathology in the current study is a sufficient risk factor for development of FGR with OR 4.2 (95% CI 1.0-17.0).

**Table N 5.** Maternal risk factors for FGR (multivariate analyses).

| **Factor** | **FGR** | | **Control** | | **OR** | **95% CI** | **p** |
|---|---|---|---|---|---|---|---|
| | n | % | n | % | | | |
| **Age** | | | | | | | |
| ≤20 y | 3 | 33.3 | 6 | 66.7 | 1 | | |
| 21-25 | 22 | 45.8 | 26 | 54.2 | 2.2 | 0.3-15.4 | 0.4 |
| 26-30 | 24 | 42.9 | 32 | 57.1 | 1.5 | 0.2-10.8 | 0.7 |
| 31-35 | 12 | 41.4 | 17 | 58.6 | 1.3 | 0.1-11.6 | 0.8 |
| >35 | 9 | 64.3 | 5 | 35.7 | 3.6 | 0.4-36.3 | 0.3 |
| **Level of education** | | | | | | | |
| High | 34 | 44.2 | 43 | 55.8 | 1 | | |
| Secondary/professional | 11 | 52.4 | 10 | 47.6 | 1.0 | 0.3-3.6 | 1.0 |
| Secondary | 19 | 42.2 | 26 | 57.8 | 0.6 | 0.2-1.7 | 0.4 |
| Basic | 6 | 46.2 | 7 | 53.8 | 0.6 | 0.1-3.1 | 0.6 |
| **Type of residence** | | | | | | | |
| Urban | 54 | 45.0 | 66 | 55.0 | 1 | | |
| Rural | 16 | 44.4 | 20 | 55.6 | 0.8 | 0.3-2.1 | 0.7 |
| **Employment status** | | | | | | | |
| Employed | 59 | 45.4 | 71 | 54.6 | 1 | | |
| Unemployed | 11 | 42.3 | 15 | 57.7 | 0.9 | 0.3-2.5 | 0.8 |
| **Marital status** | | | | | | | |
| Married | 39 | 42.9 | 52 | 57.1 | 1 | | |
| Unmarried | 28 | 45.2 | 34 | 54.8 | 1.0 | 0.5-2.2 | 1.0 |
| **Smoking** | | | | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Smoking in current pregnancy active | 13 | 72.2 | 5 | 27.8 | **3.5** | **0.9-13.5** | **0.02** |
| Smoking in current pregnancy passive | 9 | 30.0 | 21 | 70.0 | 0.4 | 0.1-1.4 | 0.1 |
| Smoking until current pregnancy | 10 | 71.4 | 4 | 28.6 | **5.8** | **1.4-23.5** | **0.01** |
| No | 38 | 40.4 | 56 | 59.6 | 1 | | |
| **STI/RTI in current pregnancy** | | | | | | | |
| Yes | 10 | 76.9 | 3 | 23.1 | **4.9** | **1.1-21.6** | **0.03** |
| No | 60 | 42.0 | 83 | 58.0 | 1 | | |
| **Interval between pregnancies** | | | | | | | |
| Less 17 month | 8 | 66.7 | 4 | 33.3 | 2.5 | 0.6-10.7 | 0.2 |
| More 60 month | 11 | 68.8 | 5 | 31.2 | **4.5** | **1.2-17.1** | **0.01** |
| **Extragenital pathology** | | | | | | | |
| Yes | 10 | 71.4 | 4 | 28.6 | **5.0** | **1.2-20.9** | **0.03** |
| No | 60 | 42.3 | 82 | 57.7 | **1** | | |
| **Gynecological anomalies** | | | | | | | |
| Yes | 8 | 72.2 | 3 | 27.3 | 2.2 | 0,4-11.4 | 0.4 |
| No | 62 | 42.8 | 83 | 57.2 | 1 | | |
| **≥3 or more miscarriages or TOP** | | | | | | | |
| Yes | 5 | 83.3 | 1 | 16.7 | 4.3 | 0.3-67.6 | 0.3 |
| No | 65 | 43.3 | 85 | 56.7 | 1 | | |
| **Use of medication for therapeutic reasons** | | | | | | | |
| Yes | 17 | 73.9 | 6 | 26.1 | **5.7** | **1.7-18.7** | **0.004** |
| No | 53 | 39.8 | 80 | 60.2 | **1** | | |

**Table N 6.** Maternal risk factors for FGR (multivariate analyses).

| Factor | FGR | | Control | | OR | 95% CI | p |
|---|---|---|---|---|---|---|---|
| | n | % | n | % | | | |
| **Pregnancy anemia** | | | | | | | |
| Yes | 11 | 44.0 | 14 | 56.0 | 0.9 | 0.3-2.8 | 0.8 |
| No | 59 | 45.0 | 72 | 55.0 | 1 | | |
| **Bleeding in early pregnancy** | | | | | | | |
| Yes | 13 | 68.4 | 6 | 31.6 | **4.1** | **1.2-13.9** | **0.02** |
| No | 57 | 41.6 | 80 | 58.4 | 1 | | |
| **Threatened premature delivery** | | | | | | | |
| Yes | 5 | 62.5 | 3 | 37.5 | 1.7 | 0.2-11.3 | 0.6 |
| No | 65 | 43.9 | 83 | 56.1 | 1 | | |
| **Gestational hypertension** | | | | | | | |
| Yes | 6 | 85.7 | 1 | 14.3 | 2.9 | 0.2-46.7 | 0.5 |
| No | 64 | 43.0 | 85 | 57.0 | 1 | | |
| **Pre-eclampsia** | | | | | | | |
| Yes | 13 | 81.2 | 3 | 18.8 | 3.4 | 0.7-16.9 | 0.1 |
| No | 57 | 40,7 | 83 | 59,3 | 1 | | |
| **Weight gain during pregnancy** | | | | | | | |
| ≤10 kg | 38 | 88.4 | 5 | 11.6 | **29.8** | **9.0-98.7** | **<0.0001** |
| 11-15 kg | 22 | 38.6 | 35 | 61.4 | **2.9** | **1.2-7.2** | **0.02** |
| ≥16 kg | 10 | 17.9 | 46 | 82.1 | 1 | | |

**5. Management and outcome**

Gestational age of FGR group upon inclusion in the study was 25 weeks of gestation. Mean gestational age and birth weight at delivery were $36.3 \pm 3.4$ weeks and $2020 \pm 622$ g, respectively, in the study group, and $39.8 \pm 1.1$ weeks and $3623 \pm 515$ g, respectively, in the control group ($p<0.001$, see *Table N 7 and Fig. 2).* Women with FGR had lower birth weights of their babies at any gestational age.

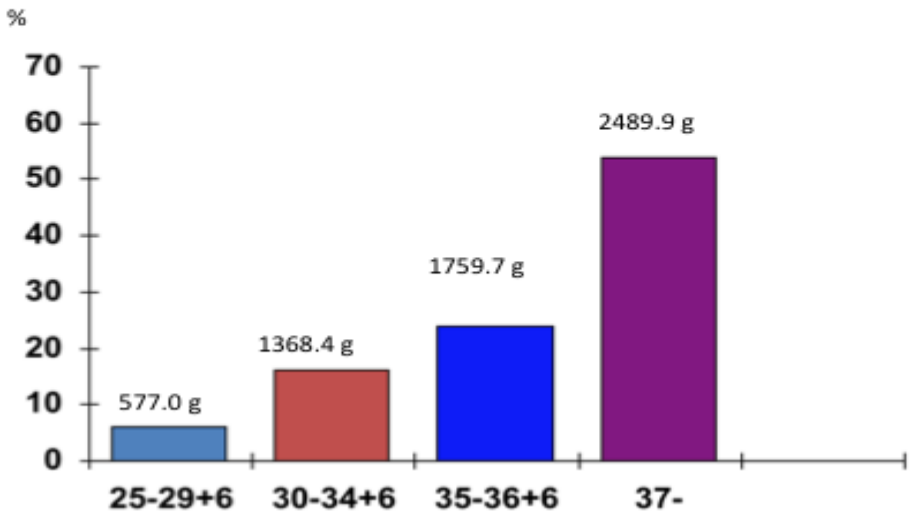


**Figure N 2.** The distribution of growth restricted newborns according to their gestational age and birth weight

**Table N 7.** Clinical characteristics of patients from FGR and control groups.

| | **FGR (70)** | **Control (152)** | **p value** |
|---|---|---|---|
| **Gestational age at birth** (weeks, mean ± SD) | 36.3 ± 3.4 | 39.8 ± 1.1 | <0.001 |
| **Birth weight** (grams, mean ± SD) | 2020 ± 622 | 3623±515 | <0.001 |
| **Length of hospital stay** (days, mean ± SD) | 5.0 ± 1.7 | 3.7 ± 1.2 | <0.001 |
| **The mode of delivery**<br>Spontaneous delivery<br>Labor induction/vaginal | <br>14 (20.0)<br>10 (14.3) | <br>99 (65.1)<br>22 (14.5) | <br><0.001<br>0.97 |

| | | | |
|---|---|---|---|
| Emergency Cesarean Section | 6 (8.6) | 8 (5.3) | 0.38 |
| Elective Cesarean Section | 40 (57.1) | 23 (15.1) | <0.001 |

Data are given as numbers, mean and SD, percentage in parenthesis

The incidence of spontaneous deliveries in the FGR group was significantly lower than in the controls (20% vs. 65.1%; p<0.001), whereas rate of elective cesarean section before onset of labour was significantly higher (57.1% vs.15.1%; p<0.001). The differences between the groups in rates of induced labor and secondary cesarean delivery during labor were not statistically significant (see *Table N7*).

Fetal distress, abruption of placenta and pre-eclampsia were all significantly more frequent indications for cesarean section in FGR (p=0.001; 0.003; 0.001, respectively) than in controls, whereas uterine scar from previous CS (p=0.07), fetal breech presentation (p=0.9) or maternal indications (p=0.22) were not different between the groups (*Table N 8*)**.**

The 6 emergency cesarean deliveries were performed because of progressive intrapartum fetal distress (n=1), failure to progress (n=2), HELLP syndrome (n=1), and uncontrolled hypertension (n=2).

Low biophysical profile score (n=5), polyhydramnios (n=1), non-reassuring CTG (n=1), spontaneous rupture of membranes (n=5) and prolonged pregnancy involved induced labor in the control group.

