Low birthweight

Definition, screening and prediction

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AALBORG UNIVERSITY
DENMARK

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ENGLISH SUMMARY

The definition of low birthweight (BW) varies worldwide, as different BW curves are used as references. Screening for low BW is an important part of the antenatal care, as low BW may be a result of fetal growth restriction (FGR) due to placental dysfunction. In these high-risk fetuses, correct antenatal identification of small-for-gestational-age (SGA) allows for timely delivery and rational use of obstetric interventions, which improves the obstetric outcome. Unfortunately, SGA screening is challenged by low sensitivity and high false positive rates (FPR).
Therefore, the aim of this project was to compare various definitions of low BW. The performance of the antenatal SGA screening was investigated in a local clinical setting and the prediction of low BW and other placenta-related outcomes was investigated using T2* weighted placental magnetic resonance imaging (MRI) in a cohort of suspected SGA pregnancies. Finally, the use of low BW as a proxy of placental dysfunction was critically discussed.

In this project, we demonstrated that the definition of low BW was markedly different when comparing a universal standard BW curve with a Danish standard BW curve. This finding does not support the idea that one universal BW curve can be used in all populations. Low BW was associated with an increased risk of adverse neonatal outcomes. However, the majority of adverse outcomes occurred in non-SGA pregnancies (***Study I***). The performance of the Danish antenatal SGA screening has improved over the last 20 years. However, in a local clinical setting, the sensitivity at term remains rather low. Antenatal classification of SGA increased the risk of obstetric interventions in SGA neonates as well as in normal weighted neonates (***Study II***). Finally, in a cohort of suspected SGA pregnancies with normal fetal Doppler flows, placental dysfunction is frequent. In this cohort, T2* weighted placental MRI was a strong biomarker of placental dysfunction regardless of clinical manifestations such as low BW (***Study III***).

In conclusion, the antenatal detection of low BW is challenged by different reference curves, and low sensitivity of the SGA screening programs. In addition, fetal size alone does not perfectly reflect placental function. Therefore, new direct markers of placental dysfunction are of outmost clinical importance to improve the antenatal identification of placental dysfunction.

DANSK RESUMÉ

Definitionen af lav fødselsvægt varierer internationalt, da der anvendes forskellige referencekurver og forskelligt cut-off. Screening for lav fødselsvægt er en vigtig del af svangreomsorgen, da lav fødselsvægt kan være resultatet af vækshæmning forårsaget af placentadysfunktion. Korrekt antenatal identifikation af disse høj-risiko fostre gør det muligt at forløse rettidigt samt at sikre rationel brug af obstetriske interventioner, hvilket forbedrer det obstetriske udfald markant. Screeningen for small-for-gestational-age (SGA) er dog udfordret af lav sensitivitet og høje falsk positive rater.

Formålet med dette projekt var at sammenligne forskellige definitioner af lav fødselsvægt. Performance af den danske antenatale SGA-screening blev undersøgt i en lokal, klinisk kohorte. Desuden blev prædiktionen af lav fødselsvægt og andre placenta-relaterede udfald undersøgt ved hjælp af T2* vægtet placenta magnetisk resonans (MR-) skanning blandt graviditeter med mistænkt SGA. Afsluttende findes en kritisk diskussion af anvendelsen af lav fødselsvægt som proxy for placentadysfunktion.

I dette projekt demonstrerede vi, at definitionen af lav fødselsvægt var markant forskellig ved brug af en universel standardkurve sammenlignet med en dansk standardkurve for fødselsvægte. Dette fund støtter ikke idéen om én universel standardkurve for fødselsvægte til brug i alle populationer verden over. Lav fødselsvægt var associeret med øget risiko for dårlige neonatale udfald. Dog fandtes størstedelen af disse udfald blandt normalvægtige fostre ***(Studie I)***. SGA-screening i Danmark er forbedret væsentligt over de seneste 20 år, dog ses et markant fald i sensitiviteten til terminen. Forventet SGA medførte en øget risiko for obstetriske interventioner uanset barnets fødselsvægt ***(Studie II)***. Forekomsten af placenta-dysfunktion var høj iblandt fostre med mistænkt SGA og normale Doppler flow målinger. T2* vægtet MR-skanning var en stærk biomarkør for placentadysfunktion uanset de kliniske manifestationer så som lav fødselsvægt ***(Studie III)***.

Det kan konkluderes, at den antenatale detektion af lav fødselsvægt er udfordret af forskellige referencekurver og screeningsmodeller med lav sensitivitet og høje falsk positive rater. Desuden er fosterets størrelse alene ikke en perfekt markør for placentas funktion. Derfor er der et stort klinisk behov for nye markører som afspejler placentas funktion direkte, for på den måde at forbedre identifikationen af placentadysfunktion under graviditeten.

