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RĪGAS STRADIŅA UNIVERSITĀTE

Natalija Vedmedovska

Fetal growth restriction

For obtaininga degree of
a Doctor of Medicine
Speciality – Obstetrics and Gynecology

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Speciality: Obstetrics and Gynecology

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ABBREVIATIONS

AC	Abdominal circumference
ACM	Arteria Cerebri Media
AEDFV	Absent end-diastolic flow velocity
AFI	Amniotic fluid index
AFV	Amniotic fluid volume
AGA	Appropriate-for-gestational age
APS	Antiphospholipid syndrome
ARED	Absent or reversed end-diastolic flow velocities
ASA	Acetylsalicylic acid
AU	Arteria umbilicalis
BPD	Biparietal diameter
BFP	Biophysical profile
BV	Bacterial vaginosis
CA	Celiac artery
CD4	Cluster of differentiation 4 antibodies
CMV	Cytomegalovirus
CTG	Cardiotohography
DA	Ductus arteriosus
DV	Ductus venosus
DNA	Deoxyribonucleic acid
EFW	Estimated fetal weight
ELISA	Enzyme-linked immunosorbent assay
FGR	Fetal growth restriction
FHR	Fetal heart rate
FL	Femur length
FTV	Fetal thrombotic vasculopathy
HA	Hepatic artery
HC	Head circumference
HIV	Human immunodeficiency virus
IGF	Insulin growth factor

IL	Interleukin
IUGR	Intrauterine growth restriction
IVC	Inferior vena cava
LBW	Low birth weight
LeLL	Left liver lobe
LPV	Left portal vein
NICU	Neonatal Intensive Care Unite
NO	Nitric oxide
NST	Nonstress test
PAPP-A	Pregnancy-associated plasma protein –A
PCR	Polymerase chain reaction
PE	Pre-eclampsia
PGE2	Prostogalndin E2
PI	Pulsatility index
PIGF	Placental growth factor
PSV	Pick systolic velocity
PV	Portal vein (main stem)
RiLL	Right liver lobe
RI	Resistance indices
RNA	Ribonucleic acid
S	Stomach
SA	Splenic artery
SGA	Small for gestational age
STI	Sexually transmitted infections
T	Thalamus
UtA	Uterine artery
UTI	Urinary tract infections
UV	Umbilical vein
VEGF	Vascular endothelial growth factor
VUE	Villitis of unknown etiology

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1. INTRODUCTION

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 10th 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.

2. RELEVANCE OF THE SUBJECT MATTER OF THE STUDY

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.

2.1. Fetal biometry

Ultrasound measurements of fetal parts to assess gestational age or evaluate fetal size and growth play a fundamental role in FGR diagnosis, identifying fetuses at risk.

Ian Donald first introduced ultrasound imaging in the obstetric context in 1958 when he visualized the outline of the fetal skull and twin pregnancy (*Donald, 1958*). In 1961 Donald and Brown described the measurement of biparietal diameter (BPD). Willocks (1964) suggested a correlation between BPD and fetal weight. In the second and third trimester BPD, head circumference (HC), femur length (FL), and abdominal circumference (AC) are the most frequently measured parameters.

2.2. Fetal size and growth

Charts for fetal biometry can be used for three different purposes: to evaluate size, growth, and to estimate gestational age. To assess fetal growth *in utero*, the estimated fetal weight (EFW) is a way of transferring ultrasound measurements of different parts in a more comprehensible expression. Fetal biometrical measurements are expressed as a regression equation to derive fetal weight. In recent years appropriately designed studies have been published. Owen *et al.* (1998) presented conditional biometric growth charts based on a study of 274 pregnancies analyzed by multilevel models. In 2000 longitudinal unconditional growth charts were published by researchers from Italy (*Di Batista, 2000*). According to Bakketeig (1998), growth charts ought to be standardized and population-specific.

Birth weight percentile charts are commonly used to assess intrauterine growth (*Skjaerven 2000-A; Alshimmiri 2004*). Marsal *et al.* (1996) presented intrauterine growth charts based on EFWs in 86 pregnancies from four Scandinavian centers. Pregnancies complicated by preterm delivery, pre-eclampsia and FGR were excluded from this study. However, Altman *et al.* (1993) found that reference data should relate to normal fetuses and therefore it is important to have as unselected group as possible. Norwegian scientists (*Johnsen, 2006*) published new percentile charts for fetal weight assessments between gestational weeks 20 and 42, which were used to determine the effect of fetal and maternal factors.

2.3. Fetal circulation

General hemodynamic aspects

Fetal circulation displays specific features: the placental circuit with the umbilical arteries (*AU*) and vein (UV) connecting the fetus and placenta and the three fetal shunts-the *ductus venosus* (*DV*), the *foramen ovale* and the *ductus arteriosus* (*DA*).

The *AU* originates from the fetal internal iliac arteries and returns one third of the cardiac output (one fifth after 32 weeks) to the placenta (*Sutton, 1991; Kiserud, 2006a*). The placental vasculature is compliant and relatively non-responsive, and the placental compartment constitutes a large blood volume.

Both ventricles of the fetal heart work in parallel. While the left ventricle perfuses the upper part of the body, including the myocardium and the brain, the right ventricle through the *DA* supplies the lower part of the body and the placenta. The *DA* is a short vessel connecting the pulmonary artery to the descending aorta and is a part of the arterial outlet to the lower part of the body. The right ventricle output is larger than the left cardiac output (*Mielke and Benda, 2001; Kiserud, 2006a)*; see *Figure N 1*.

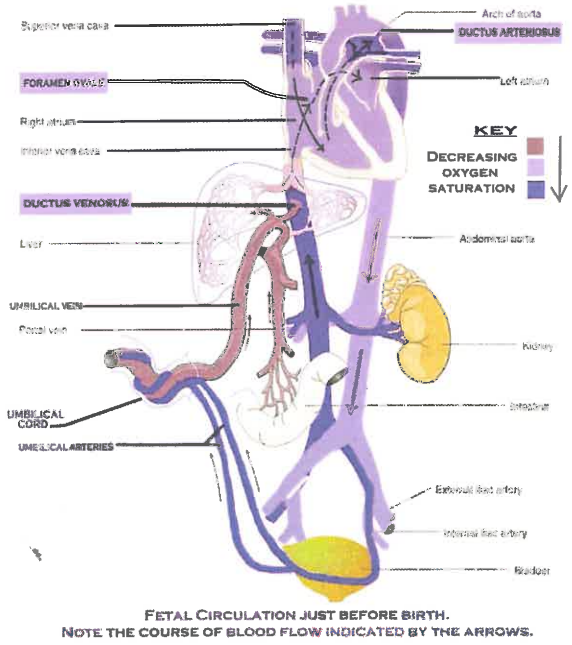


Figure N 1. Fetal circulation

(adapted from faculty.lagcc.cuny.edu/ pdillon/FetalCirc/)

2.3.1. Venous circulation

Oxygen and nutrient rich blood from the placenta returns to the fetus *via* the UV, that first gives off branches to the left lobe of the liver. A fraction of the umbilical blood is then shunted through the *DV*, while the remaining part is directed to the right liver lobe (RiLL) *via* the left portal vein (LPV) (*Figure N 2*).

The highest oxygen saturation is found in the UV and *DV* (80%), the lowest in the IVC (35%) and the PV (30%) (*Bristow et al., 1981; Rudolph, 1985*). Therefore, the left liver lobe (LeLL) is perfused by highly oxygenated umbilical blood, in contrast to the right liver lobe (RiLL), which perfused by a mixture of umbilical and portal blood with a lower saturation.

DV in the human fetus is a slender, trumpet like vessel from 5 to 15mm in length (*Kiserud et al., 1994*), connecting the UV to the IVC. *DV* directs oxygenated UV flow towards the *foramen ovale*, through it to the left atrium, left ventricle and aortic arch, ensuring a steady supply of highly oxygenated blood to the heart and brain. Human studies of low-risk pregnancies showed a shunt fraction of 30% at mid gestation and 20% at term (*Kiserud et. al., 2000; Haugen et al., 2004*). Consequently, 70-80% of the umbilical venous returns in human pregnancies perfuse the liver, which illustrates the high developmental priority given to this organ.

The LPV connects umbilical circulation with the portal circulation (*Figure N 2*) and the flow velocity in the LPV directly reflects UV supply to the right liver lobe (*Kessler et al., 2007*). The flow is orthograde during the second half of pregnancy, but may be reversed during fetal respiratory movements, therefore affecting venous liver flow (*Marsal et al., 1984*). Apart from local regulatory mechanisms, maternal diet and body compositions may modify the fetal liver blood flow (*Haugen et. al., 2005*).

Venous blood from the spleen, stomach, pancreas and intestine drained into the portal vein, which perfuse the RiLL in fetal life under physiological conditions (*Figure N 1*).

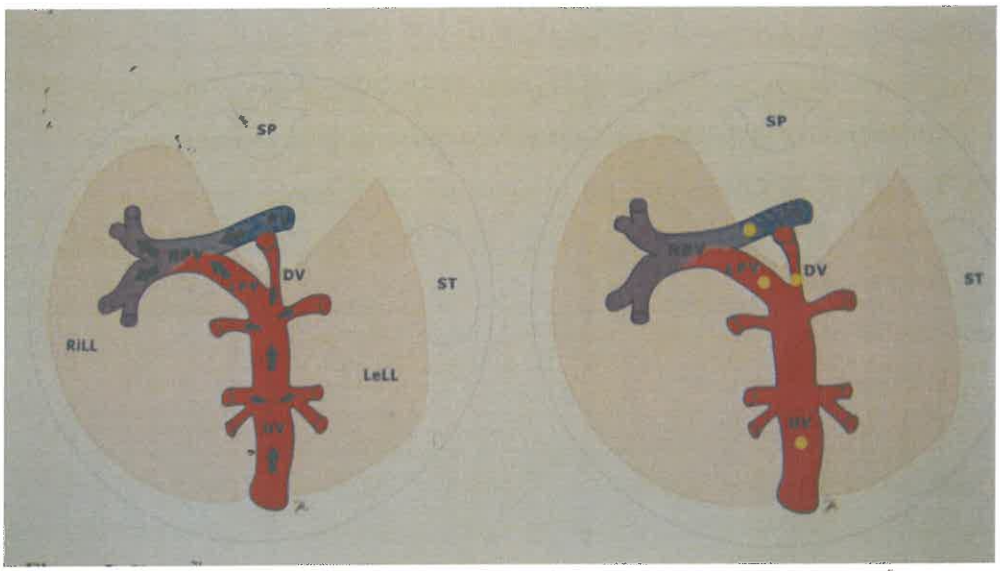


Figure N 2. Venous blood supply of the fetal liver with level of oxygenation, flow direction (blue arrows). Red colour: high oxygen saturation, blue colour: low oxygen saturation. UV: umbilical vein; *DV*: *ductus venosus*; LPV: left portal vein; RPV: right portal vein; PV: main stem of the portal vein, Lell: left liver lobe; RiLL: right liver lobe; St; stomach; Sp: spine. (Adapted from J. Kessler, 2007).

2.3.2. Arterial circulation

The most fundamental principle of cardiovascular regulation is to match each tissue's blood flow to its metabolic demands. The capacity to sustain the perfusion of an organ when blood pressure or cardiac output is altered, depends on the ability for autoregulation and the responses to neural and hormonal stimuli.

The sympathetic nervous system is the main mediator of neural control of the blood vessels (arterioles). Increased sympathetic tone induces vasoconstriction in peripheral beds such as the skin and guts. However, it causes vasodilatation and increased blood flow in

the heart, brain and adrenal glands. The change in the distribution of cardiac output to the various vascular beds is called redistribution.

Local control of the circulation, autoregulation, has two functions: to keep the blood flow constant in the face of changes in blood pressure, and to adapt the blood flow in the organ to local changes in metabolism independently of the blood pressure. The ability to regulate may be impaired after sustained hypoxia (*Bristow et al., 1985*).

The heart, brain, intestine and kidney have highly efficient autoregulation mechanisms. Recent experimental research has revealed that the liver has a highly specialized system for regulating intrahepatic blood volume and flow. The portal perfusion is a key component in the intrinsic regulation of the hepatic artery (HA) (*Lautt, 2007*).

The *AU* coils around the UV in a clockwise manner (90%) to enter the placenta. The *AU* returns deoxygenated blood from the fetus to the placenta. The *AU* is assessable for Doppler interrogation. Towards term with increased vascularisation in the placenta decreases downstream impedance of the *AU*. This is reflected in linear decline in the pulsatility index (PI) of the *AU* through gestation (*Acharya et al., 2005a*).

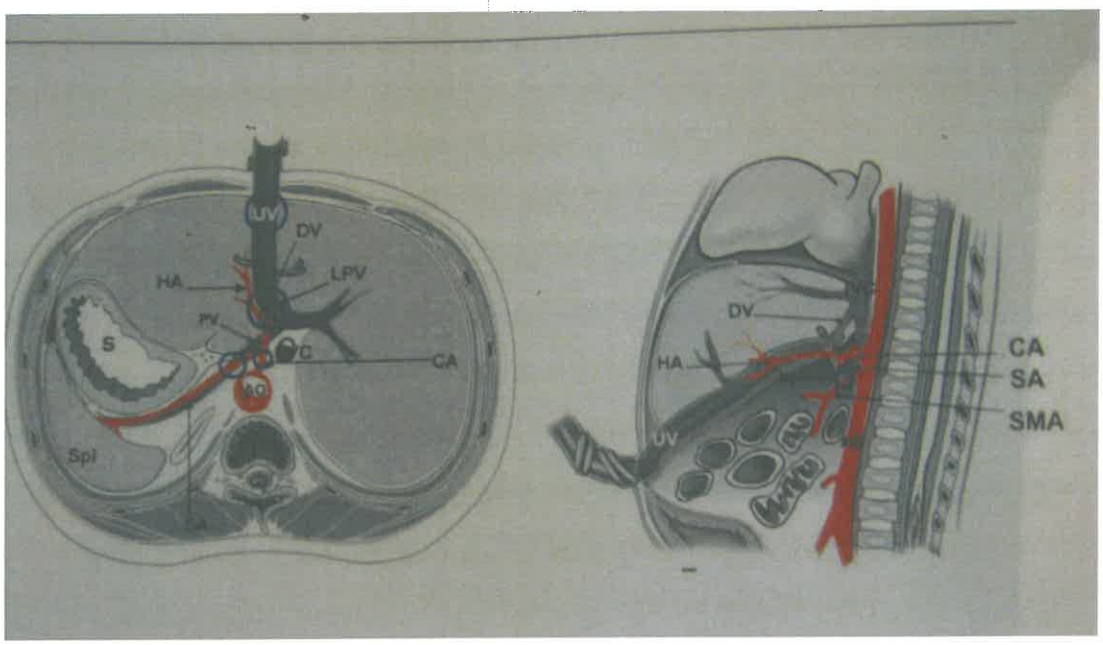


Figure N 3 Left. Oblique horizontal section of the upper fetal abdomen. Right. Sagittal section of the fetal trunk. AO: aorta; CA: celiac artery; IVC: inferior vena cava; SA: splenic artery; HA: left hepatic artery; SMA: superior mesenteric artery; *DV*: *ductus venosus*; UV: umbilical vein; PV: portal vein; LPV: left portal vein; Spl: spleen; S: stomach. (Adapted from C.Ebbing, 2008)

The middle cerebral artery (*ACM*) is the cerebral vessel of choice for Doppler assessment. The *ACM* supplies 80% of the cerebrum. Assessment of the flow velocity and PI of the *ACM* has become the integral part of the monitoring of fetuses with placental compromise (*Mari and Deter, 1992*).

The splenic artery (SA) courses on the cranial border of the pancreas to the spleen. It supplies the pancreas, stomach and spleen and is a major contributor to hepatic portal flow. Stress induces splenic contraction (*Aoki et al., 1992*) and in adults, this is considered to be a compensatory mechanism to increase the venous return from the spleen to the central circulation during stress (*Froelich et al., 1988)*; *see Figure N 3*.

Hemodynamic aspects of fetal growth restriction

The etiology and pathogenesis of FGR is complex and not completely understood. However, morphological changes in the placenta are well documented (Mayhew et al. 2003). This results in a progressive impairment of placental function and an increase of resistance in the placental capillary bed, which is reflected in changes of the flow velocity pattern in the umbilical artery. As the capillary bed is reduced by more than 50%, enddiastolic blood flow in the umbilical artery becomes absent or reversed (*Giles et al., 1985*).

As a consequence of increased placental resistance, umbilical venous blood flow reduced (*Boito et al., 2002; Di Naro et al., 2002; Kiserud et al., 2006a*) in a graded fashion depending on the severity of placental compromise (*Dubiel, 2003a; Kiserud et al. 2006b*). The vascular shunts on the venous and vascular sides enable the fetus to rearrange the circulation in order to compensate the deficit of nutrient and oxygen supply and to give priority to the vital organs: myocardium and brain. During experimental hypoxia and hypovolemia, the *DV* shunting fraction increases (*Edelstone et al., 1980*), and an increased degree of shunting is seen in fetuses with placental compromise and intrauterine growth restriction (*Tchirikov et al., 1998; Bellotti et al., 2004; Kiserud et al., 2006a*). However, *DV* shunting increases at the expense of the liver perfusion with potential short and long term consequences affecting liver growth and function. (*Tchirikov et al., 2002*). The findings of the Norvegian scientific group (*Kessler, 2009*) suggested that this would affect predominantly the right lobe. Furthermore, exposure of the liver to hypoxia *per se* may also induce reduced fetal growth: human hepatocytes cultured in hypoxic conditions increase their expression of IGF binding proteins, which inhibits the IGF action (*Popovici,*

2001). Impaired liver growth has been previously demonstrated in human FGR (*Boito, 2003; Latini, 2004*).

Furthermore, increased *DV* shunting may shift the umbilical-portal watershed towards the LeLL and finally reverse the blood flow direction in the LPV (*Kilavuz et al., 2003; Kiserud et al. 2003; Belloti et al., 2004*).

Redistribution to cerebral circulation is seen as a response to hypoxia (*Jensen et al., 1991; Cheema, 2006),* and low PI in the middle cerebral artery is associated with low fetal ρO_2 (*Vyas et al., 1990*). The effect is termed "brain-sparing": an increase in the diastolic flow velocity and reduced downstream vascular impedance (*Wladimiroff et al., 1986*). Resent studies showed that cerebral resistance gradually declines with reduced growth, also before the stage of apparent growth restriction (*Verburg et al., 2008*).

FGR restriction affects the flow velocity and PI of the fetal SA. Doppler assessment of the SA provides information on the risk of adverse outcome in high-risk pregnancies (*Capponi et al., 1997; Bahado-Singh et al,. 2000*). Reference ranges based on longitudinal data have been established for the flow velocity and PI of the fetal SA. (Ebbing, 2008). Relationships between fetal AS and local or general hemodynamic have not been extensively explored.

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.

2.4. Ultrasound physics

Ultrasound has frequencies above the audible range of the human ear, *i.e.* beyond 15-20 kHz. Diagnostic medical ultrasound normally operates in the range of 2-10 MHz for transcutaneous and transvaginal imaging. A source of ultrasound, a transducer, is placed in contact with the skin or in the vagina and ultrasound pulses are sent into the body. As the waves travel into tissues, they are reflected and scattered, generating echoes. Some of the echoes travel back to the transducer, where they are detected. Echoes detected by the transducer are used to generate the image.

Doppler ultrasound is used to evaluate blood flow quantitatively and qualitatively. Christian A. Doppler first described the Doppler effect in 1842. It is defined as the observed changes in the frequency of transmitted waves when a relative motion exists

between the sources of the wave and the observer. In diagnostic Doppler ultrasound, the red blood cells act as moving receivers and as moving sources. Different Doppler techniques may be applied in diagnostic ultrasound, such as pulsed-wave Doppler, colour Doppler and continuous-wave Doppler. The pulsed-wave Doppler and colour Doppler techniques will be discussed here since these modes were applied in this study.