**Table N 8.** Comparison of indications for cesarean delivery between FGR and control groups

| | **FGR n=70** | **Control n=152** | **p value** |
|---|---|---|---|
| Fetal distress* | 28 (36) | 8 (5.2) | <0.001 |
| Maternal indications | 0 | 4 (2.6) | 0.22 |
| Abruption of placenta | 5 (6.5) | 0 | 0.003 |
| Pre-eclampsia | 7 (9) | 1 (0.65) | <0.001 |
| Scar | 1 (1.3) | 11 (7.2) | 0.07 |

| | | | |
|---|---|---|---|
| Breech presentation | 4 (5.2) | 8 (5.2) | 0.9 |
| HIV | 1 (1.3) | 2 (1.3) | 0.68 |
| Other indications | 0 | 5 (3.2) | 0.15 |

Data are given as numbers, percentage in parenthesis

*Fetal distress defined as biophysical profile < 6 and olighydramnios, abnormal Doppler studies, or non-reassuring CTG.

Among FGR fetuses, the most common indications for elective cesarean section before onset of contractions were: abnormal fetal Doppler studies, low biophysical profile score, non-reassuring fetal heart rate on cardiotocography and oligohydramnios (36% vs. 5.2%, for the control group $p<0.001$).

All FGR patients with trisomies had an abnormal pulsatility index or absent diastolic flow in the umbilical artery on Doppler studies, as well as low BPP scores.

Perinatal outcomes are shown in *Table N 9*. The boy-to-girl ratio was slightly lower among growth-restricted newborns than in controls (0.79 vs. 1.02), but this difference was not significant ($p=0.6$).

There was a significantly higher perinatal mortality rate in the study group than among the controls ($p=0.01$). Two perinatal deaths occurred antenatally—one because of placental abruption and one owing to progressive pre-eclampsia. Pre-eclampsia was also involved in two intranatal deaths. Three neonatal deaths in the study group were related to multiple fetal malformations (n=1) and trisomy 18 (n=2): two of them died within seven days after delivery and another one before the $28^{th}$ day.

Neonates in the study group who survived birth had a greater likelihood of developing serious morbidity than did controls ($p<0.001$). Five-minute Apgar score below 7 (12.9% vs. 3.9%, $p=0.02$), admission to neonatal intensive care unit (17.1% vs. 1.9%, $p=0.001$) and transfer to pediatric hospital for further treatment (32.9% vs. 1.9%, $p<0.001$) occurred more frequently in the study group than in the control group. Preterm birth (40% vs. 1.3% $p<0.001$), respiratory distress (20% vs 1.9%, $p<0.001$) and necrotizing enterocolitis (2.9 vs. 0%, $p=0.1$) were more common in the study group than among controls. Twenty-seven of 28 cases of premature delivery in FGR group involved induced labor or cesarean for medical reasons.

Four infants in the study group developed severe intraventricular hemorrhage grade III or IV, whereas there were no such cases in the control group (p=0.003). Sepsis was considered to be clinically likely in 12.9% of neonates in the study group showing high C-reactive protein and abnormal leukocyte counts, compared with 3.9% in the control group (p=0.02). *Staphylococcus aureus* was isolated in two cases in the study group. *Streptococcus agalactiae* in culture was identified with similar frequencies in FGR (n=1) and control (n=1) groups. Bacteriological examinations were performed in all neonates admitted in NICU or on clinical indications.

Overall, mean length of hospital stay after birth for patients with FGR pregnancies was 5 days (± 1.7), compared to 3.7 (± 1.2) in the control group (p<0.001).

The Spearman's correlation test shows that perinatal outcome in FGR group correlated significantly with gestational age and birthweight. Perinatal mortality (p=0.002), five-minute Apgar score <7 (p<0.0001, NICU admission (p<0.0001), and transfer to pediatric hospital for further treatment (p<0.0001) occurred more frequently in the extremely low weight group. Neonatal morbidity and cesarean deliveries correlates significantly (p=0.05 and 0.07) with the very low weight group (see *Figure N 3 and 4*)

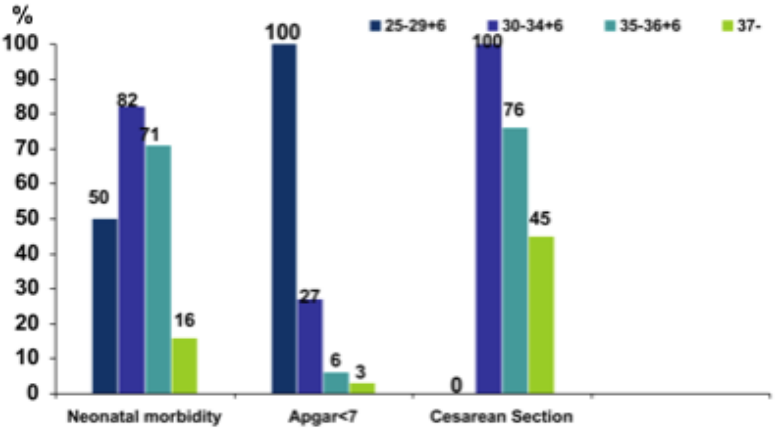


**Figure N 3.** Perinatal outcome in FGR group according gestational age and birth weight

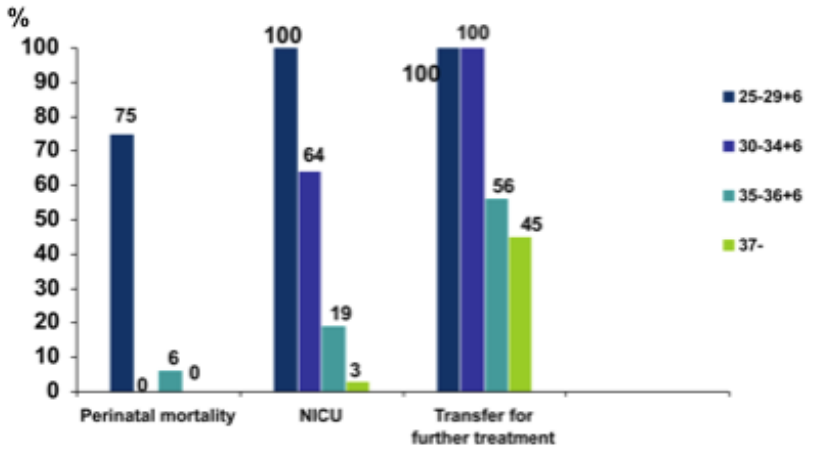


**Figure N 4.** Perinatal outcome in FGR group according gestational age and birth weight

We were able to follow-up on 10 out of 29 compromised infants (34%) at the age of one year, who had been transferred to the university children hospital. Respiratory system complications (n=2), malabsorption syndrome (n=8), neonatal encephalopathy (n=6), and retinopathy due to prematurity were the most common morbidities among infants up to 1 year of age in the study group.

**Table N 9.** Perinatal outcome FGR and control groups

| | **FGR (70)** | **Control (152)** | **p value** |
|---|---|---|---|
| **Newborn gender** | | | |
| Male | 31 (44.3) | 77 (51.4) | 0.6 |
| Female | 39 (55.7) | 75 (48.6) | 0.6 |
| Newborn serious morbidity | 23 (32) | 4 (2.6) | <0.001 |

| | | | |
|---|---|---|---|
| Five-minute Apgar score <7 | 9 (12.9) | 6 (3.9) | 0.02 |
| NICU admission | 12 (17.1) | 3 (1.9) | 0.001 |
| Transfer to pediatric hospital for further treatment | 23 (32.9) | 3 (1.9) | <0.001 |
| Antenatal death | 2 (2.9) | 0 (0.0) | 0.1 |
| Perinatal mortality | 4 (5.7) | 0 | 0.01 |
| Intranatal death | 2 (2.9) | 0 (0.0) | 0.1 |
| Infections/Septicemia | 9 (12.9) | 6 (3.9) | 0.02 |
| Preterm birth* | 28 (40.0) | 2 (1.3) | <0.001 |
| Respiratory distress syndrome | 14 (20) | 3 (1.9) | <0.001 |
| Intraventricular hemorrhage (grade III or IV) | 4 (5.7) | 0 (0.0) | 0.003 |
| Fetal alcohol syndrome | 1 (1.4) | 0 (0.0) | 0.31 |
| Neonatal heroin abstinence syndrome | 1 (1.4) | 0 (0.0) | 0.31 |
| Necrotizing enterocolitis | 2 (2.9) | 0 (0.0) | 0.1 |

Data are given as numbers, percentage in parenthesis

* Twenty-seven cases of premature delivery were due to induced labor or cesarean delivery for medical reasons

After adjusting for covariates, it is clear that prematurity, not FGR is responsible for the most of the neonatal outcomes, including RDS (OR 45.5; 95% CI 7.0-294), NICU admission (OR 37.8; 95% CI 5.5-259.4) and referral to the children hospital for the further treatment (OR 62.9; 95% CI 14.4-275.2). The results of analyses are shown in *Table N 10 and N 11*.

**Table N 10.** Risk assessment of adverse outcomes

| **Outcome** | Elective cesarean section | Emergency cesarean section | Labor induction/ vaginal | Spontaneous delivery | **Apgar <7** | Respiratory distress syndrome |
|---|---|---|---|---|---|---|
| **FGR** | | | | | | |
| Yes | **OR 5.0 (1.8-13.9) p=0.002** | OR 1.9 (0.6-6.6) p=0.3 | OR1.0 (0.4-2.6) p=1.0 | **OR 0.2 (0.1-0.5) p<0.0001** | OR 2.1 (0.5-8.0) p=0.3 | OR 1.3 (0.2-9.1) p=0.8 |

| | | | | | | |
|---|---|---|---|---|---|---|
| No | 1 | 1 | 1 | **1** | 1 | 1 |
| Preterm birth | | | | | | |
| Yes | **OR 4.3 (2.1-9.0) p<0.0001** | OR 0.7 (0.1-3.9) p=0.7 | OR 0.9 (0.2-3.3) p=0.9 | **OR 0.1 (0.03-0.7) p=0.01** | OR 3.1 (0.8-12.5) p=0.1 | **OR 45.5 (7.0-294.0) p<0.0001** |
| No | 1 | 1 | 1 | **1** | 1 | 1 |

95% CI are given in parenthesis

**Table N 11.** Risk assessment of adverse outcomes

| **Outcome** | Infections/Septicemia | NICU admission | Transfer to pediatric hospital | Hospital stay >4 days |
|---|---|---|---|---|
| **FGR** | | | | |
| Yes | OR 1.5 (0.4-6.4) | OR 1.2 (0.2-8.4) 0,9 | OR 3.5 (0.7-17.9) 0,1 | **OR 2.9 (1.4-5.8) 0.004** |
| No | 1 | 1 | 1 | **1** |
| Preterm birth | | | | |
| Yes | **OR 5,3 (1.2-22.7) p=0.03** | **OR 37,8 (5.5-259.4) p<0.0001** | **OR 62,9 (14.4-275.2) p<0.0001** | **OR 12,6 (3.4-46.7) p<0.0001** |
| No | 1 | 1 | 1 | **1** |

95% CI are given in parenthesis

In 7 of the 77 newborns with fetal growth restriction, congenital anomalies were diagnosed postnatally: 2 with trisomy 21; 2 with trisomy18; 1 with multiple anomalies; 1 with gut malrotation; and 1 with Hirschsprung's disease. In three cases of fetal growth restriction with trisomy delivery was by cesarean section.