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LIST OF PAPERS

This PhD thesis is based on three studies presented in the following papers:

Study I
Construction of a Danish Birthweight Standard Curve and the comparison with the Intergrowth Newborn Standard: A nationwide register-based cohort study.

Ditte N Hansen, Henriette S Kahr, Christian Torp-Pedersen, Jan Feifel, Niels Uldbjerg, Marianne Sinding, Anne Sørensen.

Submitted to British Journal of Obstetrics and Gynaecology.

Study II
Screening for small-for-gestational-age fetuses.

Ditte N Hansen, Helle S Odgaard, Niels Uldbjerg, Marianne Sinding, Anne Sørensen.

Published in Acta Obstetricia et Gynecologica Scandinavica, 2020: 99(4); 503-9. DOI: 10.1111/aogs.13764.

Study III
T2 weighted placental MRI: A predictor of placenta-related outcomes in small-for-gestational-age pregnancies.*

Ditte N Hansen, Marianne Sinding, Astrid Petersen, Ole B Christiansen, Niels Uldbjerg, David A Peters, Jens B Frøkjær, Anne Sørensen.

In preparation.

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ABBREVIATIONS

AC	Abdominal circumference
AGA	Appropriate-for-gestational-age
ATC	Anatomical Therapeutic Chemical Classification System
AUC	Area under the (ROC) curve
B_{1+RMS}	Root mean square of the MRI effective component of the B(1) field
BMI	Body mass index
BPS	Biophysical profile score
BW	Birthweight
CI	Confidence interval
CPR	Cerebroplacental ratio
CPR-number	Civil registration number
CTG	Cardiotocography
dB	Decibel
DV	Ductus venosus
EFW	Estimated fetal weight
EFW_{us}	Estimated fetal weight by ultrasound scan
FGR	Fetal growth restriction
FHR	Fetal heart rate
FOV	Field of view
FPR	False positive rate
FVM	Fetal vascular malperfusion
GA	Gestational age
HELLP	Hemolysis elevated liver enzymes low platelet
ICD	International Classification of Diseases and Related Health Problems
ICNIRP	International Commission on Non-Ionizing Radiation Protection
IG-21	International Fetal and Newborn Growth Standards for 21st Century (also abbreviated as Intergrowth-21st)
MCA	Middle cerebral artery
MoM	Multiples of Median
MRI	Magnetic Resonance Imaging
MS	milliseconds
MVM	Maternal vascular malperfusion
M_0	Equilibrium magnetization
NICU	Neonatal intensive care unit
OR	Odds ratio
OR_{adj}	Adjusted odds ratio
PAPP-A	Pregnancy-associated Plasma Protein-A
PHE	Placental histological examination
PI	Pulsatility Index
PlGF	Placental growth factor
PPROM	Preterm premature rupture of membranes

REDCap	Research Electronic Data Capture
ROC	Receiver operating characteristics
ROI	Region of Interest
SAR	Specific absorption rate
SD	Standard deviation
s-Flt-1	Soluble fms-like tyrosine kinase-1
SGA	Small-for-gestational-age
T	Tesla
T1	Longitudinal relaxation time
T2	Transversal relaxation time
T2*	Transverse relaxation time
TE	Echo time
TR	Repitition time
UA	Umbilical artery
US	Ultrasound scan
UtA	Uterine artery
VUE	Villitis of unknown etiology
W	Watt
WHO	World Health Organization

CHAPTER 1. INTRODUCTION

A neonate is born with low birthweight (BW) when BW is below normal for gestational age (GA). This definition may sound obvious. However, it remains a matter of debate, as the definition of normal BW tends to vary between centers and countries.[1–4] Different definitions of normal BW may lead to different proportions of low BW neonates and different rates of obstetric interventions. In order to improve the obstetric outcome, it is important to agree on basic definitions and select rational BW curves for the population of interest.
Screening for low BW is an important part of antenatal care, as low BW is associated with an increased risk of adverse neonatal outcomes.[5–9] Fetal growth can be assessed by an external clinical examination and symphysis-fundal height measurement[10] or by ultrasound assessment of fetal biometries.[11–14] Antenatal identification of small-for-gestational-age (SGA) fetuses allows for timely delivery and obstetric interventions, which improves the obstetric outcome by up to 4-fold.[5] Unfortunately, the antenatal screening for SGA is challenging. In large centers, with routine ultrasound screening in the third trimester, the sensitivity of the screening program is reported to be 77% at a false positive rate (FPR) of 13%[12], whereas selective screening on indication has a lower sensitivity of 32% at a FPR of 3%.[12] In Denmark, ultrasound screening for SGA is on indication only; however, the number of women referred for ultrasound scans and the performance of SGA screening remains unexplored.