Pulsed-wave Doppler technique

Pulsed-wave Doppler provides the ability to select Doppler signals from specific depth. Short pulses of ultrasound are sent out and received. Each Doppler signal is sampled once for every pulse transmission, and the time interval for the pulse transmission and reception determines the distance or range of the target area from the transducer. Spectral analysis is a quantitative analysis showing the distribution of frequencies (*i.e.*, velocities) to be presented as a function of time.

Colour Doppler technique

Colour flow imaging combines data from moving reflectors (the Doppler shifts) with gray-scale images and provides anatomical details along with information on flow (high or low velocities and direction).

2.5. Ultrasound safety

Since US is a form of energy, it has the potential to produce biological effects that can constitute a health risk. Theoretically, US could produce harmful effects through sonically generated heat, cavitation and radiation force (*Stratmeyer, 1982a*). Animal studies suggest that US may produce adverse effects in the neurological, immunological, hematological, developmental and genetic status of the exposed fetuses *(Stratmeyer, 1982b*). However, human epidemiology studies fail to show adverse outcome in fetuses exposed to ultrasound (*Itskovitz and Israel, 2005*) and according to a WHO conducted systematic review and meta-analysis, exposure to diagnostic ultrasonography during pregnancy appears to be safe (*Torloni, 2009*).

The international guidelines based on the ALARA (as low as reasonably achievable) principle underscore the operators' responsibility to use ultrasound prudently (*Barnett, 2000; Barnett, 2001*)

3. REVIEW OF THE LITERATURE

Of the estimated four million neonatal deaths worldwide each year, over 60% are associated with low birth weight due to intrauterine fetal growth restriction and/or preterm delivery (*Lawn, 2005*). According to the definition of the American College of Obstetricians and Gynecologists (*ACOG, 2001*), intrauterine growth restricted fetuses are those who "fail to reach their potential growth" and are defined as having a birth weight below the given the 10th percentile for gestational age. FGR is mostly detected by a diminished growth velocity of the fetus on serial ultrasound examinations with the AC measurement deviating 10% or more from the expected growth curve.

3.1. Maternal factors

A number of risk factors have been identified, including maternal smoking (Andres, 2000), low maternal size/weight gain, primiparity, race, and hypertension in pregnancy, infections, alcohol and other drugs (*Maina, 2008; Abeysena, 2009; Seror, 2009; Romo, 2009*).

3.1.1. Socioeconomic determinants

An Australian study (*Beard et al. 2009*) analyzed the data of 877,951 singleton births occurring in New South Wales between 1994 and 2004. They included pregnancies between 22 and 43 weeks gestation without congenital malformations. Multilevel models were developed to determine the factors associated with babies weighing less than the 3rd percentile for gestational age and gender. Beard *et al.* found that the risk of SGA increased with increasing socioeconomic disadvantage. Smoking in their study accounted for approximately 40% of the increased risk, and delayed antenatal care approximately 5%. The risk of SGA in mothers living in the most disadvantaged areas increased from 1.7 to 2.2. Mothers over 35 were at increased risk. Children born in autumn were also at risk.

Rachdi *et al.* (2005) defined the etiological factors in relation to low birth weight. They conducted a retrospective study of 124 cases at the military hospital of Tunis from 1999 to 2001. Forty seven percent of the pregnant women in the study were primiparous. The mean

age of patients was 30 years. The etiology was predominantly renovascular syndromes, urinary infections, and idiopathic hypotrophy.

In 2006 a Canadian group (*Dubois et al., 2006*) published the results of the multivariate analyses of 2103 children born in 1998 in Quebec. The mean birth weight was higher for boys than girls. The birth weight improved with birth rank, mother's body mass index and family socioeconomic status; and was lower for children of smoking mothers. When maternal smoking status and mother's body mass index were combined, socioeconomic status had a positive effect on mean birth weight. Except for overweight or obese smoking mothers, among whom the relationship between socioeconomic status and mean birth weight was reversed.

In the Frisbie *et al.* (1997) study, intrauterine growth restriction was significantly more likely to occur among women who relied on government aid to pay for delivery, those who experienced vaginal bleeding, those who smoked during pregnancy and who were giving birth to their first child. They acquired information via an extensive questionnaire completed by a sample of mothers who gave birth in 1998. The chances of intrauterine growth restriction were significantly lower for women who received the support of a Special Supplemental Food Program for Women, Infants and Children, and for those who gained 41 (18.6 kg) or more pounds during pregnancy.

McCowan (2009) described the factors associated with SGA babies which included short stature, low weight, Asian or Indian ethnicity and primiparity. According to the authors, reduced risk of SGA or increased birth weight included high maternal milk consumption and high intakes of green leafy vegetables and fruit.

Romo *et al.* (2009) evaluated multiple factors as possible contributors to the FGR risk: race, parents' age, mother's height, mother's birth weight and her weight before pregnancy, ponderal gain and blood pressure during pregnancy and previous SGA. Social class, parent's profession, habitual residence, salary and diet were also evaluated. In their study, the most significant etiological factors were: active and passive tobacco consumption, mother's stress level, increase of total months worked during pregnancy, total daily working hours and the time mother spent standing up and finally, the parent's height. The authors recommend the exclusion of tobacco in all forms, including passive smoking, and improving the working conditions of the pregnant mothers, lowering the number of daily hours worked and trying to avoid and cope with stressful situations.

Thompson-Chagoyan (2008) through a case-controlled study in 376 neonates identified the risk factors implicated in the growth-restricted neonates born in a social security

institution in Mexico. Low weight gain at pregnancy was among 30 variables of socioeconomic disadvantage related to the risk of FGR in the population.

Another study from Iran was published in 2008 (*Vahdaninia, 2008*). They conducted a retrospective study using data from 15 maternity hospitals in Tehran. Data on all singleton term births in these hospitals were extracted from case records during one calendar year. Study variables included maternal age, maternal educational level, history of LBW deliveries, history of preterm labor, cigarette smoking during pregnancy, and number of deliveries, chronic diseases and residential area. They found that significant risk factors for LBW were: history of LBW deliveries, smoking during pregnancy and chronic diseases.

Interpregnancy interval appeared to affect fetal intrauterine growth and may be an independent risk factor for low birth weight. An interval of 18-23 months may be the optimal time between pregnancies (Simeonsson, 2003). Extreme interpregnancy intervals (less than 17 month) and long interpregnancy intervals (more than 60 months) have the highest risk for low and very low birth weight outcomes (Zhu et a., 1999; Fuentes-Afflick and Hessol 2000).

3.1.2. Chronic diseases and pregnancy complications, infections and gynecological abnormalities

Maternal chronic diseases may interfere with fetal growth. Baptiste-Roberts *et al.* in 2008 published data on maternal factors for placental weight, placental thickness and chorionic plate area. They conducted an analysis of 24,135 mother-placenta pairs enrolled in the National Collaborative Perinatal Project. The researchers observed an increased likelihood of growth restriction for placental weight and chorionic plate area among mothers with hypertensive disease at or beyond 24 weeks. At the same time anemia was associated with a reduced likelihood of growth restriction for placental weight and chorionic plate area. Pre-pregnancy body mass index and pregnancy weight gain in their study were associated with a reduced likelihood of growth restriction and an increased likelihood of hypertrophy for all three dimensions of placental growth.

Chou *et al.* (2009) assessed the perinatal outcomes in neonates born to mothers with Antiphospholipid syndrome (APS). Between January 1997 and July 2007 maternal and perinatal histories including demographic data, medications, obstetric histories, and neonatal clinical manifestations and laboratory data were analyzed. Eleven women

diagnosed with primary APS were included. Among the pregnancies, two had intrauterine growth retardation (18.1%). Researchers suggested that neonates born to mothers with primary APS are at risk being small for their gestational age.

Thyroid disorders are among the common endocrine problems of pregnant women. Women with hypothyroidism are more likely to deliver low birth weight babies than those in the general population (Leung, 1993; Idris, 2005) Overt hypothyroidism was associated with pregnancy-induced hypertension, intrauterine growth restriction and intrauterine demise as compared to control (Sahu, 2010).

Some studies aimed to determine whether there is an association between urinary tract infections (UTI) during pregnancy, among patients in whom antibiotic treatment was recommended. Recently Mazor-Dray *et al.* (2009) published the results of the retrospective population-based study comparing all singleton pregnancies of patients with and without UTI and maternal and perinatal outcome. They found that patients with UTI had significantly higher rates of intrauterine growth restriction and pre-eclampsia.

Hypertensive disorders are present in 30-40% of pregnancies complicated with FGR. These conditions include pre-eclampsia, chronic hypertension with or without superimposed pre-eclampsia, and pregestational diabetes complicated with vasculopathy. Pre-eclampsia was found to be associated with a four-fold increase in the risks of IUGR development in the fetus (*Odegård, 2000; Zupan-Simunek, 2010*), The greater the severity and the earlier the onset of pre-eclampsia, the lower the birth weight (Long, 1980). Mild chronic hypertension in pregnancy with or without proteinuria was associated with variable increases (8-40%) in the birth of SGA infants (*Sibai, 2005; Gilbert, 2007*).

An abruption of the placenta is an important cause of vaginal bleeding in the latter half of pregnancy. Once the placental tissue has separated from the uterine lining, that portion of the placenta can no longer receive effective uteroplacental blood flow, reducing fetoplacental oxygen availability (Wigglesworth, 1991). It may independently relate to FGR or associates with pre-eclampsia (Hall, 2009). Some studies showed three-fold increased risk of abruption associated with the fetus being small for gestational age (*Ananth, 1999; Lindqvist, 2006*), another study found no association between abruption and small for gestational age births (*Nath, 2008*)

In 1995 Hillier *et al.* (*1995*) reported the association between bacterial vaginosis (BV) and preterm delivery of a LBW infant. In this cohort study, they enrolled 10,397 pregnant women from seven medical centers without known medical risk factors for preterm delivery. At 23 to 26 weeks' gestation, bacterial vaginosis was determined to be present or

absent on the basis of the vaginal pH and the results of Gram's staining. Bacterial vaginosis was detected in 16 % of the 10,397 women. The women with bacterial vaginosis were more likely to be unmarried, to be black, to have low incomes, and to have previously delivered low birth weight infants. In a multivariate analysis, the presence of bacterial vaginosis was related to preterm delivery of a low birth weight infant. Other risk factors that were significantly associated with such deliveries in this population were the previous delivery of a low birth weight infant, the loss of an earlier pregnancy, primigravidity, smoking, and black race. Among women with bacterial vaginosis, the highest risk of preterm delivery of a low birth weight infant was found among those with both vaginal bacteroides and *Mycoplasma hominis*. The authors concluded that bacterial vaginosis was associated with the preterm delivery of low birth weight infants independently of other risk factors.

Donders *et al.* (2008) have taken Pap smears and vaginal cultures from 222 pregnant women at their first prenatal visit and compared these with the pregnancy outcome. The total score, reflecting the health of the vaginal microflora, was termed "Vaginal Ecology Score" (VECO score). The finding of epithelial cellular abnormalities resulted in lower birth weight in their study. The fetal head circumference was also lower in the group with high VECO-scores. Its connection with decreased birth weight suggests prematurity rather than dysmaturity to be the cause of lower birth weight due to the "brain-sparing" effect. The authors concluded that intrauterine growth retardation would be marked by a reversed abdomen to head circumference ratio.

Recent findings that periodontal diseases may lead to small for gestational age births provide a modifiable etiology that could potentially reduce the frequency of FGR. In a prospective longitudinal study (*Dasanayake, 2001*) it was shown that those with higher levels of *Porphyromonas gingivalis*-specific IgG at midtrimester had higher risk of giving birth to low-birth-weight infants. These data were confirmed by another group from Western Australia that studied 10 000 pregnant women. They found that the disease is often not diagnosed and has been associated with preterm birth, small for gestational age newborns, and pre-eclampsia. They also had shown in the smaller number of women, that treatment of periodontal disease might reduce the rate of preterm birth.

Association of maternal human immunodeficiency virus (HIV) infection with low birth weight in their offspring was studied during the last decade. There were five times more low-birth-weight infants in the HIV seropositive group than in the controls in the Iroha *et al.* study (2007). It appeared that severity of maternal HIV disease as indicated by the CD4 count predicts FGR (*Iqbal, 2010*).

A few reports have been found in the literature concerning FGR and gynecological abnormalities (Akar, 2005; Reichman, 2009). Heinonen *et al.* (2000) evaluated reproductive performance of women with didelphic uterus and its possible long-term consequences associated with this uterine anomaly. The presence of other anomalies, gynecologic disorders, fertility and outcome of pregnancies were reviewed. The long-term clinical implications associated with a didelphic uterus were evaluated during the follow-up period of 9.1 years. Heinonen's study revealed 75% of fetal survival rate, prematurity in 24%, fetal growth restriction in 11%, perinatal mortality in 5.3% and cesarean section rate in 84% of cases.

3.1.3. Therapeutic agents

The associations between prenatal exposure some of the medical agents and fetal growth restriction are controversial and poorly understood (*Magee, 2000; Pennell, 2002 Toh, 2009*). Tendon *et al.* (2002) reviewed the epidemiological and clinical studies concerning *in utero* exposure to immunosuppressive (IS) drugs. These IS drugs cross the placental barrier and enter into the fetal circulation, which poses a risk for fetal development. Experimental data have shown that IS drugs often have deleterious effects on fetuses, while human data have reported an increased rate of abortion, prematurity, intrauterine growth retardation and low birth weight, without significant increases in malformation rates. The authors concerns are that long-term effects of IS drugs on fertility, immune response and renal function, as well as the consequences of prematurity and IUGR, should be monitored in spite of reassuring fetal and neonatal data.

Another group from USA (*Tabakoba et al. 2003*) evaluated human and animal evidence of the ß-blocker Atenolol developmental toxicity. They proved manifestations of Atenolol prenatal toxicity such as placental changes, intrauterine growth retardation and changes in fetal weight in the absence of structural malformations and recommended its use following the risk/benefit consideration.

3.1.4. Lifestyle determinants

Numerous prior studies supported the association between smoking and fetal growth (*Anres, 2000; Suzuki, 2008; Król, 2009*). Chechowska *et al.* (2006) estimated the effect of tobacco smoking on serum nitric oxide (NO) concentration in 40 healthy pregnant women and umbilical cord blood and birth weight. They also examined the relation between serum

NO and number of cigarettes consumed by the mother. The current smokers were defined as those who had smoked five cigarettes per day for two years before conception and continued to smoke during pregnancy. The analysis revealed negative correlation between the number of cigarettes consumed and serum nitric oxide in smoking women as well as in their children. Birth weight in infants born of smoking mothers was lower in average by 260 g as compared with non-smoking ones. The authors indicated that tobacco smoking during pregnancy reduced serum nitric oxide concentrations in mothers and their children, and correlated with number of cigarettes daily consumed. In smoking women, lower concentrations of NO co-exist with smaller birth weight than in tobacco abstinent, which may suggest a correlation between these parameters.

Chiolero *et al.* (2005) gathered data for all births in the Canton of Vaud, Switzerland over a twelve-month period from 1993 to 1994. Of a total of 6284 singleton births, 303 (4.8%) were LBW, and 731 (11.7%) were SGA. Mean birth weight, adjusted for maternal age, parity, parents' occupation and neonates' sex and nationality, was lower by 190 g in babies of smokers than those of non-smokers. Comparing smokers to non-smokers in their study, the likelihood was 2.7 times higher for LBW, and 2.1 for SGA. According to the authors, maternal smoking during pregnancy accounted for 22% of all LBW babies in the population and 14% -of SGA.

Shancaran *et al.* (2006) examined the association between intrauterine growth restriction status at birth among full-term infants, exposure to substance use during pregnancy, and risk of hypertension at six years of age. They enrolled 1,388 infants in the study (600 cocaine exposed, 781 nonexposed, and seven indeterminate, matched by gestational age, race, and sex). Nine hundred fifty children (415 exposed, 535 nonexposed) were followed up for six years. They revealed that 144 (28%) of the 516 children had a diagnosis of IUGR at birth. Of them 35 (24%) had hypertension, compared with 58 (16%) of 372 children without IUGR. Intrauterine growth restriction status at birth was significantly associated with hypertension.

3.2. Fetal factors

3.2.1. Aneuploidy and fetal malformations

Fetal chromosomal abnormalities are strongly associated with fetal growth restriction (*Hall, 2010*). Recently, Robertson *et al.* (2009) published data on comparative genomic hybridization from each of 61 placentas referred with maternal pre-eclampsia and/or IUGR, and 85-control placenta. Trisomy was observed in four placentas between IUGR group (n = 43). None of the 84 control placentas showed mosaic trisomy. The authors confirmed the necessity of testing using uncultured cells from multiple placental biopsies for the accurate diagnosis of trisomy mosaicism in a case of severe IUGR.

Dashe *at al.* (2000) demonstrated that almost 14 % of symmetrical SGA had anomalies compared to the 4% of symmetrical SGA infants. They investigated this issue among 1,364 infants who were SGA and 3,873 infants who were in the 25-75th percentile Apropriate for Gestational Age (AGA). Earlier studies (*Khoury, 1988*) demonstrated that over 22% of infants with congenital malformations are growth restricted with the relative risk 2.6. Multiple malformations increased from 20% in infants having two defects to 60% in those having nine or more defects. In the last study there is a predominance of heart defects, anencephaly, and abdominal wall defects. The cardiac defects more often associated with SGA include Tetralogy of Fallot, hypoplastic left heart, pulmonary stenosis, and ventricular septal defect.

In another study (*Snijders, 1993*) blood karyotyping was performed on 458 fetuses that were referred for further assessment of growth retardation at 17 to 39 weeks' gestation in Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, in London. The fetal karyotype was abnormal in 89 (19%) of the cases. The most common chromosomal defects were triploidy and trisomy 18. Ninety-six percent of chromosomally abnormal fetuses had multisystem fetal defects that were characteristic for the different types of chromosomal abnormalities.

3.2.2. Perinatal infections

Toxoplasma gondii, parvovirus B19 and cytomegalovirus (CMV) cause most of the non-bacterial infections of the fetus. These infections are complicated by the fact that maternal toxoplasmosis and cytomegalovirus infections are almost always symptomless (*Marcinek, 2008; Anna, 2010*). Congenital toxoplasmosis is the cause of hydrocephalus,

chorioretinitis and intracranial calcifications and associated with growth restriction. Researchers tried hard to find the explanation of slower fetal development in infected women. Chronic pathogenic processes can affect the circulation of blood in the uterus and maternal part of the placenta with consequent fetal malnutrition (*Moore, 2003; Kankova, 2007*). A recent retrospective study on congenital CMV infection (*Pinillos-Pisón, 2009*) has shown that screening neonatal test by detecting DNA in blood from the filter paper should be considered in the presence of severe symptoms and different clinical pictures, such as delayed intrauterine growth, microcephaly, intracranial calcifications and/or cortical dysplasia and malformations of the temporal lobe or the hippocampus.

A prospective study was undertaken by Deorari *et al.* (2000) on the incidence of intrauterine infections by screening 1,302 cord blood samples for total IgM by radial immunodiffusion. Specific IgM against cytomegalovirus, rubella and *Toxoplasma gondii* were estimated in cord blood samples found to contain total IgM > 20 mg/dl. All these neonates were examined at birth and at discharge. Cord blood samples with total IgM > 20 mg/dl were further screened for specific IgM against rubella, CMV and *Toxoplasma gondii*. Incidence of prematurity and low birth weight were not statistically different in babies with raised cord blood IgM when compared to those with low cord blood IgM levels. The incidence of idiopathic intrauterine growth restriction was similar in two groups as well.