In controls 2 cases of Down syndrome were diagnosed postnataly (p=0.03). One of the mothers was young 25 years old and another-36 years old. One renal aplasia and congenital ichthyosis were confirmed in control neonates after birth (anomalies combined vs. controls, p=0.01).

To enable comparison with data from other studies, these cases were not included in the outcome analyses (*Table N 9*).

**6. Hemodynamic changes and perinatal outcomes related to FGR**

Doppler waveforms were recorded within seven days of delivery (ranges 2h -7 days). The outcome variables were compared with the results of the Doppler examinations.

The mean gestational age at delivery was higher in Group I (38.2± 3.3) compared with other groups (37.2± 4.2; 36.2± 2.6; 36.4± 3.6 and 31.1± 3.1, respectively, $\chi^2 = 60.33$; $p < 0.001$).

The Pearson correlation test showed an overall significant decrease in birth weights among the groups ($p< 0.001$). The mean birth weight in the first group was 2530± 473 g compared to the 1229± 403 g in Group V (*Table N 12*). Placental resistance and low *ACM* PI (Group IV) was associated with reduced birthweight.

Adverse perinatal outcome was lowest when Doppler study profile was normal and highest when Doppler examination on both maternal and fetal side was abnormal (*Table N 12)*. Neonatal morbidity was 67 % for Group III neonates. This was largely attributable to prematurity (nine cases) and RDS (nine cases) see *Table N 12*.

Oligohydramnios was associated with increasing severity of Doppler vascular changes (Normal →Abnl UtA→Abnl *AU* → Abnl *AU* and *ACM* →and *AU* ARED and Abnl *DV*, $p<0.001$). Unexpectedly, delivery by cesarean section, preeclampsia and the incidence of placental abruption were all observed more often in the group with increased umbilical artery PI without "brain-sparing" than in other groups (*e.g*. in Group III) ($p=0.007$; $p=0.25$; $p=0.62$, respectively). In all cases of placental abruption the presence of a "notch" was demonstrated in the UtA Doppler flow patterns.

Absent or reverse diastolic flow of *AU*, abnormal *ACM* and *DV* PI showed a direct correlation with five-minute Apgar scores below 7, transfer to NICU, transfer to pediatric hospital for further treatment and intranatal mortality ($p=0.01$; $p=0.01$; $p=0.02$; $p=0.03$, respectively, *Table N 12*).

Perinatal mortality occurred only in Group IV (2/12, 16.7%) and in Group V (2/8, 25%). Three of four deaths occurred during delivery, one due to placental abruption, and three due to severe pre-eclampsia. Three of eight fetuses of group V were born prematurely, had respiratory distress syndrome and developed severe intraventricular hemorrhage Grade III or IV. The mean length of stay of FGR infants in the NICU was 6 (± 1.6) days, statistically different amongst groups ($p=0.016$).

Thirteen women from FGR group were smokers and in 10 women reproductive tract infections (*C. trachomatis* n=4; BV n=6) were confirmed during pregnancy. Women with genital infections (p=0.02) had four times more frequent Doppler flow abnormalities compared to women without any other preventable risk factor (genital infections, smoking), p=0.018 (*Table N 13*). Smoking women with FGR have no different Doppler profile compared with normal women (p=0.09).

**Table N 12.** Characteristics of FGR groups according to Doppler profile

| | **Group I (n=18)** | **Group II (n=14)** | **Group III (n=18)** | **Group IV (n=12)** | **Group V (n=8)** | **p value** |
|---|---|---|---|---|---|---|
| ▪ Preventable risk factors | | | | | | |
| Nl* (n) | 12 | 3 | 7 | 4 | 0 | Ref |
| Smoking (n) | 3 | 2 | 3 | 2 | 3 | 0.09$_{\mu}$ |
| RTI (n) | 1 | 4 | 1 | 2 | 2 | 0.018$^{\mu}$ |
| ▪ Acute pregnancy complications | | | | | | |
| Pre-eclampsia | 1 (6) | 1 (7) | 4 (22) | 0 | 1 (13) | 0.25 |
| Placenta abruption | 0 (0) | 1(7) | 3(16.6) | 1 (8) | 0 | 0.62 |
| C-section | 7 (39) | 9 (64) | 17(94) | 7(58) | 6 (75) | 0.007 |
| ▪ Perinatal outcome | | | | | | |
| Birth weight | 2530±473 | 2270±364 | 1945± 111 | 1746± 516 | 1229± 403 | 0.001 |
| Gestational weeks | 38.2± 3.3 | 37.2± 4.2 | 36.2± 2.6 | 36.4± 3.6 | 31.1± 3.1 | 0.001 |
| Amniotic fluid index <5 | 2(6) | 2(14) | 4(22) | 3(25) | 3(38) | 0.001 |
| Perinatal mortality | 0 (0) | 0 (0) | 0 (0) | 2 (16) | 2 (25) | 0.01 |
| 5-min Apgar score<7 | 0 (0) | 1 (7) | 1 (6) | 2 (17) | 5 (63) | 0.01 |
| Neonatal morbidity | 1 (6) | 4 (29) | 12 (67) | 3 (10) | 6 (100) | 0.0004 |
| Days in NICU | 4.17±1.14 | 4.86±1.29 | 5.43±1.70 | 5.83±1.19 | 5.63±2.0 | 0.016 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Transfer to NICU | 0 (0) | 1 (7) | 5 (28) | 1 (10) | 5 (83) | 0.01 |
| Transfer to pediatric hospital | 1(6) | 4 (29) | 8 (44) | 4 (40) | 6 (100) | 0.02 |

Data are given as numbers and SD, percentage in parenthesis

**non smoking, at term, no genital infection*
μ: p value versus reference group of women without preventable risk factors only (non smoking, no RTI)

### 7. Adaptive changes in splenic artery and left portal vein in fetal growth restriction

Twenty of 42 FGR (62%) fetuses had increased PI of the *AU* above 95th percentile versus none of the 42 controls (p<0.0001). In 12 of FGR fetuses (54%) "brain-sparing" was confirmed by Doppler velocimetry (low PI of the *ACM*). Clinical characteristics of control women and FGR women with and without abnormal *AU* flow are shown in *Table N 13.* Gestational age at birth was 35.4 (± 3.3) weeks in FGR with elevated *AU* PI, comparing to either 38.1 (± 2.8) weeks in women with FGR with normal *AU* PI or 39.7 (± 1.3) weeks in controls (p<0.05). Time span to delivery varied between groups and was the shortest in the abnormal *AU* PI FGR group (1.3± 1 days, versus 13 ± 7.8 and 23.6 ± 17.3, p<0.05 compared to the other groups). Compared with controls (3584 ± 473 g), birth weight was 2440 (± 528) g in normal *AU* PI FGR (p<0.001) and 1643± (626) g in elevated *AU* PI FGR group (p<0.001 vs. controls and p<0.001 versus FGR/normal *AU*). Cesarean section rate was also higher in FGR/Normal *AU* PI women (11/50%, p=0.006) and in FGR/Abnormal *AU* PI women (16/ 80%), p<0.0001) when compared to controls (6/42, 14%). Pre-eclampsia was present in 20% of FRG/Abnormal *AU* PI women versus none in FGR/Normal *AU* PI or control women (p=0.001).

Compared to controls, FGR fetuses showed decreased TAMXV in the left portal vein in 54.7% (23/42, p<0.0001) with reverse flow in 4 fetuses (9.5%, p=0.04) (*Table N 13)*. In fetuses with abnormal umbilical artery PI, TAMXV of the left portal vein was significantly reduced compared to controls (15/20 vs. 1/42, p< 0.001) and to FGR women with normal *AU* flow (8/22, p=0.016). In women with severe reduced flow in the LPV (<5%) this trend was confirmed, and the incidence of reverse flow through the LPV was increased versus controls (4/20 vs. 1/42, p=0.034). With increasing placental compromise

in FGR pregnancies (abnormal *AU* flow) the signs of dilatation of splenic artery (low SA PI) we found significantly more often compared to FGR with normal *AU* flow, p=0.0004 (*Table N 13).*

Among 42 FGR fetuses three prenatal deaths occured and six newborns had 5 minute Apgar scores below 7. All perinatal mortality cases were associated with reduced LPV flow (p=0.04) and two out of three had SA PI less than 5% (p=0.03). Low Apgar scores were found in 14% of FGR cases versus none in controls (p=0.025). All cases with low Apgar were found in the group with reduced LPV flow and/or SA PI (p=0.018 and p=0.03, respectively) (*Table N 14)*.

**Table N 13.** Doppler pattern of the left portal vein (LPV) and splenic artery (SA) in growth restricted (FGR) fetuses, FGR fetuses with increased pulsatility index (PI) of the Umbilical artery (*AU*) and control groups.

| | **FGR** | | | **Control** |
|---|---|---|---|---|
| | **Total (n=42)** | **Normal *AU* PI (n=22)** | **Increased *AU* PI (n=20)** | **(n=42)** |
| LPV (TAMXV, cm/sec) <50centile | 23(54.7%) p< 0.0001 | 8 (36%) p=0.0005 | 15 (75%) p< 0.0001 p=0.016$^{\$}$ | 1 (2.3%) |
| LPV (TAMXV, cm/sec) <5centile | 16 (38%) p< 0.0001 | 6 (27%) p=0.0053 | 10 (50%) p< 0.0001 | 1 (2.3%) |
| LPV reverse flow | 4 (9.5%) p=0.04 | 0 | 4 (20%) p=0.034 | 1 (2.3%) |
| SA (PI) <50centile | 17 (40.4%) | 3 (14%) p=0.025 | 14 (70%) p=0.059 p=0.0004$^{\$}$ | 18 (42.8%) |
| SA (PI) <5centile | 10 (24%) | 5 (23%) | 5 (25%) | 3 (7.1%) |

Data given as number, percentage in parenthesis
Fisher exact p-values (unmarked) are given for comparison versus normal controls. $: Fisher exact p-values for comparison versus FGR women with normal *AU*.