Among low BW neonates, some are constitutionally small.[6] These neonates have good outcomes with no need for obstetric interventions or early delivery. However, low BW may also be a result of placental dysfunction.[1,6,15–17] In case of placental dysfunction, the neonates have failed to reach their genetic growth potential; they are suffering from fetal growth restriction (FGR). This is a pathological condition associated with intrauterine fetal hypoxia and acidosis[9,18,19], which leads to an increased risk of obstetric complications.[5–8] FGR fetuses will benefit from early delivery, and very often they need obstetric interventions in labor. Unfortunately, it is an obstetric challenge to identify FGR among SGA fetuses, as both fetuses have small size. However, direct markers of placental function may improve the antenatal identification. Over the last decade, T2* weighted placental magnetic resonance imaging (MRI) has demonstrated the ability to identify placental dysfunction during pregnancy.[20–25] However, the method has never been described in a clinical well-defined SGA cohort.

CHAPTER 2. BACKGROUND

This chapter contains four parts. First, the definition of low BW is discussed. The second part explains the antenatal screening of low BW including current methods and their performance. The third part introduces placental dysfunction as a cause of low BW, and the last part addresses the challenges of antenatal identification of placental dysfunction. Current clinical markers of placental dysfunction are described, and T2* weighted placental MRI is introduced as a new predictor of placenta-related obstetric complications.

2.1. LOW BIRTHWEIGHT

In Denmark, 2.8% of neonates have low BW, when defined as BW≤ -22% of the expected for GA using the Scandinavian reference BW curve by Maršál et al.[26] At term, the low BW cut-off is 3634 grams for males and 3522 grams for females.[26] However, SGA definitions tend to differ between countries and centers all over the world.[1–4,27] Basically, there are two different types of BW curves[28]: ***reference curves,*** based on unselected populations describing how neonates have grown at a specific time and place, or ***standard curves,*** based on selected pregnancies describing how normal, healthy neonates should grow. Some centers claim to have universal BW curves that are relevant for all populations, while others use local or national BW curves. ***Customized BWs*** include maternal characteristics such as height, weight, parity and ethnicity for estimating individiualized normal BW curves.[29] Very often BW is given as deviation (percentage) rather than absolute size (gram). BW deviation provides information on how much the neonate deviates from normal weight at that exact GA.

Accordingly, the weight curves used to determine BW deviation is very different worldwide.[26,27,30–32] Some weight curves are based on ultrasound estimated fetal weight (EFW)[26], others on BW[27,30–32]. At term, BW may represent normal growth. However, neonates that are born preterm tend to be smaller than intrauterine fetuses at equivalent GA. Therefore, curves based on BW may underestimate normal growth at early gestation[33], and weight curves based on EFW may be more valid. However, this approach is challenged by inaccurate ultrasound estimates of fetal weight.[34,35] Hence, at term, BW curves may be valid for describing normal fetal growth.

2.2. ANTENATAL SCREENING OF LOW BIRTHWEIGHT

It is well known that low BW is associated with adverse obstetric outcomes, such as stillbirth, asphyxia in labor and admission to neonatal intensive care units.[5–9] The more BW deviates from normal, the higher the risk of adverse obstetric outcomes.[8] Because of this risk, antenatal assessment of fetal growth is an important part of the antenatal care. Antenatal identification of SGA may lead to timely delivery and allow for obstetric interventions, which may improve the neonatal outcome considerably.[5,36]

Fetal size can be assessed by clinical examination and symphysis-fundal height measurements.[14] Moreover, ultrasound EFW are provided by fetal biometries (fetal head circumference, abdominal circumference and femur length) using the Hadlock formula.[34] The standard deviation of EFW based on the Hadlock formula is 8%[34], which implies that fetuses with weight close to the SGA cut-off may not be identified antenatally. Estimation of fetal biometries may be inaccurate particularly in cases of maternal obesity or suboptimal fetal positions. In addition, the Hadlock formula was developed in symmetric, normally grown fetuses, so the precision of the formula is reduced in cases of extreme weight such as SGA or large-for-gestational-age fetuses.[35]