3.2.3. Preterm deliveries

An association between fetal growth restriction and prematurity has been recognized (*Lackman, 2001; Zaw, 2003*). Bukowski *et al.* (2001) tested the hypothesis that fetuses destined to deliver preterm do not reach their individual growth potential. In a case control study on 44 preterm deliveries before 35weeks that were compared with control group of successive consecutive term deliveries, they observed a significantly higher number of fetuses with birth weight under the 10^{th} percentile in the preterm group compared with that in the control group. There were no significant differences in variables defining growth potential between the case and control groups.

Recently Espinoza *et al.* (2007) compared the frequency of small for gestational age neonates among patients with an episode of increased uterine contractility that delivered at term and those who delivered preterm. They included in the retrospective cohort study 849 patients. Inclusion criteria were 1) regular uterine contractions that required

hospitalization, 2) intact membranes, and 3) gestational age between 20 and 36 weeks. According to the results, patients who delivered at term had a significantly higher number of SGA neonates than those who delivered preterm. They found that term delivery after an episode of regular preterm uterine contractions was associated with an increased risk to deliver an SGA neonate.

3.3. Management and outcomes

3.3.1. Intervention

Fetal growth restriction constitutes one of the major complications of pregnancy associated with an increased risk of perinatal mortality and morbidity, iatrogenic prematurity. Fetal growth restriction is associated with both intrapartum and neonatal complications. Up to 50% of growth restricted fetuses exhibit abnormal heart rate patterns during labor, and such fetuses have an increased cesarean delivery rate. According to the American College of Obstetricians and Gynecologists (*ACOG, 2001*), the general approach to management of the fetus with ultrasonographically suspected growth restriction is risk factor modification when possible, and antepartum surveillance, ultrasonography and delivery when the risks of continued *in utero* development outweigh the benefits. Still, timing and mode of delivery for growth-restricted fetuses are controversial issues. Currently, we can quite precisely depict the fetal effect of placental failure. But because there is no effective therapy for the failing placenta, we are left with only two management options: to wait for organ maturation, accepting an increasing risk of fetal acidemia and stillbirth, or to deliver and accept the risk of permanent injury from prematurity (*Baschat, 2007; Brodszki, 2009; Maslovitz, 2009*).

A randomized trial in 69 hospitals in 13 European countries on 548 women (*Grit Study Group, 2003*) compared the effect of delivering early to pre-empt terminal hypoxemia with delaying for as long as possible to increase maturity. The intervention was "immediate delivery" or "delay until the obstetrician is no longer uncertain". They did not find any difference in overall mortality (total death was 10% in the immediate vs. 9% in delay group). The median time-to-delivery was 0.9 days in the immediate group and 4.9 days in the delay group. Total cesarean sections rate was 91% vs. 79% in the delay group.

Later, a group from the Netherlands (*Van den Hove, 2006*) tested the hypothesis that in pregnancies at term complicated by fetal growth restriction, induction of labor is as safe as expectant management. 33 women with a clinically suspected FGR were randomly

allocated. The gestational age of induction group was lower, 38 vs. 40 weeks, compared to the expectant group with a tendency to the lower birth weight (2428 g vs. 2651g), but on the other hand there was reduced need for antepartum surveilllance. They did not find any significant difference in obstetrical interventions and neonatal morbidity (50% vs. 35%).

Ben-Haroush *et al.* (2004) from Israel evaluated the mode of delivery and immediate neonatal outcome in pregnancies with suspected fetal growth restriction and normal antenatal assessment following induction of labor with vaginal application of prostaglandin E2 (PGE2). Ninety women with suspected FGR having normal oxytocin contraction test, biophysical profile, and reassuring fetal heart rate underwent induction of labor with vaginal application of PGE2 tablets. The findings were compared with 115 women admitted for induction of labor because of decreased fetal movement and with 510 women with normal spontaneous onset of labor. The rate of cesarean section in the study group was similar to the rate in both other groups (8.9% vs. 14.8% and 9.0%, respectively). The incidence of non-reassuring fetal heart rate pattern leading to cesarean delivery was higher in the FGR group, but the rate of low 5-min Apgar scores (<7) was similar in all groups.

Another group from Israel (*Maslovitz, 2008*) reviewed the computerized files of 836 parturients who underwent inducted labor because of IUGR and compared these parameters to 711 control patients whose deliveries were induced for other medical reasons. Forty three percent of FGR women have been delivered by non-elective cesarean section. Growth restricted neonates born after labor induction had higher rates of low Apgar scores and NICU admissions compared to growth-restricted neonates delivered by elective CS. The researchers suggest re-considering the vaginal route of delivery in FGR cases.

Results of the new study on 126-vertex singleton birth were presented recently during 58 th annual ACOG meeting. The researchers analyzed patients admitted between 1998 and 2003. The mode of delivery does not affect the neonatal morbidity or mortality with gestational age ranged from 23 to 30 weeks. The neonatal death rate and rates of neonatal sepsis, necrotizing enterocolitis, and respiratory distress syndrome did not differ significantly between groups. There was a higher rate of intraventricular hemorrhage in the vaginal group, although the authors concluded this did not reach statistical significance (*Uxer, 2010*).

3.3.2. Neonatal outcomes

Fetal growth restriction is associated with prematurity and a number of complications, such as poor neurodevelopment and childhood and adult disease (*Henriksen, 1999; Procianoy, 2009; Fortes Filho, 2009; Evensen, 2009*).

Garite *et al.* (2004) reviewed the date of 29, 916 singleton neonates who were born between 23 and 34 weeks. They compared the newborn infants with and without growth restriction. 22,790 neonates without any markers for growth restriction were used as controls. Up to 4.8% of their sample population was reported to have an antenatal diagnosis of FGR. Neonates with all categories of growth restriction were more likely to die, have necrotizing colitis, and require respiratory support at 28 days of age. The occurrence of severe intraventricular hemorrhage was similar between the groups. According to the researchers, poor intrauterine growth at all gestational ages up to 32 weeks was associated with increased mortality and morbidity with adverse long-term problems.

A Swedish group (*Brodszki et al., 2009*) described the outcome of FGR fetuses with absent or reversed end-diastolic flow in the umbilical artery delivered on the fetal indication before 30 weeks of gestation. They reviewed the data on 42 live born fetuses with FGR and ARED in the umbilical artery, who were born between 1998 and 2004. They compared the data with 413 control newborns. In this study, one infant died in the neonatal period, and three during the first year of postnatal life (90% of two-year survival). The incidence of chronic lung diseases was higher in ARED group than in controls. There were no differences between the groups in the occurrence of necrotizing enterocolitis, cerebral hemorrhage or retinopathy of prematurity. According to this study, severly preterm growth restricted fetuses with ARED showed high two-year survival and low morbidity.

Bachat et al. (2009) in prospective observational study including 113 pregnancies complicated by intrauterine growth restriction evaluated different variables (birth weight and neonatal morbidity, including bronchopulmonary dysplasia, intraventricular hemorrhage, or necrotizing enterocolitis) with a two-year neurodevelopmental delay. There were 38 (52.8%) infants with neurodevelopmental delay, 20 (27.8%) with speech delay, 23 (31.9%) with an abnormal neurological examination, eight (11.1%) with a hearing deficit and six (8.3%) with cerebral palsy. They concluded that gestational age and birth weight remain the predominant factors for poor neurodevelopment in growth restricted infants with *AU*-ARED as an independent contributor to poor neurodevelopment in IUGR.

3.4. Monitoring of fetal growth and health

Effective monitoring of fetal growth is of major importance in antenatal care. A primary role of obstetric ultrasound is to determine gestational age and to assess fetal growth. Assessment of fetal growth should not be viewed in isolation. Other methods for assessing fetal health include fetal cardiotohography, assessment of amniotic fluid, the biophysical profile, and Doppler studies (*Scifres, 2009; Korzun, 2002; Gudmundsson, 2001*)

3.4.1. Assessment of fetal growth

According to the practice bulletin (*ACOG, 2001*), the two steps involved in antenatal recognition of FGR are elucidation of maternal risk factors with clinical assessment of uterine size, including abdominal palpitation, measurement of symphyseal fundal height, ultrasound biometry, and ultrasound estimated fetal weight and ultrasound Doppler flow velocimetry. Standard assessment relies on ultrasound measurement of the head, abdomen, and one or more long bones. From these measurements, the fetal weight could be estimated and compared with the expected weight. Fetal weight estimation is thought to be helpful for making effective management decisions regarding very low birth weight group (*Medchill, 1991; Nyberg, 2004*; *Bluemenkampt, 2005*).

In high-risk women, AC at less than the tenth percentile has sensitivities of 73-95% and specificities of 51-84% in the prediction of fetuses with birth weight at less than the tenth percentile. The respective figures for EFW are sensitivities of 33-89%, and specificities of 54-91% (*Royal College of Obstetrician and Gynecologists, 2002*). Dudley (2004) concluded in the systematic review on the ultrasound estimation of fetal weight that no consistently superior method has emerged. Four databases were searched for studies comparing ultrasound estimated fetal weight with birth weight. The volumetric methods provide some theoretical advantages. Analyses of a large Swedish (*Clausson, 2001*) dataset between 1992 and 1995 (n = 326,377) showed that SGA defined by a customized birth weight percentile was more closely associated with stillbirth, neonatal death or low Apgar scores than the unadjusted population percentile. This study concludes that customized birth weight standards improve identification of fetuses at risk of stillbirth, neonatal death and Apgar score fewer than 4 at 5 minutes, probably due to more precise identification of fetal growth restriction.

3.4.2. Assessment of fetal hemodynamic (surveillance tests)

Prenatal identification of isolated placental dysfunction as a cause of fetal growth restriction is critical. Ultrasound biometry documenting small fetal size implies placental dysfunction, while Doppler ultrasonography directly demonstrates placental vascular abnormality. Two studies showed the evidence of association between first trimester uterine artery Doppler resistance indices (RI) and the subsequent development of FGR (*Albaiges, 2003; Melchiorre, 2009*) and proved the hemodynamic changes in uteroplacental circulation.

The combination of a small fetal abdominal circumference with elevated umbilical artery Doppler blood flow resistance provides the most specific diagnosis of placenta-based fetal growth restriction (*Brodszki, 2002; Dubiel, 2003a).* Doppler evaluation of fetal cerebral and venous circulation has amplified our understanding of placental dysfunction, providing evidence that placenta-based fetal growth restriction manifests progressive cardiovascular signs heralding fetal acidemia and stillbirth (*Dubiel, 2002; Ferrazzi, 2002; Dubiel, 2003b; Cheema, 2004;).* Doppler surveillance of the fetal circulation represents an important tool for management of the growth-restricted fetus and provides the information about fetal circulation characteristics *(Korzun, 2002; Harman, 2003).*

Cosmi *et al.* (2005) published data on 145 singleton growth restricted fetuses with abnormal *AU* PI. They assessed the Doppler studies of *AU, ACM, DV*, and amniotic fluids index of fetuses with idiopathic growth restriction during the four-year period. In their report there were 4 fetal and 50 neonatal deaths. There were no significant differences between fetuses with abnormal BPP or non-reasuring nonstress test (NST) and those with all normal measures. Neonatal death was increased in fetuses with *AU* reversed flow and *DV* reversed flow. The conclusions were that in fetuses with idiopathic growth restriction, low birth weight, *AU* and *DV* reversed or absent flow are associated with an increased perinatal morbidity and mortality.

Bachat *et al.* (2007) in their prospective multicenter study identified specific predictors for neonatal morbidity and mortality in early onset fetal growth restriction. From January 2000 to March 2006, 604 patients from 12 academic perinatal centers were included. Total mortality was 21.5%, and 58.3% survived without complications. *DV* Doppler parameters and cord artery pH predicted neonatal mortality, and *DV* Doppler alone predicted intact survival.

In 2007 a group from Michigan (*Mari et al., 2007*) proposed the classification of staging based on severity of Doppler changes of IUGR fetuses delivered at 32 weeks or earlier. 74 singleton fetuses were included and Doppler studies of *AU,* UV, *ACM,* and DV were obtained within 72 hours before delivery. Perinatal outcome end points included perinatal and total mortality. There was a direct correlation between the stage and both perinatal mortality and mortality before neonates were discharged from the hospital. This new staging system allows comparison of outcome data for IUGR fetuses and may be valuable in determining more timely delivery for these high-risk fetuses. One year later Mari and Picconi (2008) assessed the new Doppler parameters in a case of IUGR. They performed a cross-sectional and a longitudinal assessment of the *ACM*-PI and *ACM*-PSV. Their data showed that the *ACM*-PSV predicts perinatal mortality more accurately than the *ACM*-PI. This finding reflects hypoxemia and hypercapnia, and thus the brain auto-regulation.

Another staging system was proposed by researchers from Sweden (*Ghosh, 2009*). They compared umbilical and uterine artery Doppler in predicting the outcome of pregnancies suspected of fetal growth restriction. 353 pregnancies suspected of FGR diagnosed by ultrasound fetal biometry during a five-year period were included in the prospective study. There was a statistically significant correlation between abnormal Doppler of both the umbilical and uterine arteries and adverse outcome of pregnancy. The two vessels were compared in predicting adverse outcome. Women with normal umbilical artery Doppler (251) were analyzed separately. Abnormal uterine artery Doppler, seen in 61 (24.3%) of those women, showed a statistically significant correlation for adverse outcome of pregnancy.

Kiserud *et al.* (2006b) determined the degree of *DV* shunting in fetuses with IUGR and the effect of various degrees of umbilical circulatory compromise. They performed the cross-sectional, observational study on 64 fetuses with IUGR. In fetuses with compromise average *DV* shunting was 39% compared with 25% in the reference group (212 low-risk pregnancies). Fetuses with normal *AU*-PI did not shunt significantly more than did the reference group. Those with *AU*- PI^{95th} and particularly those with ARED did shunt significantly more. The authors concluded that fetuses distributed correspondingly less umbilical blood to the liver in a case of hemodynamic compromise.

Bellotti *et al.* (2004) designed a cross-sectional study and included in it 56 IUGR fetuses and compared them to the 137 normal control fetuses. Researchers evaluated the changes in the distribution of the umbilical venous blood flow to the liver and the ductus

venosus in IUGR fetuses in relationship with dilatation of the *DV* inlet diameter. They found that the DV flow was increased significantly in IUGR fetuses compared with controls. As a consequence, *DV* shunting in IUGR was also increased. The percentage of blood flow to the right lobe showed a significant reduction, with the evidence of reversed flow from the portal system into *DV*. The same observations have been described earlier by Kilavuz *et al* (2003). They measured blood flow velocity in *AU*, the extra-abdominal umbilical vein, *DV*, and the first part of the left portal vein in 28 IUGR fetuses. Researchers suggest that absent or reversed flow in the *AU* may coincide with elevated resistance to blood flow in the right liver lobe and elevated pressure in the right atrium blood flow.

A few studies described splenic artery flow velocity waveforms in relation to the FGR fetuses. Abuhamad *et al.* (1995) prospectively obtained SA flow velocity waveforms from 95 appropriate and 15 growth-restricted fetuses. They found decreased resistance at the level of the splenic artery. In 14 of 15 compromised fetuses, the splenic artery resistance index was below the mean, and five (33%) had values <2SEMs. They explain this finding by the stimulating effect of hypoxemia on the erythropoietin and offered a new diagnostic tool in the management of the high-risk pregnancies. Later on Capponi *et al.* (1997) assessed the association between splenic artery flow waveforms and pH and blood gas levels in FGR fetuses, and compared their data with fetal arterial and venous vessels Doppler measurements in predicting acid-base status. 42 FGR and 316 AGA fetuses were included in the cross-sectional study. According to the results, splenic artery PI was lower in all FGR fetuses with abnormal *AU* PI, but not in the FGR group with normal *AU* PI. The amplitude of the decrease was significantly associated with fetal hypoxemia, academia and hypercapnia. At the same time authors agreed that the *ACM* and inferior vena cava are better predictors of hypoxia and acidosis.

Finally, some researchers tried to evaluate whether reduced fetal growth is associated with fetal circulatory changes and cardiac dysfunction (*Verburg, 2008*). Fetal circulation variables were assessed in 1,215 healthy women in a population-based, prospective cohort study. They found that decreased fetal growth is associated with adaptive fetal cardiovascular changes. This phenomenon began to occur before the clinically apparent fetal growth restriction and may contribute to the increased risk of cardiovascular disease later in life.

3.5. Placental factors

FGR is an important clinical problem. Among the known causes of FGR, there are few with no obvious fetal and maternal interfering factors. The placentas of these "idiopathic" intrauterine growth restricted babies might give a clue to the etiology of the growth retardation. Different placental factors may be involved, such as anatomical, vascular, chromosomal and/or morphological abnormalities (*Maulik, 2006b*).

Physiological studies on humans placentas demonstrate several features that are relevant to the understanding of placental disease and the possible development of improved noninvasive prenatal assessment of placental function. In the literature, gross morphological changes of placenta (*Heinonen, 2001; Fox, 2003; Biswas, 2008*), placental weight (*Pardi, 2001; Biswas, 2008)*, foetal/placental weight ratio, type of placental insertion of umbilical cord, length, diameters and presence of knots of the umbilical cord (*Osak, 1997; Sornes, 2000; Hitschold, 2001*) and structural abnormalities of placenta in cases of FGR have all been associated with fetal growth restriction (*Rayburn, 1989; Salafia, 1992; Salafia, 1995; Salafia, 2003; Wee, 2006; Egbor, 2006*).

3.5.1. Gross changes of placentas

Biswas *et al.* (2008) published data on 28 placentas delivered from FGR pregnancies and compared them with 22 control placentas. Gross examinations of full term singleton live FGR placentas were performed. The position of insertion of umbilical cords, placental weights, volumes and diameters were elaborately examined. This study revealed that the control placentas were bigger in diameter, with the normal insertion of umbilical cord. The placental weight and the volume were significantly lower in the case of FGR placentas with the more often abnormal position of the umbilical cord (marginal in 7.14% and velamentous in 11%). The mean placenta -fetal weight ratio of FGR placentas in the Biswas study was 0.156 versus 0.138 in control group.

Pryse Davies *et al.* (1973) reported mean placental weight of FGR group as 383g (SD± 90) and that of control group as 489 (SD± 97), whereas the mean placental coefficient for normal-weight babies was recorded as 0.14 (SD± 2.2). Murthy *et al.* (1976) observed that the mean placental weight of the study group was 296g and that of the normal weight group was 416g, and the mean placental volume of the FGR group was 272ml (SD± 31.6) and that of the normal weight group was 402ml (SD± 37.3). Mardi and Sharma (2003) also

reported decreased placental weight (68% weighting between 300 and 400g) in FGR pregnancies.

In the study of Heinonen *et al.* (2001), placental weight, birth weight and their ratio in chromosomally normal singleton pregnancies with SGA (n=1,569) and AGA (n=15,047) infants were compared, and their determinants were analyzed. SGA infants had 24% smaller placentas than AGA infants when gestational age was used as a covariate.

Oliveira *et al.* (2002) observed that placental coefficient was significantly greater in FGR than normal weight babies, indicating that although both placentas and babies in FGR had less weight, placental size was not so small. Little *et al.* (1960) considered placental coefficient between 0.10 and 0.18 as normal. Any value less than 0.08 was considered as abnormally small and more than 0.2 as abnormally large. Fox *at al.* (2000) concluded, that even placentas of idiopathic FGR pregnancies were considered smaller, placental coefficient was usually normal. Hence, "placental insufficiency" could not explain a small fetus.

Janthanaphan *et al.* (2006) in a prospective, cross-sectional study of 238 placentas between the 36th-40th gestational weeks determined whether abnormal placental weight and its ratio are associated with poor pregnancy outcomes. The mean placental weight was 519g (SD ± 89.01g). The mean placental weight to birth weight ratio was 17.08%. They found that abnormal placental weight and its ratio below the 10th percentile were strongly associated with fetal distress.