**Table N 14.** Neonatal outcome in FGR and control groups according to blood flow through the Left Portal Vein (TAMX, cm/sec) and splenic artery flow (PI).

| | **FRG** | | | | | **Control** |
|---|---|---|---|---|---|---|
| | **Total n=42** | **LPV <50% n=23** | **LPV <5% n=16** | **LPV ≥ 50% n=19** | **SA <5% n=10** | **n=42** |
| Perinatal mortality | 3 (7.1) | 3 (13.0) p=0.04 | 2 (12.5) p= 0.072 | 0 (0) | 2 (20) p= 0.034 | 0 |
| Apgar <7 at 5 min | 6 (14.2) p= 0.025 | 4 (17.4) p= 0.013 | 3 (18.8) p= 0.018 | 2 (10.3) | 2 (20) p= 0.034 | 0 |

Data given as number, percentage in parenthesis
Fisher exact test are given for comparison versus normal controls, p values are shown only if significant

## 8. Placental factors

### 8.1 Gross changes of placentas and placental histological lesion

Macroscopic findings of placenta and umbilical cord are represented in *Table N 15.* The mean weight of the placenta in the FGR group was 412g ± 117 versus 641± 133 in the control group (p<0.001). The foetal-placental weight ratio was also lower in the FGR group than in the control group (4.87± 1.17 vs. 5.73 ± 0.95). This difference achieved statistical significance (p <0.001). There was no significant difference in the thickness or shape of the placentas between both groups.

**Table N 15.** Placentas on gross examination in FGR and control groups

| **Marcoscopic findings of placentas /umbilical cords** | **FGR (n=50)** | **Control (n=50)** | **p value** |
|---|---|---|---|

| | | | |
|---|---|---|---|
| **Placental weight** (g, mean ± SD) | 412.8±117.8 | 641.6±133.8 | <0.001 |
| **Fetal weight/placenta ratio (**mean ± SD) | 4.84±1.17 | 5.73±0.95 | <0.001 |
| **Thickness of placenta** (mean ± SD, mm) | 19.1±6.0 | 18.9±4.6 | 0.91 |

Data are given as numbers, percentages in parentheses, ± standard deviation

In the present study the mean length of umbilical cord was shorter in FGR group in comparison to control group (57.2 vs. 64.5cm, p<0.001). In FGR group entanglement of umbilical cord around fetal parts was found in 42% (21/50) versus in 34% (17/50) in controls (p=0.35, not significant). There was also no difference in implantation site of the umbilical cord between FGR and control women and all umbilical cords in both groups had 3 vessels. Macroscopic characteristics of umbilical cord are presented in *Table N 16.*

**Table N 16.** The umbilical cord on gross examination in FGR and control groups

| **Marcoscopic findings of umbilical cords** | **FGR (n=50)** | **Control (n=50)** | **p value** |
|---|---|---|---|
| **Length of umbilical cord** (mean, cm) | 57.2± 9.7 | 64.5 ± 8.2 | <0.001 |
| **Umbilical cord attachment to the chorionic disc** | | | |
| central | 30 (60) | 35 (70) | 0.62 |
| eccentric | 14 (28) | 10 (20) | 0.46 |
| marginal | 5 (10) | 2 (4) | 0.27 |
| velamentous | 1 (2) | 3 (6) | 0.32 |
| **Umbilical cord entanglement around fetal parts** | 21(42) | 17/ (34) | 0.35 |
| entanglement more than 4 circles | 1 (2) | 0 | 0.31 |

Data are given as numbers, percentages in parentheses, ± standard deviation

The histological findings are shown in *Table N 17*. There was no difference between both groups in the occurrence of perivillous fibrin deposition, cytotrophoblast proliferation or stromal fibrosis. The presence of thrombi or haematomas (p=0.01), incidence of villous infarction (p=0.02) and thickening of the villous trophoblastic basal membrane (p=0.03) was more frequent in the FGR group than in the controls. Villitis was more (13/50 vs 3/50, p=0.01) and vasculitis less common in FGR placentas than in controls (0 vs. 18%, p=0.01).

**Table N 17.** Incidences of placental histological lesions in FGR and control groups

| **Histological findings** | **FGR N=50** | **Control N=50** | **p value** |
|---|---|---|---|
| Perivillous fibrin deposition | 41 (82) | 38 (76) | 0.46 |
| Stromal fibrosis | 13(26) | 12 (24) | 0.82 |
| Cytotrophoblast prolifiration | 26 (52) | 31 (62) | 0.31 |
| Incidence of villous infarction | 17 (34) | 7 (14) | 0.02 |
| Thickening of the villous trophoblastic basal membrane | 30 (60) | 19 (38) | 0.03 |
| Intervillous thrombi/hematomas | 21 (42) | 6 (12) | 0.01 |
| Villitis | 13 (26) | 3(6) | 0.01 |
| Vasculitis | 0 (0) | 9 (18) | 0.01 |

Data are given as numbers, percentages in parentheses

### 8.2. Microscopic lesions of placenta and Doppler velocimetry

Doppler studies were abnormal in 30 of 50 FGR women versus 9 of 50 control women (60% vs 18%, p<0.0001). Eight different histologcal lesions were studied on the placentas. More abnormal histology findings were encountered in the placentas of FGR women than of women with normal fetal growth and circulation (p=0.02, *Table N 18*). Likewise, abnormal Doppler findings were more frequent in FGR patients (p=0.02). Compared to normal pregnancies with normal uteroplacental blood flow (group a, *Table N 18*) FGR patients with abnormal doppler had the highest number of placental lesions (p=0.003) with more than 50% of 4 or more different lesions, compared to 24% in the normal women (p=0.007).

Among controls 2 cases presented with elevated *AU* PI and 7 with abnormal UtA blood flow. Fifteen of the 50 FGR patients had increased *AU* PI velocities of whom 6 - ARED. 22 FGR patients had abnormal uterine artery PI and 7 had pathological Doppler velocities in both umbilical and uterine arteries.

In the presence of normal Doppler velocimetries we did not find any significant difference in most separate placental lesions studied: the frequencies of fibrin deposition (31/41 vs. 16/20), stromal fibrosis (10/41 vs. 2/20), cytotrofobalastic hyperplasia (25/41 vs. 13/20), basement membrane thickening (19/41 vs. 9/20), infarctions (3/41 vs. 5/20), or villitis (3/41 vs. 5/20) were all similar in both groups. However, intervillous haematomas or thrombi were encountered more frequently in the FGR patients with normal Doppler (5/41) than in the normal controls (7/20, p=0.04). Vasculitis was only present in controls with normal flow (8/41) versus none in the FGR with abnormal Doppler (p=0.04).

Amongst women with abnormal Doppler, FGR women had thicker placental basal membrane (p=0.006), more intervillous haematomas/thrombi (p=0.02) and less vasculitis (p=0.048) than control women. In FGR women, both abnormal uterine and umbilical arteries flow were associated with villous infarction (p=0.002 and p=0.0003, respectively) and intervillous haematoma/thrombi (p=0.01 and p<0.0001, respectively) compared to normal control women. Furthermore, in FGR women intervillous

haematoma/thrombi were primarily found in FGR women in association with abnormal *AU* (p=0.029) compared to FGR/normal flow, and not with abnormal UtA Doppler velocities. If flow was abnormal simultaneously in both *AU* and UtA arteries, more villous infarction was recorded (p=0.03 compared to control with normal flow).

Villitis was more frequent in FGR patients than in controls (p=0.01), but there was no difference between patients with different Doppler velocities. On the contrary, the presence of vasculitis was linked to 25% of control placentas, but was not encountered in FGR women, irrespective whether the flow in *AU* and/or UtA was normal (p=0.018) or abnormal (p=0.048).

**Table N 18.** Number of placental lesions according to Doppler findings in control and FGR women

| **Number of histological lesions** | **Normal Doppler** | | **Abnormal Doppler** | |
|---|---|---|---|---|
| | **a.Control (n=41)** | **b.FGR (n=20)** | **c.Control (n=9)** | **d.FGR (n=30)** |
| 0-1 | 11 (26.9) | 4 (20) | 3 (33.3) | 2(6.7) |
| 2-3 | 20 (48.8) | 11 (55) | 3 (33.3) | 11 (36.7) |
| 4 and more | 10 (24.3) | 5 (25) | 3 (33.3) | 17 (56.6) p=0.003 vs. a |

Data are given as numbers, percentages in parentheses, p value are shown only when different

Chi² trend for FGR (b+d) vs. Control (a+c): p =0.02

Chi² trend for Abnormal Doppler (a+b) vs. Normal Doppler (c+d): p=0.02

# DISCUSSION

## 1. Maternal characteristics

According to Latvian statistical data (*Ministry of Health of the Republic of Latvia)* in 2008 the prevalence in Latvia of small for gestational age newborns was 12.5/1000 for term births and 17.5/1000 for preterm births. The prevalence of FGR and its etiological factors were formerly insufficiently evaluated. Therefore we decided to study the association between FGR and a broad spectrum of medical, socio-demographic and reproductive characteristics in more detail.

In contrast to another reports (*Romo, 2009; Beard et al. 2009; McCowan, 2009*), in the present study FGR patients had the same socio-economic and medical background (age, type of residence, marital status, level of education, medical history, most current medical problems and obstetric parameters) as control group, which excludes the possibility of selection bias (*Table N 1-2*). A possible bias we could not exclude was that the Riga and Riga's regions have some economical differences from another rural parts of Latvia, although most of the FGR patients were referred to the Riga Maternity hospital as it is the biggest perinatal center in the country.