The program for SGA screening tends to vary between countries and centers. In some centers, ultrasound EFW is performed routinely in the third trimester[12,37,38], while other centers do ultrasound EFW on indication only[37,39]. Screening for SGA is not perfect, and even in a routine setting, the sensitivity is less than 77% at a FPR of 13%.[12] In Denmark, ultrasound EFW is performed on indication, and therefore the sensitivity of the program is probably even lower. However, the last investigation in Denmark of the proportion of pregnant women who are referred to third trimester ultrasound and the performance of the Danish screening program is based on data from 1996-98.[39] Back then, only 3.7% had an EFW due to SGA suspicion, which resulted in a sensitivity of 29% at an FPR of 0.26%.[39] Another matter of concern in SGA screening is the complications related to false positive cases of SGA screening. A false diagnosis of SGA leads to more obstetric interventions[40] and lower GA at birth probably due to a higher rate of labor induction[41]. On the contrary, false negative cases of SGA screening are associated with higher risk of adverse outcomes including stillbirth.[5] Nevertheless, previous literature is conflicting in regards to the benefits and potential harm of the SGA screening.[5,41–45]

2.3. ETIOLOGY OF LOW BIRTHWEIGHT

Low BW may have different etiologies. Some low BW neonates are constitutionally small. These are low risk pregnancies without an increased risk of obstetric complications. In contrast, some low BW neonates suffer from placental dysfunction.

These neonates do not reach their genetic growth potential because of an inadequate supply of oxygen and nutrients from the placenta. This is a pathological condition, with an increased risk of fetal hypoxia and acidosis[18], which leads to serious short-term and long-term complications.[46,47] Short-term complications include e.g. asphyxia[47] and intrauterine fetal death[46,48], whereas long-term complications include risk of neurological impairment[47,49,50], cardiovascular disease[51,52] and insulin resistance[53]. Currently, the explanation of long-term complications is 'fetal programming' as the abnormal intrauterine environment may change the genetic profile of the fetus by complex epigenetics.[54] This may lead to a specific metabolic phenotype in adult life. This hypothesis is also known as Barker's hypothesis.[55,56]

The placenta

The normal human placenta at term is a disc-shaped organ with an average weight of 470 g.[57] The placenta consists of a fetal side (the chorionic plate) and maternal side (the basal plate) (Figure 1). The basal plate attaches the placenta to the uterine wall, whereas the chorionic plate includes the chorionic vessels, a continuation of the umbilical cord. The branches of the villous tree are bathed in maternal blood, which enters the intervillous space through the spiral arteries. The intervillous space is bounded by the basal and chorionic plate, which at the marginal zone, in the periphery, fuse to form the chorion leave ("fetal membranes").[57] The formation of the placenta begins about day 6-7 post conception, when the blastocyst attaches to the uterine wall.[57] The syncytiotrophoblast invades the uterine endometrium to uncover the maternal capillaries. The implantation continues until invasion of the spiral arteries. The trophoblast replaces the endothelium within these vessels and destructs the muscular wall. This physiological transformation is the remodelling of spiral arteries.[57,58] The functional unit of the placenta is the villous tree in which the exchange between maternal and fetal circulation occurs.[54,59] The terminal villi are the final branches of the villous tree with a high degree of capillarization and an extremely thin placental barrier to increase the transport capacity.[57] During pregnancy, the maturation of the villous tree changes the organization of the vessels and surrounding stroma, as the vascular volume of the villous increases, the membrane gets thinner and the surface area increases.[60] This maturation of the villous tree increases the efficiency of the placenta to meet the metabolic demand of the growing fetus.