3.5.2. The umbilical cord

Numerous studies tried to clarify if examination of fetoplacental vessel may be used for prediction of the fetuses at risk for asphyxia or fetal death and how the number, diameter of vessels or obstructive lesion of the umbilical cord influence fetal and placental growth (*Hitschold, 2001; Tantbirojn, 2009; Saleemuddin, 2010; Hua, 2010)*

A study by Hansen *et al.* (1999) examined 1,146 placentas from pregnancies that resulted in the live birth of very low birth weight infants. In this study both marginal and membranous insertion were seen relatively commonly in all placentas. However Bjoro *et al.* (1981) examining 223 placentas, observed more velomentous insertion of umbilical cord in the FGR group, but did not find such prevalence with marginal insertion. At the same time among 22,012 births occurring in Akershus Central Hospital, Norway, there were 216 instances of umbilical cord knots (*Sornes, 2000*). In this study pregnancies with

knotted cords have characteristics different from those with ordinary umbilical cord encirclements. The authors concluded, that there is a 10 times higher chance of intrauterine fetal death with a knotted cord, but if this does not occur then there is no increased risk of obstetrical intervention and Apgar scores are the same as in other babies and other fetuses.

Osak *et al.* (1997), using the computerized perinatal database of St. Joseph's Health Centre, London, Ontario, obtained the birth weight, placental weight, umbilical cord gases, and nuchal cord status for all term singleton live born infants between January 1991 and December 1994. They found that umbilical cord complications at the time of birth are associated with a decrease in fetal size relative to that of the placenta.

The study of Redline (2004) tested the hypothesis that vascular stasis related to chronic umbilical cord obstruction might be a contributing factor to the fetal vascular impairment. The study population consisted of 125 impaired term infants. Clinical umbilical cord entanglement (true knots or cord loops around the neck or body parts at delivery) was significantly more common in cases with fetal thrombotic vasculopathy (FTV). Potentially obstructive pathological abnormalities of the umbilical cord (marginal/ membranous insertion, decreased Wharton's jelly, maximum cord diameter <8 mm, or hypercoiling) were also more frequent in this group (30% vs. 9% without FTV). Overall, 16 of 23 placentas with FTV had either clinical or pathological cord abnormalities. Recently Tantbirojn *et al.* (2009) confirmed that gross cord abnormalities predispose the fetus to stasis-induced vascular ectasia and thrombosis, thus leading to vascular obstruction and adverse neonatal outcome, including FGR and stillbirth.

3.5.3. Placental histological lesions

The different kind of placental lesions that interfere with the normal trophoblast function have been described before (*Rayburn, 1989; Salafia, 1992; Salafia, 1995; Oliveira, 2002; Salafia, 2003; Wee, 2006; Egbor, 2006; Salafia, 2006*). Analyzing placental pathology versus birth weight Z score, Hansen *et al.* (1999) reported that the placentas of infants with prominent growth restriction tended to have old infarcts, increased syncytial knots, intervillous thrombi, abruption, villous fibroses, chronic villitis, and increased nucleated red blood cells. They also found that growth restriction correlates with decidual vasculopathy independent of maternal hypertension but this correlation is strongest in the absence of maternal hypertension.

Salafia *et al.* (1992) conducted studies to investigate the relationship between maternal and placental factors in case of idiopathic FGR at term. Placentas examinations were carried out in 128 consecutive cases of idiopathic intrauterine growth retardation and the findings were compared with those of 179 gestational age-matched cases with normal growth. In this study FGR infants more frequently had multiple types of lesions in their placenta. Chronic villitis was found in 55% of FGR cases, placental infarction in 63% of cases and 59% of hemorrhagic endovasculitis were associated with FGR. One or more of these placental lesions were present in 71 of 128 (55%) of FGR cases. According to the researchers, different placental lesions show different patterns of related growth failure, suggesting different time of onset of intrauterine stress.

Beebe *at al.* (1996) examined 1,252 placentas from clinically selected at-risk singleton pregnancies. Placental pathology features were analyzed relative to gestational age and status of the newborn, including fetal growth restriction. These analyzes revealed the associations of ischemic changes and infarction with FGR in term infants, suggesting the need for comprehensive investigations of the effects of histopathologically apparent low placental blood flow.

Placentas in a case of intrauterine growth restriction, according to Redline (2008), demonstrate five chronic patterns of injuries. These are maternal and fetal vascular obstructive lesions, high grade VUE (villitis of unknown etiology), perivillous fibrinoid deposition and chronic abruption. Maternal vascular disorders were the most frequent finding in preterm and hypertensive mothers with FGR while VUE was the most common finding in normotensive term gestations with FGR. Furthermore, in another study of Redline *at al.* (1994) VUE in a case of FGR was also associated with oligohyramnios and chronic monitoring abnormalities including abnormal nonstress testing, abnormal pulsed flow Doppler studies and abnormal biophysical profile.

In the work of So-Yong Park *et al.* (2002) histologically, FGR was characterized by increased incidence of decidual vasculopathy (31.1%), multiple and severe infarct, villous fibrosis (31.1%), syncytiotrophoblastic knots (86.7%), and higher degree of increased perivillous fibrin deposition. They obtained placental tissue samples from 45 singleton third trimester pregnancies complicated by FGR and from 24 normal uncomplicated term pregnancies appropriate for gestational age. However, they were unable to confirm the association of fetal vessel thrombosis with FGR.

Mardi *et al.* (2003) conducted microscopic examination on one hundred placentas. These included 25 normal controls and 75 from intrauterine growth restricted pregnancies. The incidence of infarction, intervillous fibrin deposition was much higher in IUGR placentas on gross examination. Highly significant increase in the incidence of infarction, intervillous fibrin deposition, stromal fibrosis and syncytial knotting were found in IUGR placentas compared to full term normal placentas on microscopic examination. The incidence of basement membrane thickening and cytotrophoblastic hyperplasia were also higher in FGR placentas. The authors concluded that all major histological findings pointed towards reduced blood flow to the placentas resulting in the restriction of blood flow to fetus.

Either Madazli *et al.* (2006) reported significantly higher number of apoptotic nuclei in fetal growth restricted placentas (n=49), compared with the 25 control placentas. FGR pregnancies with associated pre-eclampsia showed higher staining of ICAM-3 (adhesion molecules) in placental tissue samples compared with control placental bed samples. Increased expression of ICAM-3 on the lymphocyte surface of both maternal and fetal side, suggests lymphocyte overactivation in FGR.

Egbor *et al.* in 2006 published data about placental morphology in pregnancies complicated by FGR using stereological techniques. In total, 69 women were studied. The research revealed that the morphology of the vascular and villous subcomponents in the intermediate and terminal villi was significantly influenced by late-onset FGR, whereas early-onset FGR caused a reduction in placental weight. Intermediate arteriole shape factor was significantly reduced in late-onset FGR.

Researchers from Manchester, UK (*Mayhew, 2007*) used stereological methods for estimating the surface areas of peripheral villi and their fetal capillaries, and arithmetic thickness of the villous membrane. The thickness was not affected significantly by IUGR, but villous membrane surface was significantly decreased.

3.5.4. Microscopic lesions of the placenta and Doppler velocimetry

Functional studies showed a correlation between specific Doppler flow patterns and histomorphological changes in placentas (*Kingdom, 1997; Viscardi, 2001)*. Laurini *et al.* (1994) defined the histological lesions in the placenta associated with abnormal blood flow findings in the fetal descending aorta, umbilical arteries and veins in 37 pregnant women with fetuses suspected of being growth restricted in the third trimester of gestation.

Macara *et al.* (1996) subsequently worked on the structural analysis of placental villi from growth-restricted pregnancies with abnormal umbilical artery Doppler waveforms. Terminal villi were examined structurally using transmission electron microscopy in 16 SGA pregnancies with absent end-diastolic flow velocity and in 16 controls matched for gestational age. They found that terminal villi in FGR cases were smaller in diameter than those of controls, and have increased syncyteal nuclei, reduced cytotrophoblastic nuclei, thickened basal lamina, and increased stromal deposition of collagen. The authors concluded that thickening of the basal lamina and congestion of the capillaries by erythrocytes are predicted to limit oxygen transfer from the intervillous space to the fetus and may represent an equilibration of oxygen tension between intervillous space and the terminal villi. Furthermore, despite the known reduction in uteroplacental blood flow in FGR, fetoplacental blood flow is compromised to a far greater extent in the presence of AEDFV.

Madazli *et al.* (2003) evaluated the histomorphology of 47 placentas and placental beds in correlation with the Doppler finding of the uterine and umbilical arteries in intrauterine restricted pregnancies. They compared findings with the 25 uneventful pregnancies, with appropriate for gestational age fetuses selected as controls. Doppler examinations in the study were performed within the last week before delivery. The incidences of pathologic bed biopsies in control, FGR with normal uterine artery Doppler velocimetry and FGR with abnormal uterine artery Doppler velocimetries were 0%, 16.6% and 79.3% respectively. They reported that placentas from FGR cases with abnormal umbilical artery Doppler velocimetry had significantly increased number of villous infarcts, cytotrophoblast proliferation and thickening of the villous trophoblastic basal membrane. According to their study, women with both abnormal uterine and umbilical artery Doppler velocimetries delivered earlier and had lower mean birth and placental weight; and placental pathologies were best reflected by abnormal uterine and umbilical artery velocity waveforms.

Viero *et al.* (2004) evaluated the utility of gray-scale placental ultrasound for the detection of pathological lesions in the placentas of preterm pregnancies with abnormal fetoplacental blood flow. They found that pregnancies with absent or reversed end-diastolic flow velocities in the umbilical arteries have high perinatal mortality rate associated with pathology of placental villi, mostly with decidual vasculopathy (sensitivity 91%) and accelerated villous maturation (sensitivity 93%).

Ferrazzi *et al.* (1999) tested the hypothesis that Doppler velocimetry of the ascending uterine arteries in cases of fetal intrauterine growth restriction can reflect the presence of

hypoxic-ischemic lesions of the placenta. It appeared that the total rate of placental lesions was significantly higher in the presence of abnormal uterine artery compared to the presence of normal Doppler velocimetry. The rate and severity of these lesions was not significantly different between normotensive and hypertensive pregnancies (87 vs. 93%). In their study when the uterine arteries Doppler velocimetry was normal, the rate of placental lesions was similar between FGR cases and control pregnancies (14 vs. 8%). Researchers compared 90 consecutive pregnancies with FGR and 37 uneventful controls. The overall perinatal outcome was not significantly different in any of the normotensive and the hypertensive pregnancies with growth restricted fetuses and abnormal uterine arteries Doppler.

Aaderma *et al.* (2001) conducted a study to investigate the association between uterine artery Doppler flow patterns and uteroplacental vasculopathology in normal and complicated pregnancies. They included 43 women in the study, whose pregnancies were complicated by pre-eclampsia or growth restricted fetuses, and 27 women with normal pregnancies, undergoing caesarean section. They found an absence of physiological changes in 58% of complicated pregnancies, and in 40% of normal pregnancies having abnormal uterine arteries Doppler profile. Pathological changes were seen in 58% of complicated and 53% of normal pregnancies. They occurred in spiral arteries with and without physiological changes, and there was no significant correlation with Doppler results. Their conclusions were that the absence of physiological changes is associated with abnormal uterine artery Doppler flow and pregnancy complications.

Earlier, Olofsson *et al.* (1993) had examined placental bed biopsy at caesarean section in 26 complicated by FGR and 29 uncomplicated pregnancies, and compared that with uterine artery Doppler blood flow. The uterine artery PI was significantly more often abnormally high in the study group compared with the control group. Physiological changes were found in placental beds of all controls, and were absent in 76% of study cases. Physiological changes were absent more often in SGA than in appropriate for gestational age newborns. Absence of physiological changes was significantly more often found in cases with abnormally high PI. The study supported the hypothesis of existence of a triad of defective placental bed vessel maturation, increased uteroplacental flow resistance, and hypertension. Also Dicke *et al.* (2009) compared the screening efficiency of the umbilical artery systolic to diastolic ratio, pulsatility index, and absent end-diastolic flow for adverse pregnancy outcomes and placental abnormalities in growth restricted fetuses, and found placental abnormalities significantly more common in cases with abnormal Doppler values with no overlap in the types of placental lesions in most cases.

3.5.5. Placenta and ultrasound

Given all of these potential abnormalities of the placenta, researchers have tried hard to find early placental signs predictive for FGR, by performing gray-scale ultrasound or magnetic resonance imaging (*Onge, 2004*). Sebire (2008) provided an overview of the most common prenatal sonographic techniques and their clinical relevance to the diagnostic pathologist, primarily focusing on conditions with specific placental implications. These ranged from abnormalities of placental site and cord insertion, to obstetric complications such as FGR, through sonographic placental parenchymal lesions such as subchorionic and intervillous thrombi, or chorioangiomata. In addition, the pathophysiological basis of abnormal maternal and fetal maternal Doppler indices and intrauterine growth restriction were described. The decidual vasculopathy and villous changes were all associated with reduced intervillous blood flow.

Hafner *et al.* (2003) determined placental growth between 12-22 weeks in normal pregnancies compared to pregnancies complicated by fetal growth restriction and maternal pre-eclampsia. The placentas were measured with 3D sonography at 12, 16 and 22 weeks of gestation. Placental volume growth was calculated. They found that FGR placentas were already smaller at 12 weeks but than developed in a similar way to normal placentas. If FGR goes together with PE, both placental volumes at 12 weeks as well as growth are reduced significantly.

Later on Law *et al.* (2009) compared 3D volume of the placenta at 12 weeks gestation with first- and second trimester artery and various maternal risk factors in 2,500 unselected pregnancies in the prediction of FGR. They recruited women from the Down syndrome screening clinic. They found only the first-trimester 3D placental volume and second-trimester Doppler to be significant predictors of an SGA infant.

Recently Proctor *et al.* (2009) published the data of their study on 90 normal singleton pregnancies, evaluating their second trimester size and correlation with first trimester maternal PAPP-A and α-fetoprotein at 15-18 week's gestation. Researches found that the small placental size and elevated α-fetoprotein identify women with low PAPP-A at high risk of FGR.

The studies confirmed that early measurement of placental size might contribute to the prediction of an SGA pregnancy and perhaps FGR.

3.5.6. Placental morphology and smoking

Rocha *et al.* (1998) evaluated the placental alterations in smoking mothers in the case-control stereological study. Twenty placentas were examined in their study. Ten placentas were selected from healthy primigravid women who smoked 20 cigarettes per day, at least two years before and during pregnancy. Each placenta of the study group was assigned a control placenta from a healthy primigravid patient who never smoked. While the macroscopic characteristics of the placentas showed no significant differences between smokers and non-smokers, the authors found a significant increase in stromal volume density, villus size and trophoblast thickness. Villus and vessels surface densities, total surfaces, and number/mm^2, histological fields were significantly decreased in the smokers' placentas.

In another study Salafia and Shiveric (1999) summarized the documented effect of smoking on the uterine vasculature and offered the resolution for the apparent paradox of the seemingly "protective" effect of smoking to reduce the incidence of the most serious vascular complications of pregnancy -pre-eclampsia. They also reviewed the association of smoking with early and late placental and fetal vascular complications. The researches concluded that chronic endotelial injury caused by maternal smoking may truly "protect" the mother from cataclysmic endotelial dysfunction of pre-eclampsia.

Zdravkovic *et al.* (2005) studied tissue samples from smoking and non-smoking mothers to determine wheather active or passive smoke exposure affects the balance between cytotrophoblast prolifiration and differentiation, and found that maternal smoking disregulate the balance between cytotrophoblast expression of all three types of molecules. In addition, cell columns and proliferating cells were reduced while there was a corresponding increase in cell islands.

4. 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.

5. 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.

6. 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 placental changes. On the bases of the results of the study we propose to 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, this study is among the first to investigate 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.

7. MATERIALS AND METHODS

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

7.1. 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 10th 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

7.2. 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.

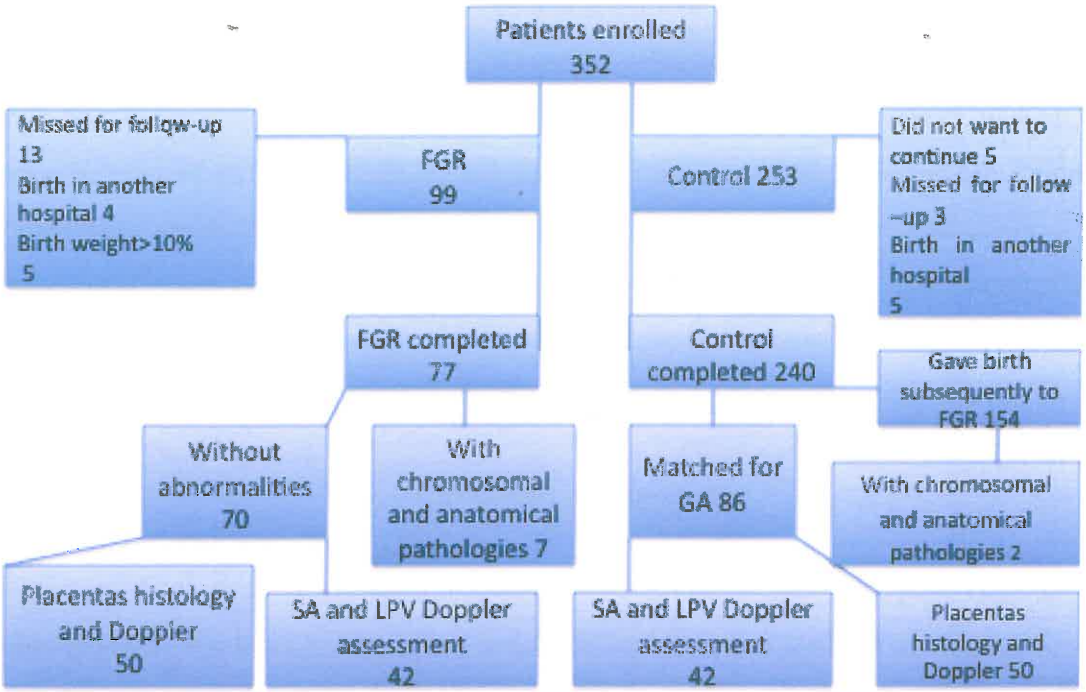


Figure N 4. Recruited patients

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.

A total of 317 patients from the sample of 352 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 4)*. 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.

7.3. Follow up

7.3.1. 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 (*Figure N 5)*. 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, for 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, and recorded a mean for each and used these data to calculate the estimated fetal weight according the formula of Combs *et al.* (1993).