Maternal chronic diseases may interfere with fetal growth. In this study we found a significant prevalence of extragenital pathologies in the FGR group. Among other things, women with FGR suffered from thyroid gland abnormalities. In the literature there are scarce data about contribution of mothers' thyroid gland diseases to the hypotrophy of the newborns (Vargová, 2008). Our cases provide new information on this aspect. Although extragenital diseases in the present study were properly controlled, they did contribute significantly to FGR. As a consequence and confirming our findings, the FGR patients used medication for therapeutic reason significantly more often than the control group. Among medications FGR patients used in the present, most were antibiotics, antihypertensive and drugs for thyroid glands hypofunction. Over the last few years, the number of pregnant women who have received medication has increased. Drugs and their metabolites can cross the placental barrier and enter into the fetal circulation. Several studies showed the association between FGR and anti-neoplastic medications (*Tendron, 2002*), anticonvulsants (*Pennell, 2002*) and β-blockers (*Magee, 2000*). The causal

relationships with FGR for other medications are uncertain, and therefore, the use of medication should always be guided by risk-benefit considerations.

The association we found between pregnancy-induced hypertension and fetal growth restriction confirms the finding of other studies (*Jain, 1997; Xiong, 1999; Odegård, 2000*) and relates to the increased likelihood of placental dysfunction in women with hypertension during pregnancy (*Long, 1980; Roberts, 2008*). Also in our study, we discovered specific vascular placental abnormalities in histological examination, such as incidence of infarcts and intervillous hematomas in the majority of FGR patients, while such abnormalities were not found in placentas of babies with normal intrauterine growth. Therefore we agree with other authors that there may be a common etiopathogenesis in pre-eclamptic disorders and fetal growth restriction (*Villar, 2006*).

The effect of smoking during pregnancy on fetal intrauterine growth was shown in a number of studies (*Frisbie et al. 1997; Vahdaninia, 2008*). Of interest, in our study, we found that not only current smoking, but also, previous smoking was related to FGR. Furthermore, none of the smoking women with FGR had PIH during their current pregnancies. This in agreement with the hypothesis, that maternal smoking reduces the risk of pre-eclampsia and has a mechanism of causing FGR which is independent of blood pressure (*Cnattingius, 1997; Zhang, 1999; Lain, 2003*). Numerous prior researches have supported the association between passive smoking and fetal growth (*Dejin-Karlsson, 1998; Fantuzzi, 2008*) but this was not confirmed by our findings; however, recall bias and underreporting of the risk factor cannot be excluded.

The most striking new finding was the strong association FGR and current STI/RTI. Although studies warn that about 5% to 10 % of the cases with FGR may be attributable to viral or protozoan infections *in utero*, its relation to STI/RTI was not mentioned (*Maulik, 2006b*). According to results of our study, it seems that genital infections may not only be involved in the causation of preterm birth and preterm rupture of the membranes (*Mårdh, 2002; Donders, 2009*), but also in FGR. Although not generally recognized as a FGR cause, there are other data suggesting that abnormal vaginal microflora, BV and mycoplasmata correlate with low birth weight and FGR (*Hillier, 1995; Donders, 2008*). Ascending genital infections by selectively damaging the invasive trophoblast components could disturb placental invasion and result in later placental

dysfunction, therefore affecting intrauterine fetal growth. Given the relatively easy and straightforward possibilities to screen, detect and treat for STI/RTI before or during pregnancy, we find this association between STI and FGR of particular interest. We feel that there is a need for further studies to understand better the nature of the associations between genital infections and FGR, as well as trials to discover the most effective therapeutic or prophylactic actions to prevent not only preterm birth but also FGR

Normal implantation and placentation is critical for pregnancy success. Some relations between bleeding in early pregnancy and adverse perinatal outcome have been previously reported (*Frisbie et al., 1997; Norwitz, 2006; Saraswat, 2010*), but were not extensively studied in correlation to the FGR. In the present study we found a significant association between bleeding in early pregnancy (with or without ultrasound signs of abruption) and impaired fetal growth. Possibly, defective placental angiogenesis in the first weeks may lead to placental insufficiency later in pregnancy. These suggestions are in line with our data as we found an association between placental pathological lesions such as an intervillous hematomas or villous infarction and FGR. We propose to include those patients in the high-risk group with the appropriate follow-up and clinical assessment. As bleeding in early pregnancy may be associated with abnormal vaginal flora, further studies are needed to reveal stronger correlations between fetal growth restriction, bleeding in early pregnancy and /or genital infections.

We did not find significant differences between groups in respect of TOP and complications in previous deliveries. But in cases of uterus anomalies, endothelial dysfunction may result in defective trophoblast development contributing to FGR. The overall prognosis of pregnancy in a case of gynecological disease is comparatively good, while fetal growth retardation indicates meticulous prenatal care. Appropriate treatment before conception should be provided for women having additional risk factors for FGR (*Zabak, 2001*). Also assessment of uterine arteries velocities at the first and second trimesters can be considered as additional prognostic factors for these risk group pregnancies.

The time period from one pregnancy to the next birth appeared to be associated with the risk of FGR in the present study. We found a correlation between long interpregnancy interval and FGR. This is in line with another reports (*Kallan, 1997*), but still, the

mechanism was not well documented. One of the possible hypotheses concerning the metabolic or anatomical factors that we did not measure may cause both delayed fertility and adverse perinatal outcomes (*Zhu, 1999*). Contrary to that, in our study short interpregnancy intervals did not influence fetal growth. This phenomenon has been described in the literature extensively and was explained by the depletion of maternal nutritional recourses (*Winkvist, 1992*). Therefore we can speculate that routine perinatal administration and the use of the vitamin substitutes in Latvia might reduce FGR related to the short interpregnancy interval, but reproductive health care providers could counsel mothers on the association between adverse perinatal outcomes and interpregnancy intervals, and on the benefits of optimizing that interval.

During the 20$^{th}$ century, recommendations for maternal weight gain in pregnancy were controversial (*Abrams, 2000*). Even though among our cases were no patients with malnutrition, our results are in line with previous data (*Windham, 2008; Tompson-Chagoyán, 2009*) about pregnancy weight gain in relation to the FGR. Low weight gain during pregnancy is another predictive factor for fetal growth impairment and should be included in the clinical national guidelines.

**2. Fetal factors and perinatal outcomes**

True fetal growth retardation occurs in 5% to 10% of all pregnancies (Lawn, 2005). Forty percent of them are at risk for potentially preventable perinatal death and in 20%, fetal diseases may contribute to their growth restriction (*Manning, 2004*). Among the latter, chromosomal abnormalities may constitute up to 7% and fetal infections up to 10% of all fetal growth restriction (*Chin-Chu, 1998*). In our study chromosomal abnormalities were encountered in 5.2% of FGR newborns. Although previous reports do not reveal the association between Doppler studies and chromosomal abnormalities in growth restricted fetuses, all our patients had abnormal Doppler studies and low BPP scores. Because the Latvian prenatal screening program implements genetic prenatal testing only for high-risk patients, chromosomal abnormalities in the study group were not diagnosed. One patient with Down syndrome was young and had no biochemical and recognizable stigmata on ultrasound. Two others having chromosome trisomies (one 21$^{th}$ and one 18$^{th}$) had

suspected ultrasound markers, but were not further confirmed as there are no present clinical recommendations to perform diagnostic amniocentesis after 22 weeks of gestation for FGR pregnancies. Our data reveal the urgent need after new protocols in order to reduce the rate of unnecessary operative interventions and perinatal mortality in Latvia.

Up to 10 % of FGR cases may be caused by viral or protozoan intrauterine infections (*Maulik, 2006b; Pinillos-Pisón, 2009*). In Latvia specific tests for toxoplasmosis, CMV and HSV are not included in the routine prenatal screening program, but all cases of compromised infants were specifically tested as a part of routine examination by admission in NICU and appeared to be negative for these infections. No characteristics typical of these fetal infections were found during prenatal ultrasound or after delivery. According to the study of another group at our hospital (*Miltina, 2008*) the CMV IgG positive pregnant women in Latvia are 86% and Toxoplasma gondii IgG positive - 40% at delivery time. Taking in account absence of congenital toxoplasmosis and CMV infections in FGR pregnancies we suppose that the yield and costs of routine examination of infants with intrauterine growth retardation for these infections may not be justifiable (*Khan, 2000*). At the same time neonatal bacteremia and septic markers were significantly more often encountered in the FGR babies compared to controls. More specifically, *S. aureus* was harvested in two newborns. Both patients were delivered by CS owing to abruption of placenta in one case and abnormal Doppler and BPP in another. All harvested bacteria were methicillin-sensitive, excluding hospital infections, but leaving the possibility for contamination of infants' blood samples. Also ascending maternal infection during pregnancy might cause intrauterine infection. Earlier studies from our group have shown that chorioamnionitis was more frequent in women with bacterial vaginosis and vaginal group D streptococci in the first trimester of pregnancy, and with aerobic vaginitis and *S. aureus* in case of funisitis (*Rezeberga, 2008*). Further research to investigate the link between primarily aerobic–maternal genital infection in early pregnancy and growth restriction and neonatal sepsis is needed.

As in other studies (*Goldenberg, 2008*), almost all preterm FGR pregnancies were terminated due to medical or obstetrical reasons. However, the prevalence of iatrogenic prematurity and its associated complications in our study was twice as high as in the

Dashe *et al.* study (*2000*). Additionally, the intervention rate for growth-restricted fetuses in our series was also higher than in theirs (*Dashe, 2000*).

The perinatal mortality rate of 64.9 deaths per 1,000 births and the neonatal mortality rate of 38.9 deaths per 1,000 live births are unacceptably high figures. Furthermore, the high rate of operative deliveries and perinatal complications contributed to significantly longer stay in the hospital and severe morbidity in infancy. These lead to the bigger financial costs (*Simell et al., 1993*), which should and can be reduced in the state with scarce economical resources.

.

**3. Doppler surveillance tests**

In the human fetuses, placental and fetal compromise are often associated with augmented PI of the umbilical artery (*Trudinger, 1995*), and redistribution of the blood flow within the fetal body in order to benefit the cerebral circulation (*Kiserud, 2006; Kilavuz, 1999; Nathanielsz, 2003*). Different staging systems were proposed in order to allow timely delivery of fetuses (*Mari, 2008; Gosh, 2009*). In the present study we report on the relation between ultrasonographic and clinical parameters of these high-risk pregnancies. Unlike in the study of Mari *et al*. (2007) we also included the maternal uterine artery flow studies in our analysis and found not only that advancing hemodynamic changes are associated with increased perinatal mortality but also that abnormal UtA flow in itself was associated with adverse neonatal outcome in surviving babies (low 5-minute Apgar, increased neonatal morbidity, as evidenced by increased transfer to NICU and pediatric hospitals). Therefore, we support Gosh *et al.*'s (2009) suggestion that the uterine artery flow studies should be included in the routine Doppler evaluation of women presenting with impaired fetal growth.

Furthermore, before delivery, the presence of a "notch" was demonstrated in the UtA flow on Doppler examinations in all five cases of placental abruption. As these events all occurred while the patients were hospitalized, four of the five neonates managed to survive.

In this study we hypothesized that fetal prognosis can be assessed by classifying Doppler abnormalities according to the severity in five different groups: from normal flow (Group I), to maternal flow abnormalities only (Group II), fetal uncomplicated flow abnormalities (Group III), abnormalities with brain "sparing effects" (Group IV) and finally to the flow indicating decompensation of the fetal circulation (Group V). We could clearly demonstrate a prognostic link between these groups and both fetal mortality and neonatal morbidity. Furthermore, we demonstrated that the most severe hemodynamic changes (Groups III-V) in FGR fetuses are achieved early in gestation, *i.e.* at the end of second or early in the third trimester. Inevitably, fetuses from Groups III-V were delivered earlier than fetuses of Groups II or I. Other studies have shown that placental compromise is indeed more pronounced if circulatory deprivation occurs before 32 weeks of gestation, and that late-onset cases have minimal placental involvement and more subtle Doppler findings (*Llurba, 2009; Crispi, 2006*).

Unexpectedly, FGR women of III Group (abnormal $AU$ without centralization) most often were delivered by cesarean section, which was even a higher rate than the compromised fetuses of Groups IV and V. We presume this might be due to the lack of specific clinical guidelines for the FGR management in Latvia. The prognosis for fetuses in Group III was actually good, with no perinatal deaths in this group. As these babies could have benefitted from delayed delivery as long as the elevated umbilical artery PI is not associated with signs of blood flow redistribution, such as in Groups IV and V, we would recommend conservative management for those fetuses, albeit under close supervision.

In a previous study, our group reported that smoking in association with fetal growth restriction showed more often intervillous hematomas and villous infarction in the placenta. In the present study, however, the pattern of Doppler velocities was similar between smoking and non-smoking women with FGR pregnancies. These findings seem to confirm the hypothesis that placental underperfusion in smokers might be periodic rather than continuous (*Newnham, 1990*).

Compared to non-smoking controls delivering at term, we found more genital infections associated with more severe flow abnormalities. Therefore, besides to the known increased risk of preterm birth (*Guaschino, 2006; Pretorius, 2007; Museva,*

*2007*), genital infections like BV are not only linked to the increased likelihood of FGR, but also constitute an increased risk for placental abruption as shown by impaired Doppler pattern in the uterine arteries. In former studies we have demonstrated that also aerobic genital infections in the beginning of pregnancy were associated with an increased risk for chorioamnionitis, but also funisitis and fetal infection (*Rezeberga et al., 2008*). The pathway of intrauterine ascending infections from abnormal vaginal flora, leading to increased intraamniotic proinflammatory cytokines, periventricular leucomalacia and cerebral palsy, was clearly documented by Yoon and coworkers (*1997).* Infants born after the diagnosis of absent or reversed end-diastolic flow in umbilical artery (Group V) are particularly at risk of central nervous system complications and need more frequent parenteral feeding (*Kornacki et al., 2009*). Several abnormal flora types are involved in the causation of such pregnancy complications (*Donders, 2009*) and therefore early screening and timely treatment with adequate antibiotics, like clindamycin, might lead to improved pregnancy outcomes (*Donders, 2000a*; *Swadpanich, 2008*). However, specific associations between the presence of genital infections and FGR have been documented only sporadically, perhaps because the emphasis of the previous studies was mostly on the prevention of preterm birth and not FGR. We hope our new data inspire researchers to perform more studies on the link between genital infections and FGR, and try to provide evidence that can help to install preventive actions to dampen the severe damage of these small babies by early screening and treatment.

**4. Adaptive response to impaired placental perfusion**

The previous studies confirmed that up to 85% of the venous perfusion to fetal liver are supplied by umbilical vein (Kiserud, 2006). Therefore umbilical venous flow to the liver is crucial for the intrauterine liver and accordingly, fetal growth (*Kessler, 2009*). In cases of placental compromise the umbilical vein volume reduces, and consequentially different adaptive mechanisms may be triggered. In one adaptive mechanism-the fetuse economizes demands and slows the growth velocity (*Haugen, 2005; Nathanielsz, 2003*). In another there is redistribution of umbilical blood flow, prioritizing the left hepatic lobe. Kessler *et al*. (2009) examined 31 growth-restricted fetuses and found an increased

*ductus venosus* shunt fraction and reduced blood flow to the fetal right lobe. This may affect the liver size and antenatal growth through decreased glycogen production (*Tchirikov, 2002*). It may also affect the liver function in adults. The association between growth restriction and insulin resistance, visceral obesity and glucose intolerance in adult life was described recently by Morrison *et al*. (2010). Another finding is that the expression of gluconeogenic genes as a result of intrauterine malnutrition is exaggerated in offspring. This change remains through adulthood and may contribute to the pathogenesis of type 2 diabetes (*Liu, 2009*). In the present study we found that blood flow through the left portal vein was significantly reduced in growth restricted fetuses, which confirmed that in a case of FGR, the liver suffers from venous hypoperfusion.

Reverse flow in the left portal vein can be observed as a physiological process in appropriately growing fetuses (*Kessler, 2007*). The present study adds that with the higher degree of compromise, the flow in the LPV becomes reversed more often than in a physiological condition. Reverse flow in the LPV supplies the ductus venosus shunting at the expense of portal perfusion, therefore mainly affecting the right lobe.
Furthermore, low Apgar scores and high perinatal mortality rates are associated with reduced blood flow through the LPV. These parameters may provide new predictive factors for perinatal adverse outcome for growth restricted fetuses. As in term FGR, surveillance tests are subtle (*Baschat, 2010*), assessment of the LPV velocity adds perspective for clinical assesment in late pregnancy.

Different theories have been offered to explain the decreased splenic artery pulsatility index in FGR. Some studies speculate that hypoxia may stimulate the fetal erythropoietin system, following the acceleration of red blood cell production and premature release of erythroblast (*Abuhamad, 1995; Capponi, 1997*). More recent studies showed compensatory vasodilatation of the splenic artery for maintaining venous perfusion of the fetal liver flow (*Dubiel, 2001; Ebbing, 2009*). In the present study we also found reduced SA resistance in the FGR group. Although the relation with reduced SA PI and FGR was less clear, low SA PI was associated with the higher perinatal mortality rate, low Apgar scores and metabolic acidosis and suggest that severe fetal deprivation causes more evident hemodynamic changes in the spleen and therefore may identify fetuses with perinatal death risk.

Relatively easy techniques of assessment and interpretation of the LPV and SA blood flow, and the fact that their velocities are not affected by gestational age (*Ebbing, 2009*) facilitate the use of these methods for fetal assessment.

A limitation of our study is that some of the sample sizes are relatively small. Possible measurement bias may result in certain cases from sub-optimal visualization due to reduced amount of amniotic fluid, and in other cases from unfavorable fetal position, or fetal movements. Interobserver variation was not assessed and that can constitute another bias. But still the regional adaptive changes in growth restricted fetuses could clearly be demonstrated in the present contribution. More studies are needed for better understanding of the underlying hemodynamic mechanism of adaptive changes in compromised fetuses.

**5. Placental macroscopic and microscopic lesions**

In the present study the association between fetal growth restriction and the presence of macroscopic and microscopic pathological changes in the placenta were investigated.

Placental weight and the fetal-placental weight ratio in FGR cases were significantly lower than in controls, corresponding to earlier studies (*Oliveira, 2002; Fox, 2003*). Placental weight in the Biswas S. *at al.* (2003) study was less than 500g in all cases, whereas Mardi *at al*. (*2003*) found placentas less than 400g in 84% of FGR cases. Low placental weight in the FGR group was related to prematurity (mean 412g ± 117) and was found appropriate for the mean gestational age, that is in line with results of the Thompson' *et al.* study (*2007*). Placental thickness marks intrauterine environmental adequacy. We did not find any difference in the thickness and shape of placentas between the two groups. This can be explained by induction of progressive branching and arborization of the villous tree in order to guarantee an adequate nutrient exchange surface and support the fetal growth (*Salafia, 2006*). Still, there is a reduced fetal-placental weight ratio (under the $10^{th}$ percentile for gestational age) (*Thompson, 2007*) indicating failed compensation. This indicator may therefore be more characteristic for FGR then placental weight alone.

We observed more cases having multiple entanglements of the umbilical cord around different fetal parts in the study group than in controls, but the difference was not statistically significant. Together with the shorter umbilical cord, this could be involved in the reduction of blood flow to the fetus and the deterioration of the fetal circulation due to chronic partial or recurrent intermittent umbilical cord compression (*Redline, 2004; Hua, 2010*). Therefore during the prenatal ultrasound examination multiple entanglements of the umbilical cord should be determined and recorded as it can predict the adverse perinatal outcomes (*Sherer, 1996; Grzesiak, 2006*).

A positive correlation between velamentous and marginal insertion of cord to the chorionic disc and FGR has been observed before (*Biswas, 2003*). In our study, however, we did not find any difference regarding the cord's insertion site, and we even had slightly more velamentous insertions in the control group. Examining 1000 placentas, Uyanwah-Akpom *at al*. (*2005*) failed to demonstrate any association of marginal and velamentous insertion of the cord with low birth weight, fetal hypoxia or intrauterine fetal death. Also Hansen *et al*. (*2000*) observed marginal and membranous insertions quite frequently, but failed to find more of these abnormalities in 1,146 placentas from pregnancies ending in the live births of very low birth weight infants.

Many morphologists have described different kinds of placental lesions that interfere with the normal trophoblast function (*Rayburn, 1989; Salafia, 1995; Salafia, 2003; Kraus, 2004*). We observed an association of FGR with the following histological findings in placental morphology: increase of incidence of infarcts and thickening of the villous trophoblastic basal membrane. The aforementioned are obstructive lesions of the placenta leading to haemostasis, vascular damage and restricted fetal circulation (*Shepard, 1980; Rayburn W, 1989; Salafia, 1995; Salafia, 2003; Mardi, 2003*). Therefore such morphologic changes may lead to FGR and adverse perinatal outcome.