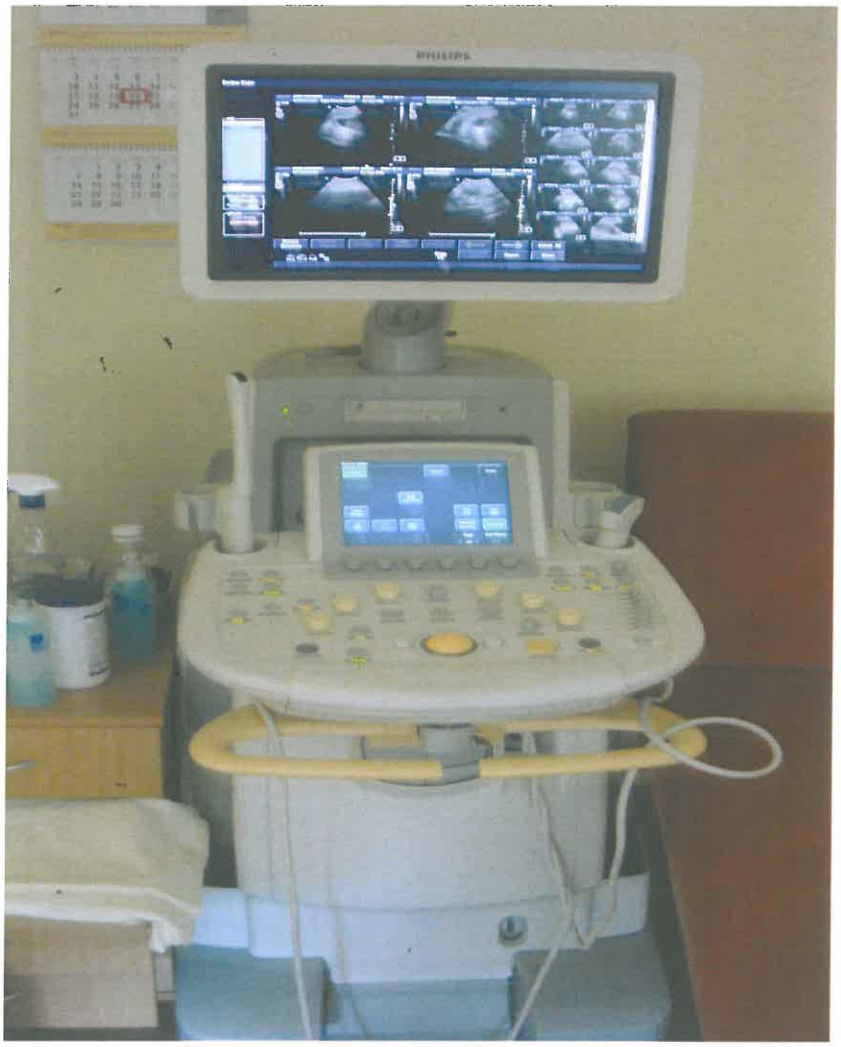


Figure N 5. Ultrasound machine Philips, AU 22, USA

7.3.1.1 Head size

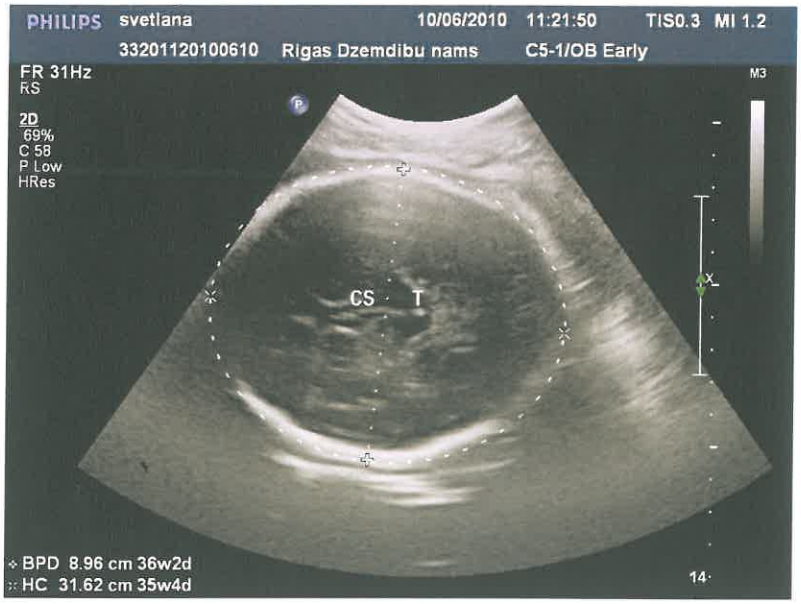


Figure N 6. Biparietal diameter was obtained in a horizontal section at the level of thalamus (T) and the cavum septi pellucidi (CS). In the present study, the measurements include the outer edges of the biparietal diameter (gestational age of 30 weeks, N.Vedmedovska). HC was obtained at the same section using an ellipse, which includes the outer surface.

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 head biometry, and the outer-outer measurements of BPD were introduced in many countries (*Hadlock et a. 1982, Hansman 1985*). In the present study we used BPD measured outer-outer (*Figure N 6*).

7.3.1.2 Abdominal size

The abdominal measurements were obtained in a transverse section of abdomen at the level where the umbilical veins enters the liver (*Figure N 7 and 8*).

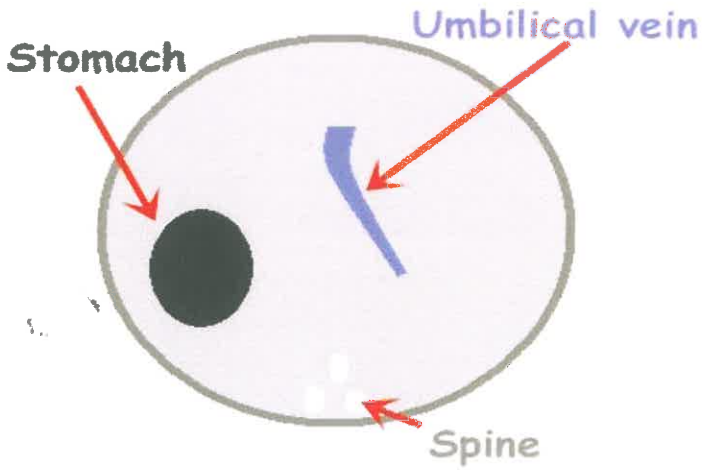


Figure N 7. The transverse section of abdomen

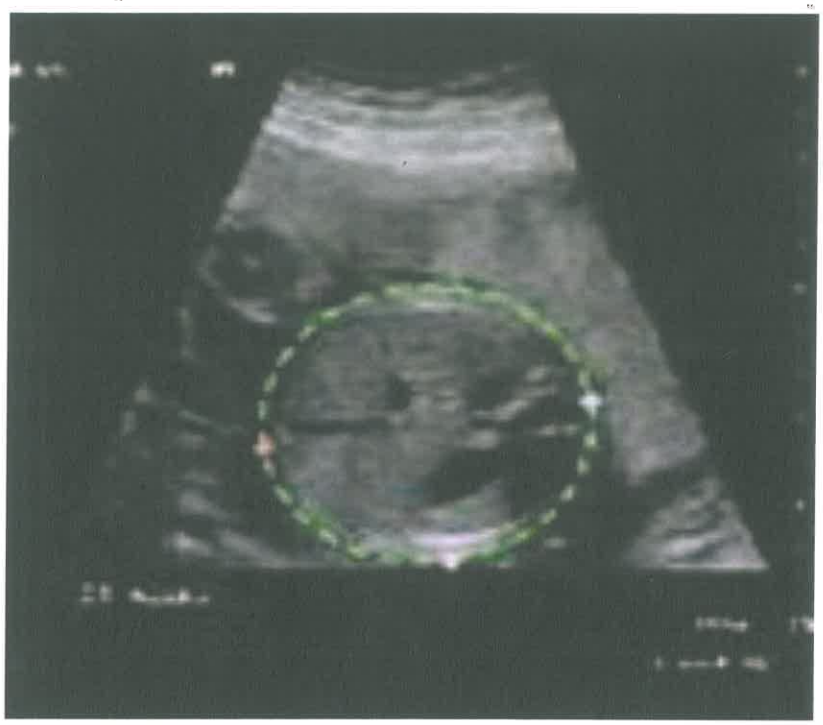


Figure N 8. In the present study AC was obtained using an ellipse, which includes the outer surface of the skin.

7.3.1.3 Femur length

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

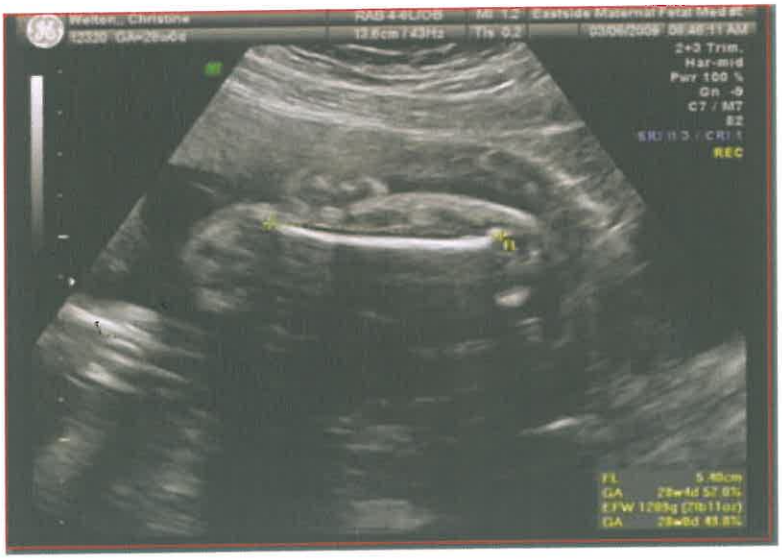


Figure N 9. To avoid over-and underestimation of this measurement it is important to make the ultrasound insonation visualize both diaphysis and the epiphyseal cartilages of the femur. Only the diaphysis was included in the measurements in the present study.

7.3.2. Doppler studies

At each session we aimed at measuring blood flow velocity in the

1) uterine artery (UtA),
2) umbilical artery,
3) middle cerebral artery,
4) left portal vein,
5) splenic artery,
6) *ductus venosus*.

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 one hour, and we placed women in a semirecumbent position.

Colour 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 values of the left and right arteries were 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.

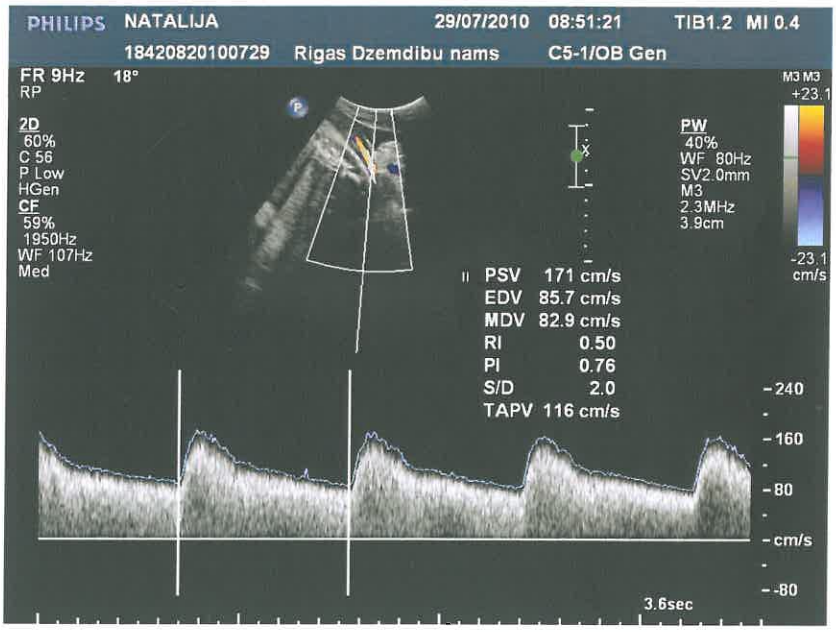


Figure N 10. Left uterine artery vizualized by transabdominal colour flow mapping and Doppler velocity waveforms at 36 weeks; absence of protodiastolic "notch" (N. Vedmedovska)

The *AU* recordings performed in a free-floating section of the umbilical cord (*Figure N 11)*. Angle correction was then applied and the signal updated until three similar consecutive waveforms had been obtained.

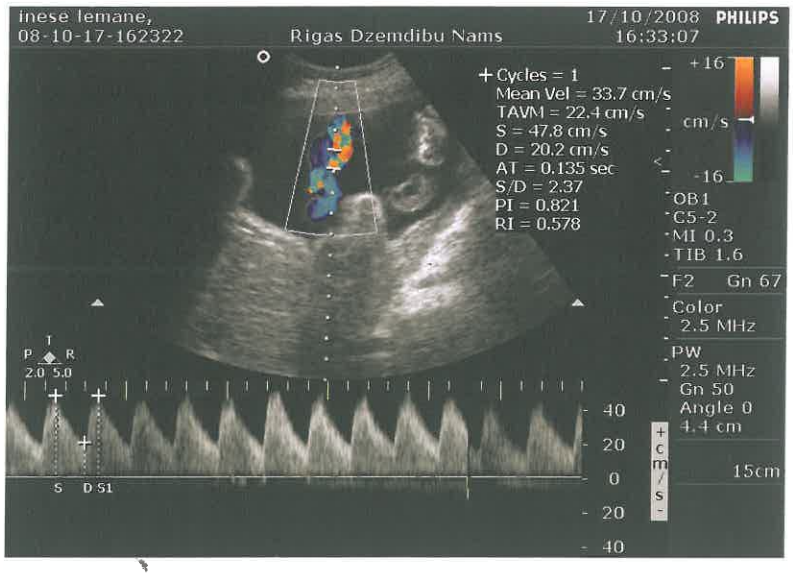


Figure N 11. Colour Doppler image of umbilical artery (N. Vedmedovska)

The *ACM* was visualized using colour 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 of Willis. When the *ACM* near the field was not able to interrogate, the *ACM* of the opposite site has been used (see *Figure N 12).*

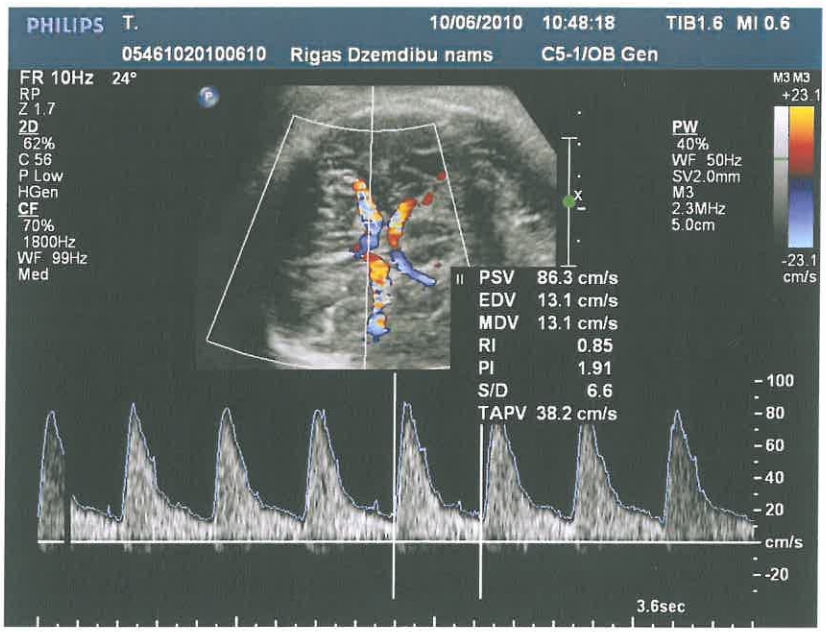


Figure N 12. Colour Doppler image of the fetal circle of Willis (gestational age 30 weeks).
Middle cerebral artery.

Using colour 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 (*Figure N 13).*

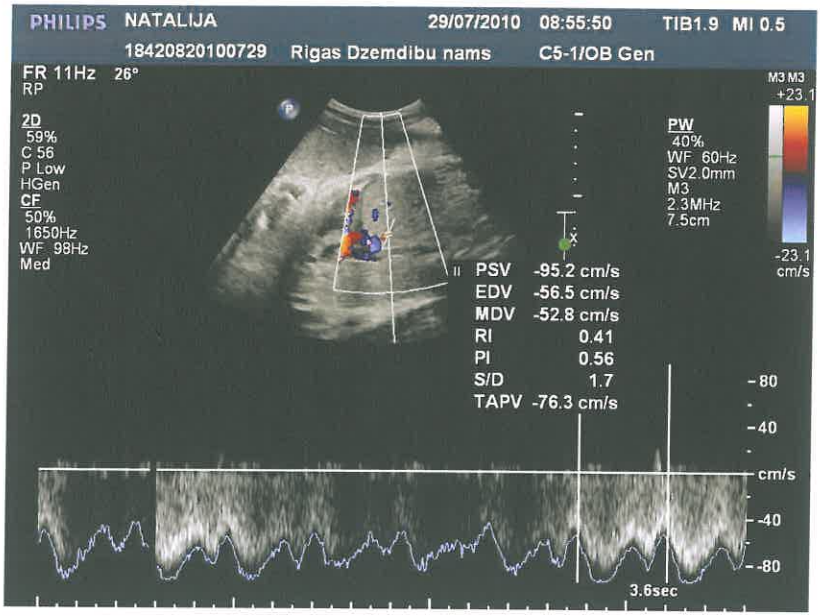


Figure N 13. Colour Doppler image of *ductus venosus* trace (gestational age 30 weeks) (N. Vedmedovska)

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 is placed over the proximal part of the vessel (see *Figure N 14).*

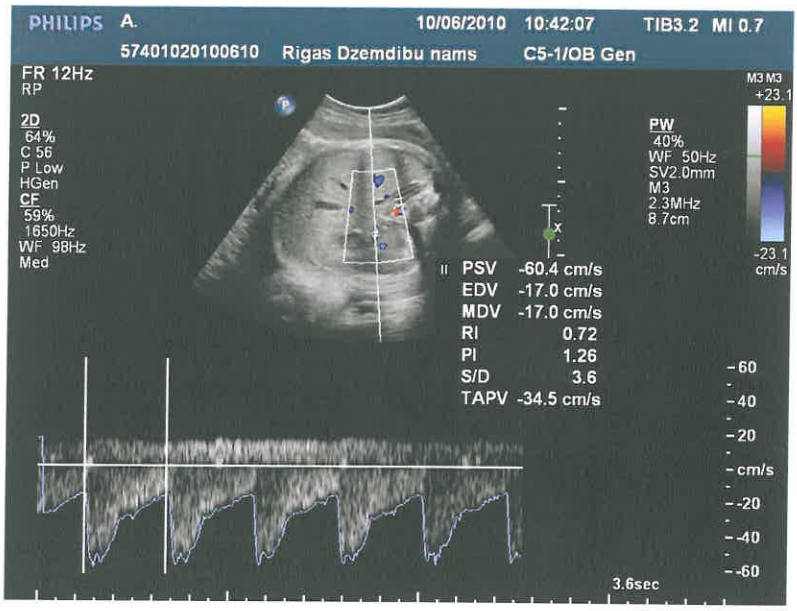


Figure N 14. Colour Doppler image of splenic artery (N. Vedmedovska)

The left portal vein was identified with colour Doppler in a transverse insonation as an extension of the umbilical vein after the branching site of the *ductus venosus* (see *Figure N 15)*.

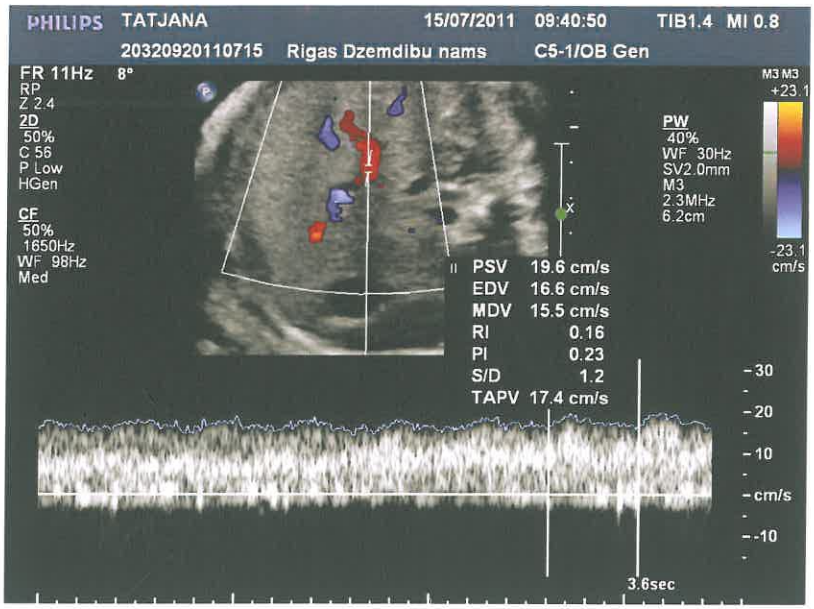


Figure N 15. Colour Doppler image of the left portal vein

7.3.3. Surveillance assessment

The biophysical profile (Manning, 1999) has five components: four ultrasound assessments and a nonstress test. The nonstress test evaluates fetal heart rate and response to fetal movement (*Figure N 16*). Amniotic fluid will be considered abnormal in the presence of amniotic fluid index less than 5 cm.