In the present study intervillous hematomas were also seen more often in FGR placentas, explaining the reduced fetoplacental oxygen delivery due to loss of integrity in the maternal circulation in patients with chronic placental abruption (*Salafia, 2006*). Five of our study patients had symptoms of acute placental abruption and one had signs of a chronic process. The latter experienced recurrent episodes of vaginal bleeding during pregnancy and histology showed old peripheral blood clots and increased chorionic-

villous macrophages. Such chronic placental abruption is strongly associated with FGR and therefore makes intensive monitoring of the fetal growth obligatory. One of the acute abruption cases resulted in perinatal death. In spite of the known association of maternal smoking and placental abruption, only one of these patients with signs of abruption admitted smoking. As intervillous hematomas and villous infarction can be associated with maternal thrombophilia (*Redline, 2006*), the screening for genetically determined tendency toward clot formation in a case of FGR should be considered. Antithrombotic therapy, such as unfractionated heparin plus low dose aspirin might improve pregnancy outcome in FGR (*Bujold, 2009*) and can be offered as a treatment's option when possible fetal and maternal factors excluded and prevented.

There were no significant differences in stromal fibrosis between groups. From one side it is known that fibrosis of stem villi is an indicator of placental maturation and together with a preterm villous hypermaturity manifests in reduced blood flow in the umbilical cord (postplacental hypoxia) (*Benirschke, 200; Faye-Petersen, 2006*). Extensive stromal fibrosis also can be found in pregnancies affected by congenital cytomegalovirus infection. In the present study, all FGR cases with villitis also had some stromal fibrosis in placentas, but none was due to congenital CMV.

Chronic villitis, found more frequently in our study patients, is a possible mechanism of fetal vascular injury in FGR with an increased risk of recurrence in subsequent pregnancies (*Redline, 2007*). As we mentioned before, there were no TORCH-related infections, recognized among our patients (*Benirschke, 2000; Redline, 2007*).

So-Young Park *et al.* (2002) examined 45 placentas from FGR pregnancies and 24 placentas from the control group, observing acute chorionamnionitis more often in control placentas than in FRG, possibly related to vaginal delivery. Also in the present study chorionic vasculitis was found significantly more often in control placentas.

Although the association of placental pathologies with maternal smoking such as abruption of placentas, impaired proliferation and differentiation of cytotrophoblast is described in other studies (*Salafia, 1999; Zdravkovic, 2005*), we failed to confirm that cytotrophoblast proliferation occurred more often in cases of FGR and smoking patients than in controls. On the other hand, in our smoking FGR patients intervillous hematomas and villous infarction were more common, implicating late uteroplacental malperfusion.

Formerly we found not only smoking during current pregnancy, but also cessation of smoking before pregnancy (previous smoking) was strongly associated with FGR (*Table N4 and 5*). In 2008 14% of all stillbirths and 10.2% of live births mothers had continued to smoke during pregnancy in Latvia (*Ministry of Health of the Republic of Latvia, 2008*). For all these reasons, even better than quitting smoking during pregnancy, it seems important to motivate women to stop smoking long before planning pregnancy. Also prophylactic administration of low molecular weight heparin (LMWH) with or without aspirin could be considered for these women in an attempt to prevent the development of FGR

**6. Correlation of Doppler velocimetry with placental microscopic lesions**

Doppler ultrasound of uterine and umbilical ateries enables us to obtain parameters to assess reduced perfusion on the maternal side of uteroplacental circulation. The relationship between placental vascular diseases and abnormal uterine and umbilical Doppler flow has been demonstrated in previous studies (*Sebire, 2001; Madazli, 2003*). Furthermore, Viscardi *et al.* (2001) showed that the presence of two or more placental lesions are associated to an increased risk of perinatal mortality and morbidity. While in some studies an association was found between perivillous fibrin deposition, cytotrophoblast proliferation, stromal fibrosis and abnormal Doppler findings in FGR pregnancies (*Aarderma, 2001 Dicke, 2009*), we failed to confirm this finding in our study. However, a significant association between villous infarction, or formation of thrombi and abnormal Doppler was found in FGR cases. This is in line with findings by Laurini *et al.* showing that placental infarction is the only valuable morphological marker of uteroplacental vascular disease related to FGR with impaired fetal and umbilical blood flow (*Laurini, 1994*). According to a recent meta-analysis on acetylsalicylic acid (ASA) for prevention of preeclampsia and intra-uterine growth restriction in women with abnormal Doppler findings of the uterine artery, ASA was linked to a significant reduction of FGR incidence if started before 16 weeks (*Bujold, 2009*). Our study adds supplementary histological evidence that FGR due to placental ischemia may be ultrasonographically predicted and sequentially prevented.

The findings that placentas from appropriate for gestational age infants with normal Doppler had similar placental lesions than normal Doppler FGR placentas suggest that compromised fetuses with normal circulation have an abnormal growth velocity which may also originate from non-placental causes. In the present study, as well as in our previus findings we found that thickening of the basal membrane and intervillous haematoma/thrombi and villitis are also associated with FGR, representing obstructive lesions due to underperfusion (*Dicke, 2009*).

As we mentioned before, villitis is a possible mechanism of fetal vascular injury in FGR and has an increased risk of recurrence in subsequent pregnancies (*Redline, 2007*). Also in our study, we encountered villitis more frequently in FGR placentas than in controls. Other authors found significant relationships between hemorrhagic endovasculitis and villitis of unknown etiology (*Sander, 2002*). The present data support that villitis of unknown etiology can be related to FGR, probably due to an ischemic, not infectious, lesion of placenta that cannot be predicted by Doppler ultrasound.

Vasculitis was found significantly more often in placentas within both control groups than in FGR and was not linked to abnormal Doppler. It confirms the previous studies (*Park So-Young, 2002*) that vasculitis may be related to vaginal deliveries that occurred more often in control group and might reflect some grade of subclinical chorioamnionitis. Doppler measurements are of no value as a predictor of the subclinical chorioamnionitis as no differences in the pulsatility indices could be attributed to it (*Santolaya, 1991; Leo, 1992*)

Vascular resistance in FGR cannot be solely explained by abnormal placental histopathology. In the case of abnormal Doppler velocimetry of uterine and umbilical arteries in FGR pregnancies, association of placental lesions is limited only to morphological changes due to vascular damage: villous infarction and intervillous hematomas or thrombi. Therefore, abnormal Doppler may predict hemorrhagic and ischemic placental lesions in FGR pregnancies and may lead to improved management in the current, and even more importantly, in subsequent pregnancies.

# CONCLUSIONS

1. The following maternal risk factors play an important role in fetal growth restriction:
   - Socio-economic deprivation: low weight gain during pregnancy, smoking before and during current pregnancy;
   - Obstetrical: long interval between pregnancies, bleeding in the 1 st trimester;
   - Genital infection: *Chlamydia trachomatis,* bacterial vaginosis
   - Others: extra-genital diseases and use of medication for therapeutic reason

We conclude that aside from refraining from smoking and decreasing the interval between pregnancies, screening and treating for gynecological abnormalities and RTI appeared to be important in the reduction of FGR. Identifying such risk factors will most likely have the greatest impact if detected before conception, or as early as possible in gestation. Previous smoking was still recognized as a risk factor, even when the mother did not smoke during the current pregnancy. Reproductive health care providers should counsel mothers on the benefits of optimizing (reducing) the interpregnancy interval.

Pre-conceptional screening for extragenital diseases and treatment may reduce the risk for FGR in the Latvian population. At the same time, the use of medication during pregnancy should always be guided by risk-benefit considerations.

2. Major fetal risk factors for FGR were unrecognized chromosomal anomalies and other fetal malformations. Therefore fetal karyotyping should be included in the Latvian clinical guidelines in severe cases of FGR.

3. This study found that the most severe hemodynamic changes in FGR fetuses occurred early in gestation (at the end of the second or early third trimester).

4. Our data suggest that growth-restricted fetuses with elevated umbilical artery PI without blood flow redistribution may be exposed to unnecessary c-section and iatrogenic preterm birth too often. We believe that these babies would benefit from close surveillance and delayed delivery in most cases, and recommend a prospective study to test this hypothesis.

5. Women having genital infections had significantly worse Doppler flow profiles than women who delivered at term without genital infections.

6. Reduced blood flow through the LPV and low splenic artery PI may be alternative predictive factors for perinatal adverse outcome for growth-restricted fetuses. Their additional predictive values need to be investigated.

7. All macroscopic and microscopic pathological changes pointed towards reduced blood flow due to vascular damage as being a contributing factor for, or cause of FGR. Low placental weight and the fetal weight/placenta ratio, as well as intervillous haematomas and infarctions are the most frequently encountered placental lesions of FGR placentas. The present study adds that smoking is a main risk factor for these placental abnormalities and emphasizes the need to persuade women to quit smoking not only during pregnancy, but even better, a long time before.

8. Abnormal Doppler may predict hemorrhagic and ischemic placental lesions in FGR pregnancies and may lead to improved management in current, and, even more importantly, in subsequent pregnancies.

## CLINICAL IMPLICATIONS AND FUTURE ASPECTS

Following the published evidence and the results of our studies, we are in the process of constructing adapted clinical guidelines for the management of intarauterine growth restriction that will be proposed to the Association of Latvian Gynaecologist and Obstetricians. We hope that this may help to decrease the rate of unnecessary operative interventions and reduce the perinatal morbidity and mortality of FGR babies in Latvia. In order to determine the best management in the specific group of growth-restricted fetuses with elevated umbilical artery PI, but without blood flow redistribution, we would welcome a prospective trial to test whether expectant management can safely guide these fetuses over the frontier of extreme prematurity, without additional risks for perinatal death or morbidity.

Latvian national birth weight charts with the 2.5 and $90^{th}$ percentiles should be established in order to enable Latvian obstetricians to diagnose intrauterine growth restriction more accurately and to reduce the number of erroneous diagnoses of FGR.

Assessment of uterine arteries velocities at the first and second trimesters should be assessed in further studies as potential additional prognostic factors for women having gynecological abnormalities.