Table N 1. Assessment of the biophysical profile

Parameter	Normal (2 points)	Abnormal (0 points)
NST/Reactive FHR	At least two accelerations in 30 minutes	Less than two accelerations to satisfy the test in 30 minutes
US: Fetal breathing movements	At least one episode of > 30s or >20sin 30 minutes	None or less than 30s or 20s
US: Fetal activity / gross body movements	At least two movements of the torso or limbs	Less than three or two movements
US: Fetal muscle tone	At least one episodes of active bending and straightening of the limb or trunk	No movements or movements slow and incomplete
US: Qualitative AFV/AFI	At least one vertical pocket > 2 cm or more in the vertical axis	Largest vertical pocket </=2cm

Adapted from Manning, 1999

Each ultrasound assessment is graded as 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 (see *Table N 1)*.

Figure N 16. The trolley with the cardiotohograph Philips Series 50A

7.3.4. Delivery

Delivery was indicated in the 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 times in 60 minutes) and/ or in a case of

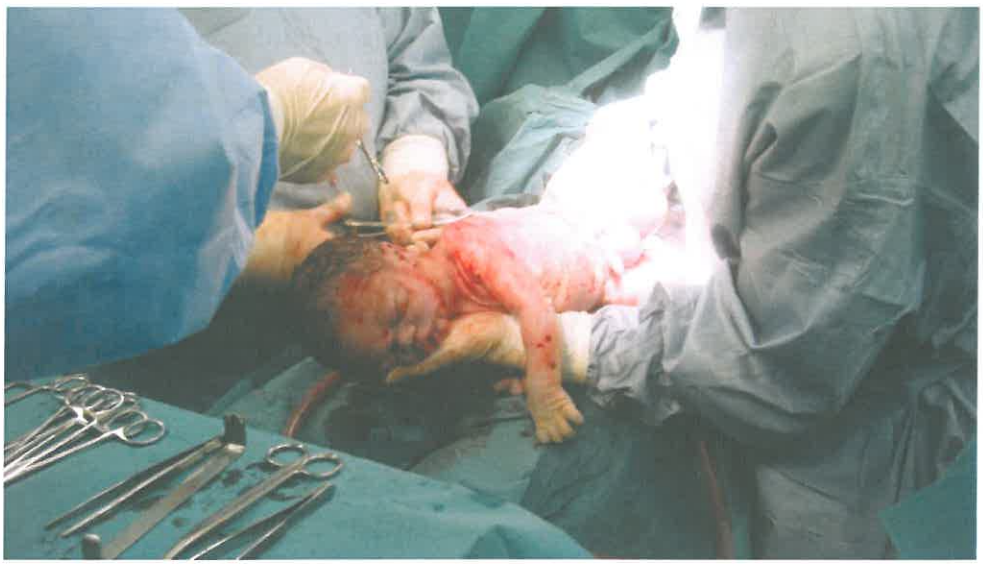


Figure N 17. Cesarean section operation, extracting of the baby

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 (*Figure N 17*).

7.4. 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 95th percentile for gestational age (GA) based reference ranges (*O.Gomez, 2008*) and/or bilateral presence of early diastolic "notching" (*T. Frusca, 1998*)
- Abnormal umbilical artery velocimetry was defined as PI above the 95th 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 2.5th percentile for GA based reference ranges (*C.Ebbing, 2007*).
- Abnormal *ductus venosus* was defined as PIV above the 95th 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 5th percentile for GA based reference ranges (*J.Kesler, 2007a*).

•Abnormal splenic artery velocity waveforms were defined as PI below the 5th 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 10th 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 pathologist was blinded to the Doppler studies' data. (see *Fifure N 18).*

The placental thickness was measured at the center of the placental tissue. The specimens were obtained from the area of cord insertion, intermediate and marginal

portions of placental plate. 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 (H-E). 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 (*Figure N 19).*

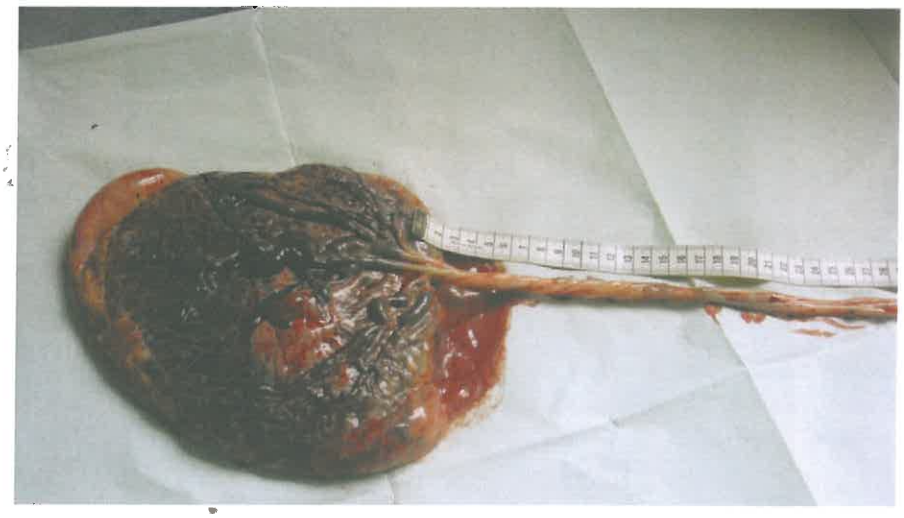


Figure N 18. Assessment of placenta and membranes after trimming, and removing the clots. Evaluation of length, insertion type and particularities of the umbilical cord.

Presence or absence of the following placental lesions were systematically recorded: perivillous fibrin deposition, stromal fibrosis, cytotrophoblast proliferation, thickening of the villous trophoblastic basal membrane, villous infarcts, intervillous thrombi or hematomas, chronic villitis and vasculitis (*Salafia, 1995*).

Villous infarction (*Figure N 20*) was defined as a localized region of ischemic necrosis of the villi, which become surrounded by coagulated blood (*Kraus, 2004*); *intervillous thrombi or intraplacental haematomas* as localized, circumscribed clots in the maternal intervillous space (*Kraus, 2004*); passage of fetal neutrophils into and through the vessel wall and oriented to the amniotic surface was defined as *vasculitis* (*Faye-Petersen, 2006*). *Chronic villitis* was defined as infiltration of the villous stroma by maternal T-lymphocytes (*Redline, 2007*) and *perivillous fibrin deposition* (*Figure N 21*) was defined as a presence of a dense meshwork of fibers measuring less than 10 mm in thickness with characteristic cross-striation of fibrin filaments with an approximately 20-nm periodicity (*Benirschke, 2000*).

Cytotrophoblast proliferation (*Figure N 22*) was defined as an increase in the number of villous cytotrophoblast, trophoblast cells and syncytium; increased thickness of villi suggestive of raised secretion of basal lamina molecules as *thickening of trophoblastic basement membrane* (*Figure N 23*) and *stromal fibrosis of stem villi* as an extensive collagen synthesis that is usually not restricted only to the stem villi (*Benirschke, 2000*). The images of placental specimens without lesions are represented in the *Figures N 24* and *N 25*.

Figure N 19. Light microscope Leica DM1000, Warburg, Germany

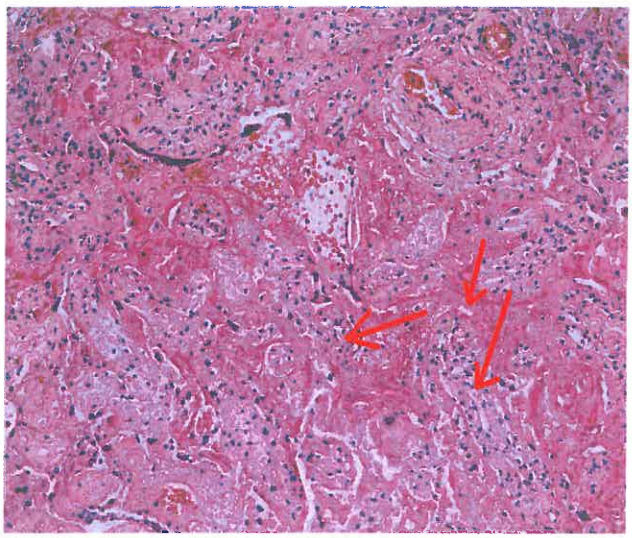


Figure N 20. Representative placental lesions with light microscopy (X10) from FGR placentas. Incidence of infarcts and necrosis (red arrows). (I.Melderis, 2008).

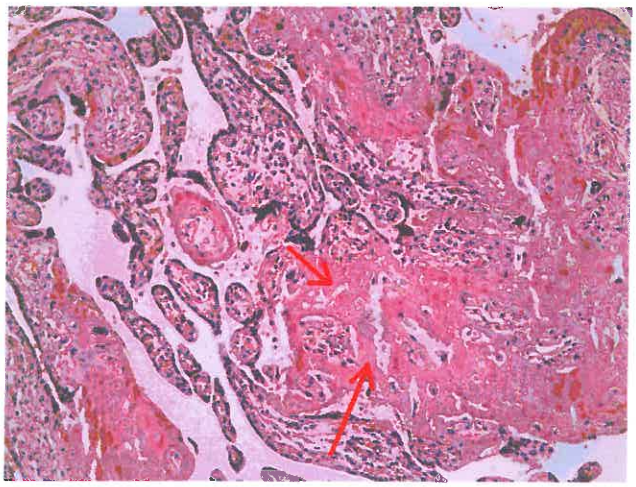


Figure N 21. Representative placental lesions with light microscopy (X10) from FGR placentas. Perivillous fibrine deposition (red arrows). (I.Melderis, 2008).

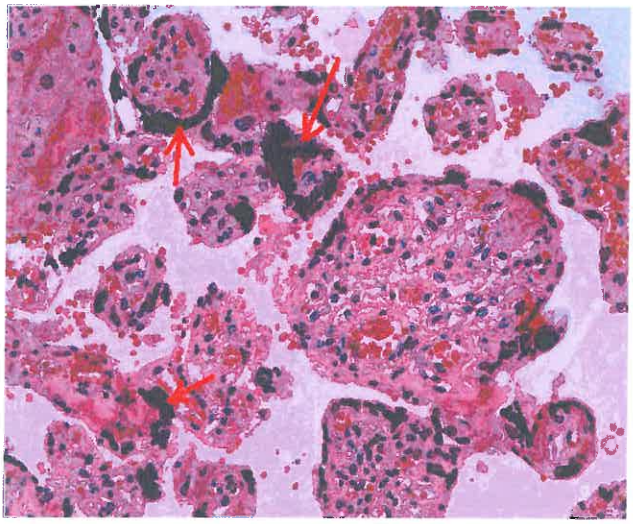


Figure N 22. Representative placental lesions with light microscopy (X10) from FGR placentas. Cytotrophoblast proliferation (red arrows). (I.Melderis, 2008).

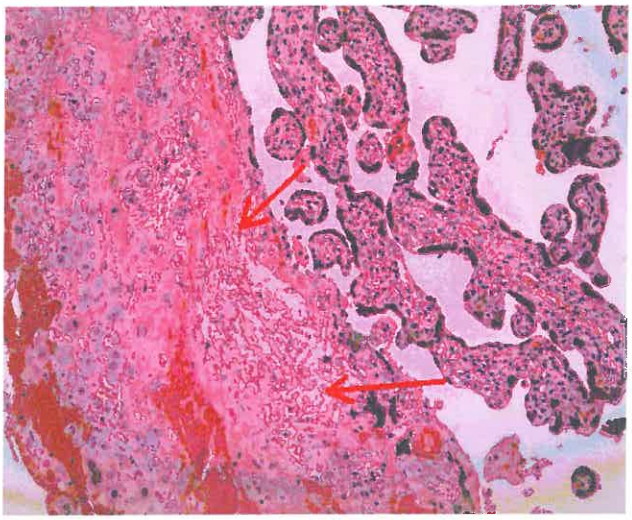


Figure N 23. Representative placental lesions with light microscopy (X10) from FGR placentas. Thickening of the villous trophoblastic basal membrane (red arrows). (I.Melderis, 2008).

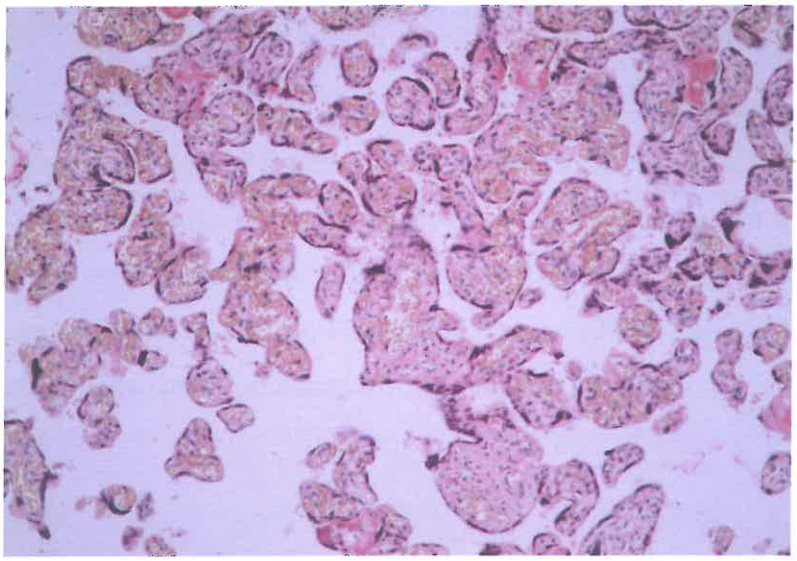


Figure N 24. Light microscopy of terminal villi from control placenta (x10) without cytotrophoblastic prolifiration and fibrin deposition (I.Melderis, 2008)

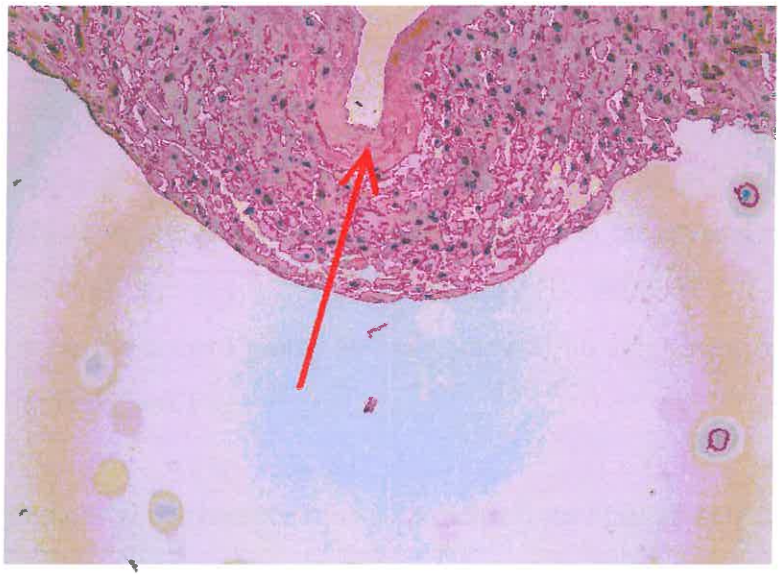


Figure N 25. Light microscopy of a terminal villus from control placenta (x10) without thickening of the villous trophoblastic basal membrane (I.Melderis, 2008)

7.5. 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.

8. RESULTS

8.1. Demographic data of patients

The demographic characteristics of women participated in the present study are shown in *Table N 2*. 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 2. 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

8.2. Socioeconomic determinants

In like manner, checking the hypothesis on the patients' place of residence, no statistical differences were found between groups (*Table N 2*). 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 diffefence (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).

8.3. Obsterical characteristics

The clinical characteristics of women are shown in *Table N 3*. 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).

Table N 3. Clinical characteristics of patients from FGR and control groups

	FGR (n = 70)	Control (n = 86)	p value
Parity			
Nulliparous	47 (67.2)	49 (57)	0.52
Multiparous	23 (32.8)	37 (43)	0.38

Data are given as numbers, percentages in parentheses

Obstetrical characteristics show no differences between the groups regarding most of the complications in current or previous pregnancies (*see Table N 4*). 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 4. Obstetrical characteristics of patients from FGR and control groups

Pregnancy complications	FGR n=70	Control n=86	p value
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
Urinary 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
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

8.4. Reproductive tract infections and extragenital diseases

Reproductive tract infections (RTI) in anamnesis (p=0.3) 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 two in whom BV was diagnosed delivered prematurely: one 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 5),* 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.

Table N 5. 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

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.

8.5. Lifestyle 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 6*).

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 6. Lifestyle determinants in FGR and control groups.

Lifestyle 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 (14.2)	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 7* and *N 8*) pre-pregnancy smoking (OR 5.8; 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 7. 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 residencé							
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 8. 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		

8.6. Management and outcome

Gestational age of FGR group upon inclusion in the study was 25 weeks of gestation. Among IUAA newborns six percent at delivery had extremely low birth weight (mean 577.0 g ± 159, gestational age 24-29+6weeks), 16%-very low birth weight (mean 1368.4 ± 345 g, gestational age 30-34+6), 24%-low birth weight at 35 till 36+6 weeks of gestation (1759.7 ± 347 g), and 54% were term infants with the mean birth weight of 2489.9 ± 248 g (*Figure N 26*)

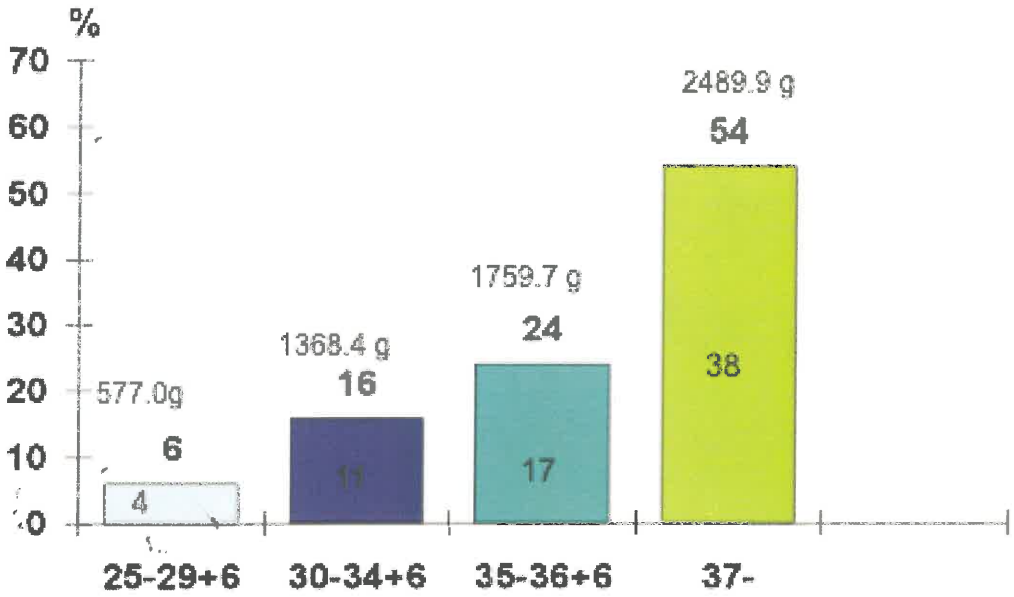


Figure N 26. The distribution of growth restricted newborns according their gestational age and birth weight (weeks of gestation on x axis)

Mean gestational age and birth weight at delivery were 36.3 ± 3.4 weeks and 2.020 ± 622 g, respectively, in the study group, and 39.8 ± 1.1 weeks and 3.623 ± 515 g, respectively, in the control group (P<0.001) *(Table N 9)*.

Table N 9. 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			
Spontaneous delivery	14 (20.0)	99 (65.1)	<0.001
Labor induction/vaginal	10 (14.3)	22 (14.5)	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 correlation between gestational age and birth weight is shown in *Figure N 27.* Women with FGR had lower birth weights of their babies at any gestational age.

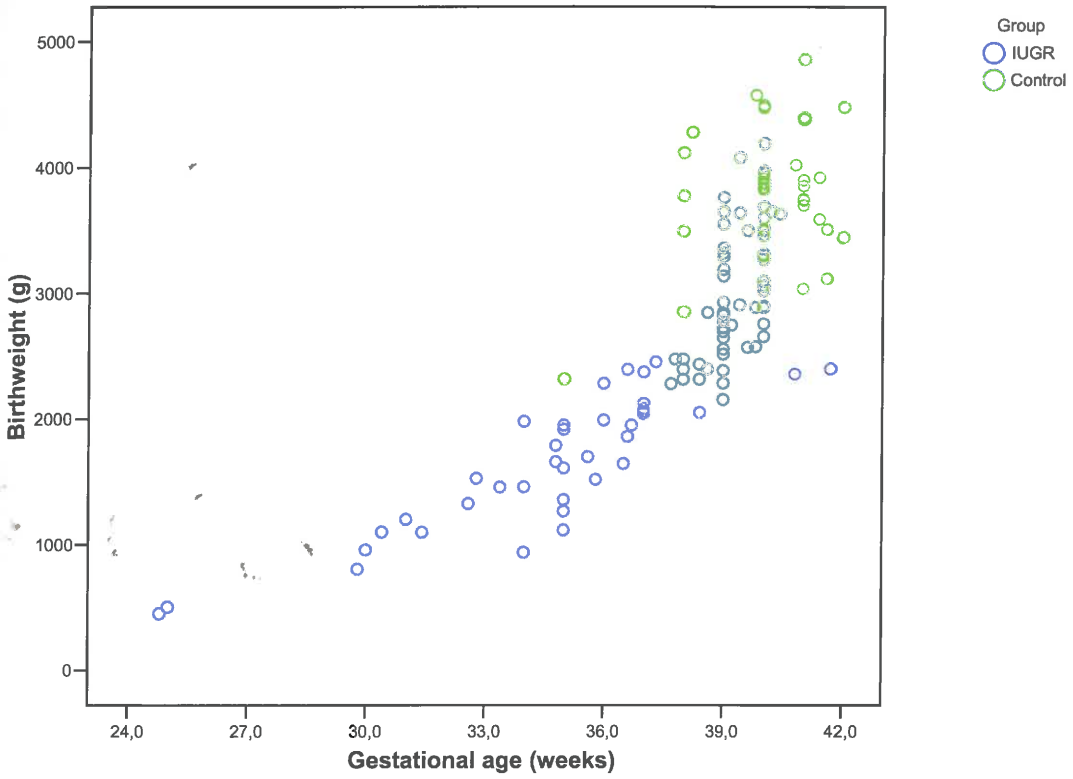


Figure N 27. The distribution of newborns according their gestational age and birth weight (blue spheres -FGR group; green spheres-control group).

8.6.1. Interventions

The incidence of spontaneous deliveries in the FGR group was significantly lower than in the controls (OR 0.1; 95% CI 0,1-0,3; p<0.001). Rate of elective cesarean section before onset of labor was significantly higher (OR 7.5; 95% CI 3,9-14,3; p<0.001). The differences between the groups in rates of induced labor and secondary cesarean delivery during labor were not statistically significant (OR1.0; 95%CI 0.4-2.2 and OR 1.7; 95% CI 0.6-5.1, respectively; see *Table N 9*).

Fetal distress, abruption of placenta and pre-eclampsia were all significantly more frequent indications for cesarean section in FGR than in controls (p=0.001, 0.003, 0.001; respectively), 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 10*).

Six 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 10. 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.

8.6.2. Surveillance tests

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.

8.6.3. Neonatal outcomes

Perinatal outcomes are shown in *Table N 11*. 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 28th 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 (OR 3.6, 95% CI 1.2-10.5; $p=0.02$), admission to neonatal intensive care unit (OR 10.3, 95% CI 2.8-37.7; $p=0.001$) and transfer to pediatric hospital for further treatment (OR 24.3, 95% CI 7.0-84.6; $p<0.001$) occurred more frequently in the study group than in the control group. Preterm birth (OR 50, 95% CI 1.4-218.5; $p<0.001$), respiratory distress (OR 12.4, 95% CI 3.4-44.9, $p<0.001$) 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 (OR 3.6, 95%CI 1.2-10.5; $p=0.003$). 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.

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 28 and 29*).

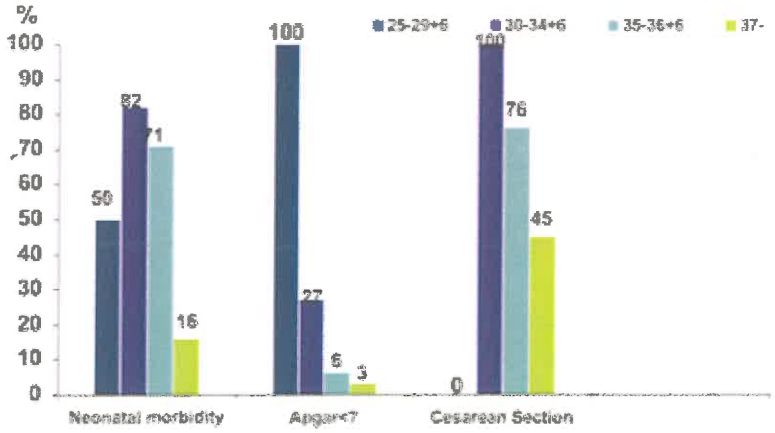


Figure N 28. Perinatal outcome in FGR group according gestational age and birth weight

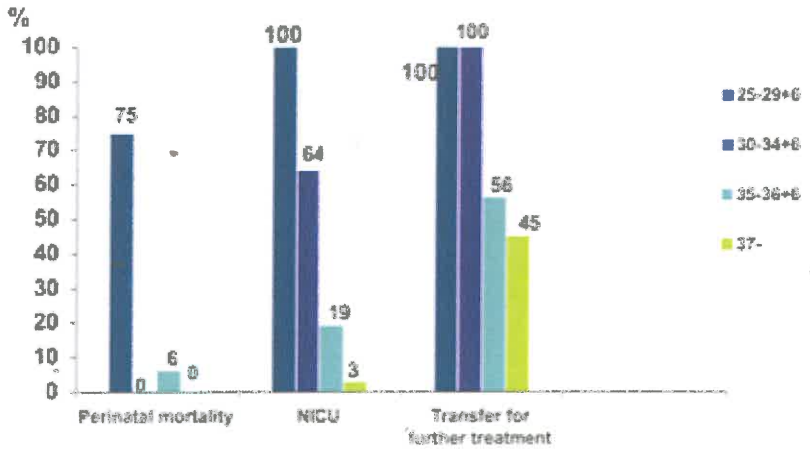


Figure N 29. Perinatal outcome in FGR group according gestational age and birth weight

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 (OR 5.9, 95% CI 3.2-10.9; $p<0.001$).

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 11. 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 12 and N 13*.

Table N 12. 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 13. 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

8.6.4. Aneuploidy and fetal malformations

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

In controls, two 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 case and

one congenital ichthyosis case were confirmed in the control neonates after birth (anomalies combined vs. controls, p=0.01).

To enable an appropriate comparison with data from other studies, these seven cases were not included in the outcome analyses (*Table N 11*).

8.6.5. Doppler studies

8.6.5.1 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.

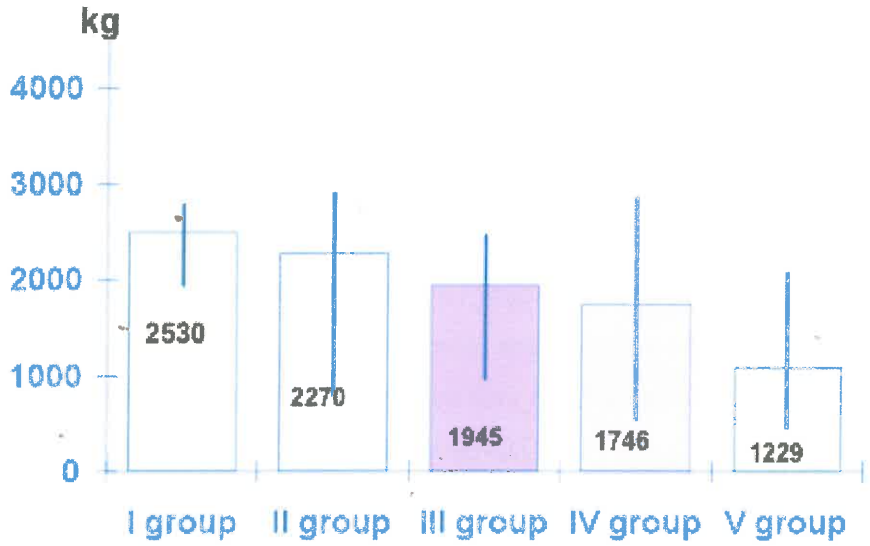


Figure N 30. Birth weight according to the Doppler profile

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 14*). Placental resistance and low *ACM* PI (Group IV) was associated with reduced birthweight (see *Figure N 30*).

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 14)*. Neonatal morbidity was 67 % for Group III neonates. This was largely attributable to prematurity (nine cases) and RDS (nine cases) see *Table N 14*.

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 14*).

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 14*). Smoking women with FGR have no different Doppler profile compared with normal women ($p=0.09$).

Table N 14. 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	0.09μ
RTI (n)	1	4	1	2	2	0.018μ
▪ **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)

8.6.5.2 Adaptive changes in splenic artery and left portal vein in fetal growth restriction

Clinical characteristics of control women and FGR women with and without abnormal *AU* flow are shown in **Table 15.** FGR group with reduced *AU* flow were recruited in the study earlier than when the flow was normal (33.8±3.2 vs. 37.2± 2.8 weeks, p= 0.005). Gestational age at birth was 35.4 (± 3.3) weeks in FGR with elevated *AU* PI, compared to 38.1 (± 2.8) weeks in FGR group with normal *AU* PI (p=0.007) or 39.7 (± 1.3) weeks in controls (p<0.0001), while control women delivered at a similar gestational age as FGR women with normal *AU* flow (39.7 vs. 38.1weeks). Time span to delivery also varied dramatically 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<0.0001 and p=0.019, respectively). 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). 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%). Preeclampsia was present in 20% of FGR/ Abnormal *AU* PI women versus none in FGR/ Normal *AU* PI or control women (p=0.001). Preeclampsia was higher in the FGR group with abnormal *AU* flow (4/20, 20%) than in FGR women with normal flow (0/22, p=ns) or normal control women (0/42, p=0.04).

Twenty of 42 FGR (62%) fetuses had increased PI of the *AU* above 95 th percentile versus none of the 42 controls (p<0.0001). In twelve of FGR fetuses (54%) "brain-sparing" was confirmed by Doppler velocimetry (low PI of the *ACM*).

Figure 31 represents the total material of the study. 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 four fetuses (9.5%, p=0.04) (**Table 16, Fig. 31A**). 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 16, Fig. 31B)**.

Among 42 FGR fetuses three prenatal deaths occured and 6 newborns had 5 minute Apgar scores below 7. In all perinatal mortality cases reduced LPV flow were recorded (p=0.04) and two out of three had SA PI less than 5% (p=0.03, **Table 17)**. Low Apgar scores were found in 14% of FGR cases versus in none in the controls (p=0.025). All cases with low Apgar were found in the group with reduced LPV flow and SA PI (p=0.01, and p=0.03, respectively). Similarly, both low LPV flow and SA PI <5% were associated with an increased risk of neonatal metabolic acidosis (p=0.004 and 0.006, respectively).

Amongst fetuses submitted to "brain sparing" redistribution, LPV flow was below the 50th percentile in 10/12 of the FGR cases, versus 1/12 of the controls (P=0.0006) and below the 5th percentile in 7/12 FGR cases versus 0/12 (P=0.005). Fetuses with "brain-sparing", however, did not express reduced flow in the splenic arteries (<50th percentile 7/12 vs. 9/12 controls, and 5 th percentile 3/12 vs. 1/12 controls).

Overall, admission to neonatal intensive care unit among FGR neonates were recorded in 5 cases, respiratory distress syndrome was diagnosed in seven newborns, necrotizing enterocolitis in one and two infants developed severe intraventricular hemorrhage grade III or IV. Sepsis was suspected in five neonates due to increased C-reactive protein and leukocyte counts, versus no cases in the control group. Twelve growth-restricted newborns were transferred to pediatric hospital for further treatment, compared to one in the control group (neonatal pneumonia).

Table N 15. Clinical characteristics of the groups

	FGR		Control (n=42)	p value
	Normal *AU* PI (n =22)	Increased *AU* PI (n=20)		
Gestational age at Investigation (wks) Delivery (wks)	37.2± 2.8 38.1± 2.8	33.8± 3.2 35.4± 3.3	34.8± 1.2 39.7 ± 1.3	<0.05 <0.05
Time span examination to delivery (days)	13± 7.8	1.3± 1	23.6 ± 17.3	<0.05
Pre-eclampsia	0	4	0	0.001
Hypertension	1	0	0	0.240
Delivery mode Cesarean section	11	16	6	0.001
Birth weight (g)	2440± 528	1643± 626	3584 ± 473	<0.0001

Data given as number and mean with SD

Table N 16. 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 (n=42)
	Total (n=42)	Normal *AU* PI (n=22)	Increased *AU* PI (n=20)	
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

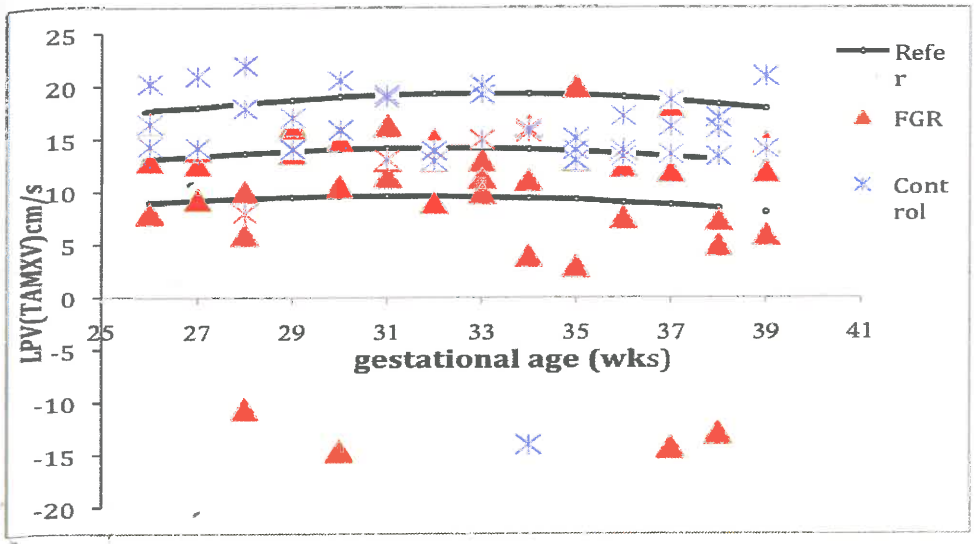
Fisher exact p-values (unmarked) are given for comparison versus normal controls. $: Fisher exact p-values for comparison versus FGR women with normal *AU*.

Table N 17. Neonatal outcome in FGR and control groups according to blood flow through the Left Portal Vein (TAMX, cm/sec) and splenic artery flow (PI).

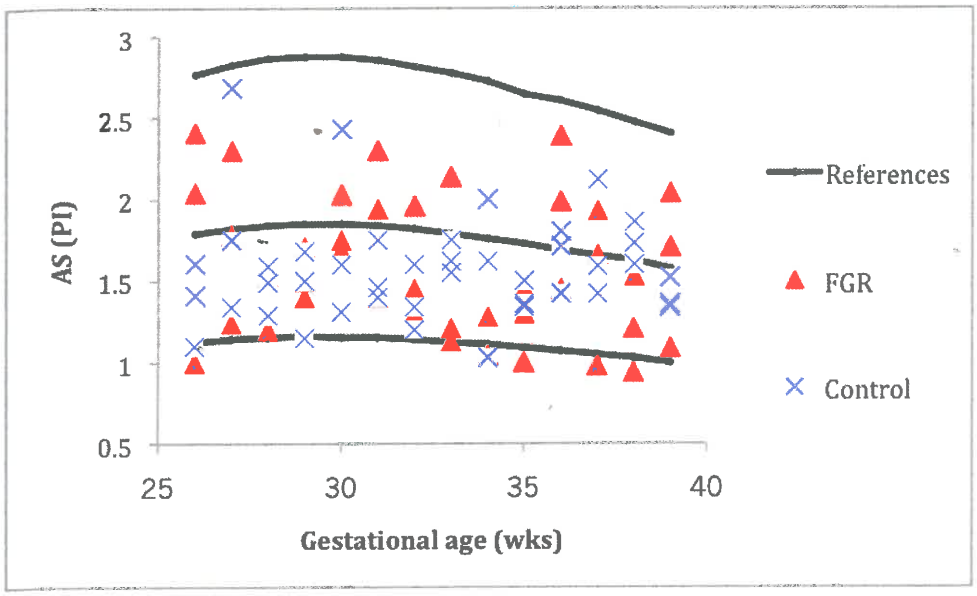
	FGR					Control n=42
	Total n=42	LPV <50% n=23	LPV <5% n=16	LPV ≥ 50% n=19	SA <5% n=10	
Perinatal mortality	3 (7.1)	3 (13.0) p=0.04	2 (12.5) p= 0.072	0 (0)	2 (20) p= 0.034	0
Apgar <7 at 5 min	6 (14.2) p= 0.025	4 (17.4) p= 0.013	3 (18.8) p= 0.018	2 (10.3)	2 (20) p= 0.034	0

Data given as number, percentage in parenthesis

Fisher exact test are given for comparison versus normal controls, p values are shown only if significant



A. Left portal vein



B. Splenic artery

Figure N 31. *The left portal vein (LPV) blood flow velocity (TAMXV, cm/s) (Panel A) and splenic artery pulsatility index (SA PI) (Panel B) in FGR (red triangles) and control (blue asterisk) groups plotted on normal ranges for gestational age with mean, 2.5th and 95th percentile*

8.7. Placental factors

8.7.1. Gross changes of placentas

Macroscopic findings of placenta and umbilical cord are represented in *Table N 18.* The mean weight of the placenta in the FGR group was 412g ± 117 versus 641± 133 in the control group ($p<0.001$). The fetal-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 18. 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

8.7.2. The umbilical cord

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 the 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 FRG and control women and all umbilical cords in both groups had 3 vessels. Macroscopic characteristics of umbilical cord are presented in *Table N 19.*

Table N 19. 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

8.7.3. Placental histological lesion

The histological findings are shown in *Table N 20.* There was no difference between FGR and control 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 20. 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

8.7.3.1 Placental histological lesion and smoking

There were nine smoking patients in FGR group (9/50, 18%), compared to none in the control group. Fifteen of the FGR women delivered preterm (30%) versus one in the control group.