As abnormal Doppler profile could predict hemorrhagic and ischemic placental lesions, one should consider screening for maternal trombophilia in FGR cases and/or test whether prophylactic administration of appropriate anticoagulant therapy can improve perinatal outcome. Ideally, randomised studies studying the effect of low molecular weight heparin with or without aspirin on Doppler profiles in cases of placental compromise or FGR should be performed to elucidate a possible effect on maternal vascularisation.

Further studies of fetal liver flow and splenic arteries might extend our knowledge on the pathogenesis of fetal growth restriction. We will continue to collect data, with the hope of developing new screening tests and individualized fetal interventions, as a larger study will improve the ability to identify the fetuses at greater risk of developing clinically relevant adverse outcomes. Another aim of future studies we envision is to study the long-term consequences which reduced oxygenation of the liver lobe may have on children, such as alterations in metabolic processes, predisposing them to disease later in adult life. To explore these aspects long-term follow-up of our FGR neonates, together with the neonatologists and pediatricians, will be necessary.

The population longitudinal specific reference ranges for splenic artery flow profiles should be constructed in order to allow differential assessment and monitoring of high-risk pregnancies.

We also plan to conduct a study to test the hypothesis that abnormal vaginal flora may influence not only the preterm timing of birth, but also the intrauterine growth.

We assume that the hemodynamic in LPV and splenic arteries might be affected by genital infections and elicit different responses and adaptation of the SA and LPV. Whether other maternal factors and influences, like smoking, are also reflected in alterations in the balance of the left portal vein and splenic arteries supply also remains to be explored.

## ORIGINAL PUBLICATIONS

1. Vedmedovska N, Rezeberga D, Teibe U, Donders G. Preventable maternal risk factors and association of genital infection with fetal growth restriction. // *Gynecol Obstet Invest.*- 2010. Nr. 70. - 219.-226.
2. Vedmedovska N, Rezeberga D, Teibe U, Melderis I, Donders GGG. Placental. pathology in foetal growth restriction and smoking. // *Eur J Obstet Gynecol Reprod Biol*.- 2011. Nr. 155. – 36.-40.
3. Vedmedovska N, Melderis I, Rezeberga D, Teibe U, Donders G G G. Microscopic lesions of placenta and Doppler velocimetry related to fetal growth restriction. // *Archives of Gynecology and Obstetrics*.-2010 (accepted. 2010 Dec 14. Epub ahead of print PMID: 21153651).
4. Vedmedovska N, Rezeberga D, Teibe U, Donders G G G. Predicting fetal prognosis by assessing fetal and maternal flow patterns in pregnancies with fetal growth restriction. // *Acta Chirurgica Latviensis.*- 2010. Nr. 10. - 42.-47.
5. Vedmedovska N, Rezeberga D, Teibe U, Donders G G G. Adaptive changes in splenic artery and left portal vein in fetal growth restriction. // *Journal of Ultrasound in Medicine*- 2011. (accepted).
6. Vedmedovska N, Rezeberga D, Teibe U, Donders, G G G, Polukarova S. Fetal Growth Restriction in Latvia. // *IJOG*- 2010. Nr. 111. – 185.-6.
7. Torloni M R, Vedmedovska N, Merialdi M, Betrán A P, Allen T, González R, Platt L L. Safety of ultrasonography in pregnancy: WHO systematic review of the literature and meta-analysis. // *Ultrasound Obstet Gynecol*- 2009. Nr. 33 (5). - 599.-608.
8. Vedmedovska N., Melderis, I., Rezeberga, D. (2009) Doppler Velocimetry and Microscopic Lesions of Placenta in Fetal Growth Restricted Pregnancies. // *IJOG*- 2010. Nr. 107. S2, pp S370.
9. Zodzika J, Rezeberga D, Jermakova J, Vasina O, Vedmedovska N, Donders GGG, Association between increased vaginal pH and flora type in pregnant women. *Acta Obstet. Gynecol. Scand - 2010* . Nr. 90. – 41.-6.

## PRESENTATIONS RELATED TO THE DISSERTATION TOPIC

1. Lisbon, Portugal, 20th EBCOG congress, “Placental macroscopic and microscopic examination of placenta in cases of fetal growth restriction”. 04-08.03.2008. N. Vedmedovska, D. Ezerina, I. Melderis, D. Rezeberga. Abstract and poster

2. Riga, Latvia, RSU 7. Scientific conference, “Placental macroscopic and microscopic lesions in FGR pregnancies”. 13-14.03.2008. N. Vedmedovska, D. Ezerina, I. Melderis, D. Rezeberga. Abstract and poster

3. Reykjavik, Iceland, 36th Congress of the Nordic Federation of Societies of Obstetricians and Gynecologists, “Maternal risk factors for fetal growth restriction (FGR) in Latvia, 14-17.06.2008, N. Vedmedovska, D. Ezerina, D. Rezeberga, I. Jermakova. Abstract and poster.

4. Chicago, USA. The 18th World Congress on Ultrasound in Obstetrics and Gynecology."Safety of ultrasonography in pregnancy: WHO systematic review of the literature and meta-analysis”. M. R. Torloni, N. Vedmedovska, M. Merialdi, A. P. Betrąn, T. Allen, R. Gonzalez, L. D. Platt. 24-28.08.2008. Abstract

5. Riga, Latvia, Baltic States Conference of Obstetrics and Gynecology. The 5th congress of Latvian gynecologists and obstetricians. “Fetal factors for FGR in Latvia”. N.Vedmedovska, D.Ezerina, I.Jermakova, D.Rezeberga. 10-11.10.2008.g. Abstract and presentation

6. Riga, Latvia, Baltic States Conference of Obstetrics and Gynecology. The 5th congress of Latvian gynecologists and obstetricians. “Epidemiologic of fetal growth restriction in Latvia. N. Vedmedovska, D. Ezerina, D. Rezeberga, I. Jermakova. 10-11.10.2008. Abstract and poster.

7. Paris, France, The 11th World congress on controversies in obstetrics, gynecology and infertility.” Fetal factors for fetal growth restriction and outcomes in Latvia”. 27-30.11.2008. N.Vedmedovska, D.Ezerina, I.Jermakova, D.Rezeberga. Abstract and poster.

8. Riga, Latvia. The 6th Latvian Doctors’ Congress. “Intrauterine growth restriction in Latvia, risk factors, diagnostic procedures”. 19-21.06.2009. N.Vedmedovska, D. Ezeriņa, I. Melderis, D.Rezeberga. Abstract and presentation

9. Cape town, South Africa, XIX FIGO (International Federation of Gynecology and Obstetrics) "Doppler Velocimetry and Microscopic Lesions of Placenta in Fetal Growth Restricted Pregnancies"**.** . 4-9.10.2009. N.Vedmedovska, I. Melderis, D.RezebergaAbstract and presentation

10. Riga, Latvia, RSU 7. Scientific conference. “Hemodynamic changes in growth restricted fetuses; outcomes”. 18-19.03.2010. N. Vedmedovska, D. Rezeberga, U. Teibe. Abstract and presentation

11. Riga, Latvia, 5. Baltic states Conference of Ultrasound in Obstetrics and Gynecology. "Safety of ultrasound in pregnancy". 19-21.06.2009. M. R. Torloni, N. Vedmedovska, M. Merialdi, A. P. Betrąn, T. Allen, R. Gonzalez, L. D. Platt. Abstract and presentation.

12. Antwerp, Belgium, 21th EBCOG congress. "The preventable maternal risk factors and association of genital infections with Intrauterine growth retardation". N. Vedmedovska, D. Rezeberga, U. Teibe, G.G. G. Donders, J. Žodžika. 05-8.05.2010 Abstract and oral poster presentation.

13.Antwerp, Belgium. 21th EBCOG congress. Association between increased vaginal pH and flora type in pregnant women. Zodzika J., Rezeberga D., Vasina O., Vedemedovska N., Bite R., Pundure I., Strazdina L., Baranovska D., Vidnere I., Dresmane A., Jermakova I., Donders G. 05-8.05.2010. *Abstract*

14. Prague, Check Republic, The 20th World Congress on Ultrasound in Obstetrics and Gynecology. "Splenic artery and the left portal vein Doppler flow velocimetry in growth-compromised fetuses". 10-14.10.2010. N. Vedmedovska, D. Rezeberga, U. Teibe. Abstract and oral presentation.

## ACKNOWLEDGEMENTS

The present study was performed at the Section for Ultrasound Diagnostics of Riga Maternity Hospital and Department of Obstetrics and Gynecology, Riga Stradins University. I have received a Grant for this project from the Latvian Ministry of Education and Science, and the European Structural Funds.

Among many people who have contributed to the success of the study I first and foremost want to thank my primary supervisor Assoc. Professor Dace Rezeberga. She managed to convert my plan of improving my sonography skills into interest for a considerably larger project finally leading to this doctoral thesis. I am very greatly indebted to her for her instructive and stimulating scientific discussions, support and encouragement during these years.

I also want to express my sincere thanks to my second supervisor Professor Gilbert Donders. His genuine curiosity, vast knowledge, impressive enthusiasm and creativity were indispensable. He always brought new valuable aspects into discussions and shared his expertise in the publication process.

Professor Uldis Teibe generously and patiently shared his skills and insight into advanced statistics. His contributions to this work have been invaluable.

Through the years, my colleagues Dace Ezerina and Iveta Bicevska backed me up when it was needed, sharing their experience.

I am grateful to Professor Torvid Kiserud, the Section of Fetal Medicine and Ultrasound Diagnostics, Department of Obstetrics and Gynecology, Haukeland University Hospital, who gave me the opportunity to share his vast knowledge of the field.

Further I would like to thank my colleagues and the midwifes at the Riga Maternity Hospital for their continuous help and their positive attitude towards my work.

My thanks to the Head of the Outpatient Department “Via Una” Natalja Bagramjan who gave me leave from clinical work when needed.

The Section for Ultrasound Diagnostics of Riga Maternity Hospital provided excellent infrastructure and working conditions for me through the course of the study. I would like to express my sincere thanks to the director of Riga Maternity Hospital Ilze Lietuviete, for the providing the opportunity to do the study.

I am indebted to all the pregnant women who unconditionally and enthusiastically participated in the study, allowing me to use several hours of their time.

I am grateful to my friend Gregory MacDonald, PhD, the Royal College of Surgeons in Ireland, who skillfully revised English language of this thesis.

Finally, my warmest thanks go to my family, Vitalij, Diana and Dana, and my parents and parents-in-law for their love, patience and support.