Table N 21. Incidences of placental histological lesions in control and FGR groups

Histological findings	Control N=50	FGR (N=50)		
		Total (% of total)	Preterm (% of FGR) N=24	Smokers (% of FGR) N=8
Perivillous fibrin deposition	38 (76)	41 (82)	19 (79)	8 (100)
Stromal fibrosis	12 (24)	13 (26)	4 (17)	4 (50)
Cytotrophoblast proliferation	31 (62)	26 (52)	13 (54)	4 (50)
Villous infarction	7 (14)	17 (34) p=0.02 vs Contrl	9 (38)	5 (63) p=0.03 vs. Contrl
Thickening of the villous trophoblastic basal membrane	19 (38)	30 (60) p=0.03 vs Contrl	13 (54)	4 (50)
Intervillous thrombi/hematomas	6 (12)	21 (42) p<0.01 vs Contrl	12 (50)	5 (63) p=0.02 vs. Contrl
Villitis	3 (6)	13 (26) p=0.01 vs. Contrl	7 (29)	1(13)
Vasculitis	9 (18)	0 (0) p=0.01 vs. Contrl	0 (0)	0 (0)

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

In an attempt to adjust for possible interference due to differences in gestational age at delivery (preterm vs. term) and smoking, we stratified the data in the FGR group. In both subgroups of women with FGR, delivering preterm or smoking, we found similar rates of morphologic placenta abnormalities as in non-smoking and term delivering women having FGR, respectively *(Table N 21)*. However, the presence of thrombi or haematomas and villous infarction were more frequent in the smoking FGR group than in the controls (p=0.02 and 0.03, respectively, see *Table N 21*).

8.7.3.2 Microscopic lesions of the 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 in women with normal fetal growth and circulation (p=0.02, *Table N 22*). 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 22*), FGR patients with abnormal Doppler had the highest number of placental lesions (p=0.003) with more than 50% of four or more different lesions, compared to 24% in the normal women (p=0.007).

Among controls, two cases presented with elevated *AU* PI, and 7 with abnormal UtA blood flow (*Table N 23*). Fifteen of the 50 FGR patients had increased *AU* PI velocities of whom 6- ARED. Twenty two 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), cytotrofobalast hyperplasia (25/41 vs. 13/20), basal 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 (*Table N 23; Figure N 32*). However, intervillous haematomas or thrombi were encountered more frequently in the FGR patients with normal Doppler (5/41) than in controls with normal Doppler (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 in the control group. 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 having 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 23; Figure N 33*).

Table N 22. 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

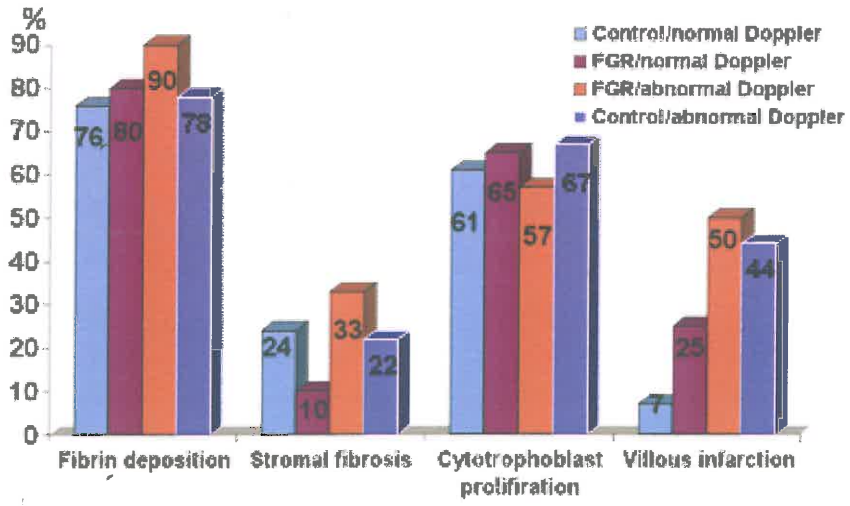


Figure N 32. Incidences of placental histological lesions (FGR with abnormal and normal Doppler velocimetry and Control with abnormal and normal Doppler profile)

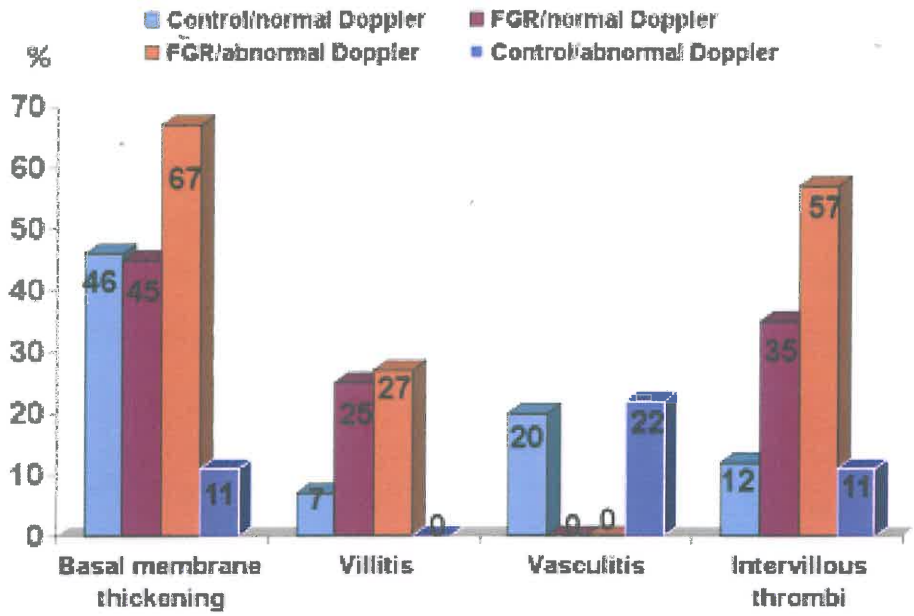


Figure N 33. Incidences of placental histological lesions (FGR with abnormal and normal Doppler velocimetry and Control with abnormal and normal Doppler profile)

Table N 23. Doppler study and placental microscopic lesions in the control and FGR groups

	Control			FGR			
	NL *UtA* & *AU* (n=41) (a)	**Abnl *UtA* (n=7)**	**Abnl *AU* (n=2)**	**NL *UtA* &*AU* (n=20) (b)**	**Abnl *UtA* (n=15)**	**Abnl *AU* (n=22)**	**Abnl *UtA* &*AU* (n=7)**
Cytotrophoblast proliferation	25 (61)	4 (67)	1 (50)	13 (65)	7 (47)	13 (59)	3 (43)
		5/9 (56)			17(57)		
Stromal fibrosis	10 (24)	1 (14)	0 (0)	2 (10)	5 (33)	8 (36)	3 (43)
		1/9 (11)			10 (33)		
Perivillous fibrin deposition	31 (76)	6 (86)	1 (50)	16 (80)	12 (80)	20 (91)	5 (71)
		7 (78)			27/30 (90)		
Villous infarction	3 (7) (a)	3 (43)	1 (50)	5 (25)	7 (47) p=0.002 vs. a	11 (50) p=0.0003 vs. a	3 (43) p=0.03 vs. a
		4/9 (44) p=0.014 vs a			15/30(50) p=0.001 vs. a		
Thickening of the basal membrane	19 (46)	1 (14)	0 (0)	9 (45)	11 (73)	13 (59)	4 (57)
		1/9 (11) (c)			20/30 (67) p=0.006 vs. c		
Intervillous haematomas/ thrombi	5(12) (a)	2 (29)	0(0)	7(35) (b) p=0.04 vs. a	7 (47) p=0.01 vs. a	16 (73) p<0.0001 vs. a p=0.029 vs. b	3 (43)
		2/9 (22) (c)			17/30 (57) p=0.02 vs. c		
Villitis*	3 (7) (a)	0 (0)	0 (0)	5 (25)	3 (20)	6 (27)	1 (29)
		0/9 (0)			8/30 (27)		
Vasculitis	8 (20) (a)	2 (29)	0 (0)	0 (0) p=0.04 vs. a	0 (0)	0 (0)	0 (0)
		2/9 (22) (c)			0/30 (0) p=0.018 vs. a p=0.048 vs. c		

Data are given as numbers, percentages in parentheses; p value shown only when significant
*Villitis was more frequent in FGR patients than in controls, p=0.01 respectively

9. DISCUSSION

9.1. 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 2-4*). 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 20th 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.

9.2. 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 21th and one 18th) 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.

9.3. Hemodynamic changes in relation to the fetal growth restriction

9.3.1. 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 (*Table N 21*). 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 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 (*Table N 5*), 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.

9.3.2. Adaptive response to impaired placental perfusion

The previous data confirm 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 normal 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 study. More studies are needed for better understanding of the underlying hemodynamic mechanism of adaptive changes in compromised fetuses.

9.3.3. 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 10th 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*). Four 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 N5*). 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

9.3.4. 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*).

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. Of course, other placental factors (immunological, metabolic and genetic) cannot be excluded. 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. So this study provides evidence that histological manifestation of FGR due to placental ischemia may be predicted by ultrasonography. Further studies relating Doppler findings during the first trimester with histological signs of placental ischemia in placentas are required to enable preventive action for FGR.

10. 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 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 in the placenta 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 profiles 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.

11. 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 90th 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.

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13. ORIGINAL PUBLICATIONS

1. Vedmedovska, N., Rezeberga, D., Teibe, U., Donders, G. G. G., Zodzika, J. (2010) Preventable maternal risk factors and association of genital infection with fetal growth restriction. *Gynecol Obstet Invest*, **70**, 219-226.

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14. SUPPLEMENTS

Appendix 1. Questionnaire

Socio-demographic variables

Age of mother, years

Type of residence

1. Urban
2. Rural

Level of education

1. Basic
2. Secondary
3. Secondary/professional
4. Higher/university

Marital status

1. Unmarried
2. Married

Employment status

1. Employed
2. Unemployed

Maternal BMI

Maternal Smoking

1. **Current smoking**
   a) yes (cigarettes per day)
   b) no
2. **Prenatal smoking**
   a) yes (cigarettes per day)
   b) no
3. **Quit smoking during pregnancy**
   a) yes (when, week of gestation)
   b) no
   c) reduce smoking (cigarettes per day)

Passive smoking (exposure to smoke)

a) yes

b) no

Maternal alcohol use (12 ounces of regular beer (5% alcohol) equals 5 ounces of table wine (12% alcohol) equals 1.5 ounces of hard liquor (40% alcohol). Beer, 12 ounces, is equivalent to 1 standard drink)

a) yes (how many drinks)

b) no

Maternal Recreational drug use

1. Current use

a) yes (type of the substances, amount)

b) no

2. Prenatal use

a) yes (type of the substances, amount)

b) no

3.Quit use during pregnancy

a) yes (when, week of gestation)

b) no

c) reduce (type of the substances, amount)

Gynecological history

1.STI/RTI in previous history

a) yes (*Trichomonas vaginalis*, *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, HIV, syphilis, vaginal herpes, BV, other _____ specify)

b) no

2.STI/RTI during current pregnancy

a) yes (*Trichomonas vaginalis*, *Chlamydia trachomatis*, *Neisseria gonorrhoeae* HIV, syphilis, vaginal herpes, BV, other _____ specify)

b) no

Gynecological anomalies

1) no

2) yes

a) Congenital uterine abnormalities (bicornuate or septated uterus, others)

b) Myoma

c) Ovary cystoma

d) Others

Gynecological operations

1) no

2) yes

a) Laparoscopy (diagnosis, y, outcome)

b) Hysteroscopy (diagnosis, y, outcome)

c) Laparatomy (diagnosis, y, outcome)

d) Others

Obstetric history

1.Total pregnancy number

2.TOP (termination of pregnancy) ≤ 12 weeks of gestation for social reason (number, y)

3. TOP (termination of pregnancy) ≤ 12 weeks of gestation for medical reason (number, y)

4. Miscarriages (number, y)

5. Stillbirth (number, y, gestational age,)

6. Vaginal delivery (number, y)

7. SC in previous history (number, y)

8. Extrauterine pregnancy in history (number, y)

9. Interval between last and current pregnancies (months)

Complications after deliveries or after TOP (termination of pregnancy)

1) no

2) yes

a) Manual ablation of the placenta

b) Instrumental ablation of the placenta or placental tissues

c) Endometritis

d) Others

Premature deliveries in previous history

1) no

2) yes (number, y, gestational age, outcome)

Concurrent medical conditions

1) no

2) yes

a) Endocrine System Diseases (thyroid gland, pituitary gland adenoma, others)

b) Heart and Vascular Diseases (congenital heart defects, peripheral vascular diseases, others)

c) Kidney Diseases (Congenital Disease, Acquired Kidney Disease (inflammation, others)

d) Respiratory Diseases (Chronic Obstructive Pulmonary Disease, Infectious Respiratory Diseases, others)

e) Neurological disorders (Back Pain, Cephalic disorder, Compression neuropathy, syndromes, others)

f) Others

Medication for therapeutic reason during pregnancy

1) no

2) yes (type, dose)

Current pregnancy

1. Natural conception

a) yes

b) no

2. Induced

a) yes (IVF/ICSI, insemination, CC induction)

b) no

Antenatal care

1. yes

a) Adequate (screening for serological and cervico-vaginal infections, including serology for antibodies (lues), wet mount microscopy (Trichomonas, bacterial vaginosis), cultures (gonorrhea, mycoplasmata) and PCR (*Chlamydia trachomatis)*

b) Intermediate

c) Inadequate

2. no

Complications in current pregnancy

1. Bleeding in early pregnancy

a) light

b) heavy

c) treatment

2. Threatened premature delivery

a) Gestational age

b) Treatment

3. Pregnancy anemiá

a) Gestational age

b) Treatment

4. Urine tract infections

a) Gestational age

b) Treatment

Viral upper respiratóry tract infection

a) Gestational age

b) Treatment

3) Pregnancy induced hypertension

a) Gestational age

b) Treatment

Pre-eclampsia

a) Gestational age

b) Treatment

Others

Appendix 2.

The list of follow-up and outcome analyzes.

Grūtniecības laiks iestājoties pētījumā/nedēļas

1.grūtniecības laiks pēc mēnešreizēm/nedēļas

2. pēc agrīnas(pirmās) sonogrāfijas/ nedēļas

3.grūtniecības laiks pēc ultrasonogrāfijas/nedēļas, iestājoties pētījumā

4.augšanas atpalicība _____ nedēļas

Biometriskie un Dopplerometriskie rādītāji iestājoties pētījumā

BPD, HC, AC, FL, EEW

A.umbilicalis

PI(cipari)

A.umbilicalis

1. neizmainīta asinsrite;

2. saglabājas beigu diastoliska plūsma, bet paaugstināts RI vai PI;

3. iztrūkst beígu diastoliskā asins plūsma;

4. reversa plūsma vēlīnā diastolē

V.umbilicalis

1. nav pulsācijas; 2. ir pulsācija

Ductus venosus

PI(cipari)

Ductus venosus

1. neizmainīta plūsma; 2. izmainīta, bet saglabājas a-viļņa plūsma; 3. iztrūkst vai ir reversa a-viļņa plūsma

Arteria Cerebri Media

PI(cipari)

PSV(cipari)

Arteria Cerebri Media

1. ir" brain sparing" efekts; 1. nav „brain sparing" efekts

A.Splenica

PI(cipari)

LPV

TAMX (cm/s, cipari)

Amniotiskā šķidruma daudzums

1. AFI>5; 2. AFI<5

Kardiotokogramma

1. reaktīva; 2. areaktīva; 3. ar decelerācijām; 4. citas izmaiņas.....

Biometriskie un Dopplerometriskie rādītāji ik 5-14 dienām(no pētījuma sākuma); (šo daļu var atkārtot ik pēc 5-10-14 dnn, atkarībā no situācijas)

grūtniecības laiks pēc ultrasonogrāfijas/nedēļas

BPD, HC, AC, FL, EEW

A.umbilicalis

PI(cipari)

A.umbilicalis

1. neizmainīta asinsrite;

2. saglabājas beigu diastoliska plūsma, bet paaugstināts RI vai PI;

3. iztrūkst beigu diastoliskā asins plūsma;

4. reversa plūsma vēlīnā diastolē

V.umbilicalis

1. nav pulsācijās; 2. ir pulsācija

Ductus venosus

PIV(cipari)

Ductus venosus

1. neizmainīta plūsma; 2. izmainīta, bet saglabājas a-viļņa plūsma; 3. iztrūkst vai ir reversa a-viļņa plūsma

Arteria Cerebri Media

PI(cipari)

PSV(cipari)

Arteria Cerebri Media

1.ir" brain sparing" efekts; 1.nav „brain sparing" efekts

A.Splenica

PI(cipari)

LPV

TAMX (cm/s, cipari)

Amniotiskā šķidruma daudzums

1.AFI>5; 2.AFI<5

Kardiotokogramma

1. reaktīva; 2. areaktīva; 3. ar decelerācijām

Izvestā ārstēšana

1. nē

2. jā

a. i/v šķidrums

b. cita (kāda)_____

Antenatāla nāve

1. nē

2. jā

a. nedēļas

Dzemdības

1 gestācijas laiks/ nedēļas (cipari)

2. grūtniecības laiks pēc ultrasonogrāfijas/nedēļas

3. augšanas atpalicība _____ nedēļas

Grūtniecības atrisināšana

1.spontānas dzemdības

2. inducētas/izraisītas

3. vaginālas asistētas (forceps, vakuumekstrakcija)

4. ķeizargrieziens pirms dzemdību darbības sākuma

5. ķeizargrieziens dzemdību laikā

Indikācijas dzemdību indukcijai

1.izmainīts BFP;

2.izmainīta KTG ;

3. izmaiņas dopplerogrāfijas rādītājos

Indikācijas SC

1.patoloģiska KTG (decelerācijas);

2. kritiski izmainīti dopplerogrāfijas rādītāji

Apgares novērtējums 1 minūtē pēc dzimšanas

1.>7balles; 2.<7balles

Apgares novērtējums 5 minūtē pēc dzimšanas

1.>7balles; 2.<7balles

Apgares novērtējums 10 minūtē pēc dzimšanas

1.>7balles; 2.<7balles

Jaundzimušā ķermeņa masa

grami

Jaundzimušā dzimums

1. meitene

2. puika

Dzimis iznēsāts

1.nē

2.jā

Dzimis neiznēsāts

1. nē

2. jā

Piedzimis retardēts

1. nē
2. jā

Piedzimis mazs ģestācijas laikam

1. nē
2. jā

Jaundzimušā veselības stāvoklis pēc dzemdībām

1. ar māti;
2. bērnu nodaļā;
3. JITN;
4. miris JITN

Jaundzimušā ārstēšanas ilgums JITN

1. nebija; 2. 1-3dnn; 3. 4-7dnn

Jaundzimušais tālākai terapijai pārvests uz Bērnu slimnīcu

1. nē 2.jā

Jaundzimušā veselības stāvoklis

1. nav nepieciešama terapija;
2. RDS;
3. mekonija aspirācijas sindroms;
4. pulmonāla asiņošana;
5. sepse;
6. iedzimtas anomālijas (kādas)
7. Citas komplikācijas

Placentas makroskopiska apskate

Placentas biezums cm_____

Placentas svars g _____

augļa/placentas koeficients

Nabassaites garums, cm

Nabassaites īpatnības

1. nē
2. jā
    a. īstais mezgls
    b. ļoti tieva
    c. izteikts savijums

Nabassaites apvijums ap bērna kaklu

1. nē
2. jā
    a. 1 reízi
    b. 2 reizes
    c. 3 un vairāk reizes

Nabassaites asinsvadu skaits

1. 3 as/v
2. 2.as/v
3. 1.as/v

Nabassáites piestiprināšana

1. centrāla
2. laterāla
3. plēvēs

Apendix 3.

Placentas histoloģiska izmeklēšana

Placentas mikroskopiska apskate

Placentas biezums mm_____

vai ir konstatēts starpbārkstiņu fibrīna nogulsnējums/depozīcija

1.nē
2.jā

vai ir konstatēta stromāla fibroze

1.nē
2.jā

vai ir konstatēta citotrofoblasta hiperplāzija

1.nē
2.jā

vai ir konstatēti infarktu perēkli

1. nē
2.jā

vai ir konstatēts bazālās membrānas sabiezējums

1.nē

2.jā

Vai ir konstatēts iekaisums

1.nē

2.jā (villīts ( ); intervilīts ( ); vaskulīti ( )

Vai ir konstatētas hemorāģijas

1.nē

2.jā

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