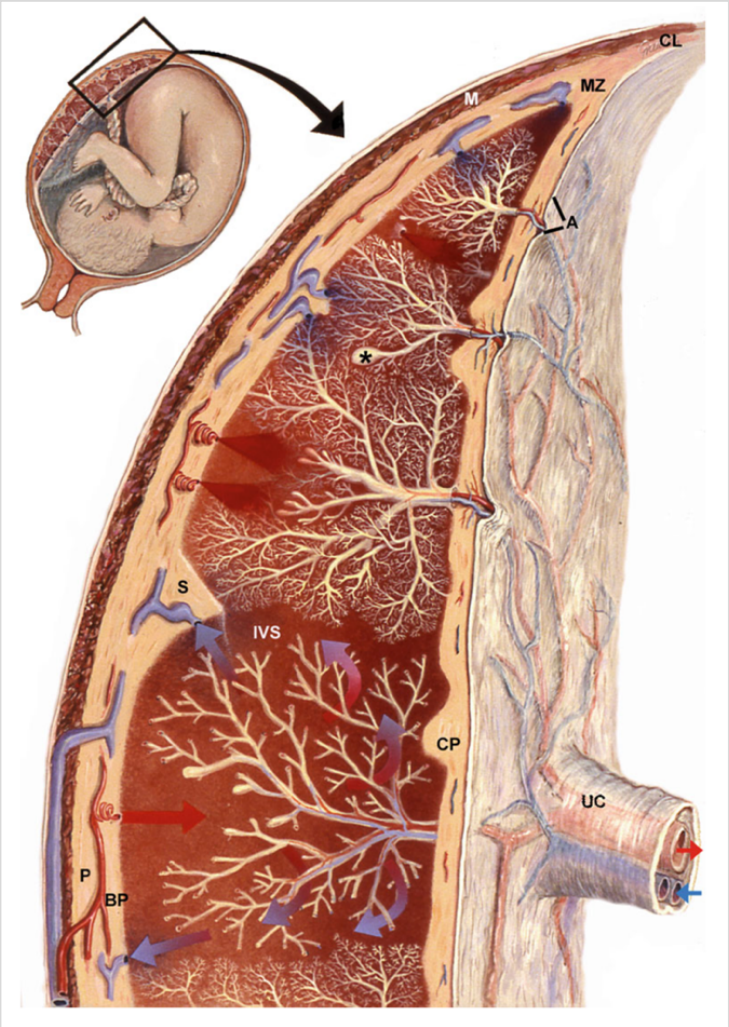

Figure 1 The mature human placenta.

A, amnion, BP, basal plate, CL, chorion leave, CP, chorionic plate, IVS, intervillous space, M, myometrium, MZ, marginal zone, P, placental bed, S, placental septum, UC, umbilical cord. From Kaufmann and Scheffen[61], with permission from publisher (Elsevier).

Placental dysfunction

The true prevalence of placental dysfunction is not known, but it is likely 5-10% depending on definitions.[1–4] In case of placental dysfunction, the insufficient remodeling of spiral arteries causes placental hypoxia through maternal vascular malperfusion (MVM).[62] MVM leads to hypoplasia of the villous tree, resulting in reduced surface area for the maternal-fetal exchange.[63] Moreover, the terminal villi may have a thickened the placental barrier and decreased number of transport molecules, which reduces the exchange capacity.[64] These changes may lead to placental dysfunction.

Placental dysfunction may be diagnosed post partum, when the placental histological examination (PHE) reveals placental lesions associated with dysfunction. According to an international consensus statement[63], the pathological lesions of the placenta are subdivided into 1) vascular processes and 2) inflammatory-immune processes.[63,65] The vascular processes are further subdivided into MVM and fetal vascular malperfusion (FVM).[63]

MVM is a consequence of abnormal spiral artery flow and includes both macroscopic and microscopic findings.[63,65] The macroscopic findings are placental hypoplasia (weight <10th centile according to GA), infarcts (in preterm placenta or >5% non-peripheral infarction at term) and retroplacental hemorrhage (corresponding to the clinical diagnosis of placental abruption).[63] Microscopic findings include distal villous hypoplasia, which is more common <32 weeks of gestation, and accelerated villous maturation. Distal villous hypoplasia is the paucity of villi in relation to the surrounding stem villi affecting >30% of all distal villi[63,65], whereas accelerated villous maturation is the presence of hypermature villi for gestation with an increase in syncytial knots (knots on more than >33% of villi at term).[63,65]

FVM is most likely caused by the obstruction of fetal blood flow and includes findings of thrombosis, avascular villi and delayed villous maturation. Thrombosis may be of both arterial or venous origin. Avascular villi are the endpoint after degeneration of villi with a total loss of capillaries within the terminal villi and fibrosis of villous stroma. Delayed villous maturation is rarely seen before 34 weeks of gestation. It is characterized by excessive villous stroma, lack of syncytial membranes surrounding the capillaries and decreased fetal-placental weight ratio.[63,65]

The inflammatory-immune processes include all types of infectious inflammatory responses and villitis of unknown etiology (VUE).[63,65] VUE may be caused by a type of graft-versus-host reaction.[66] VUE is subdivided into low grade (affecting <10 contiguous villi with more than one focus) or high grade (multiple foci on more than one section with at least one area with > 10 contiguous villi affected). The grading of VUE is important since the severity affects the recurrence risk and long-term consequences for the infant.[66]

Placental dysfunction is divided in two subtypes, which may have different pathophysiology and clinical phenotype: early-onset before 32 weeks of gestation and late-onset ≥ 32 weeks of gestation.[15] In case of placental dysfunction, a certain timeline of physiological changes in the fetus is associated with the progression of fetal hypoxia, leading to acidosis and cardiac failure, fetal hypotension, vascular collapse, and ultimately intrauterine fetal death.[9,67] Hypoxia leads to a redistribution of oxygenated blood, leading to an increased cerebral blood flow caused by a

reduction in the resistance of the middle cerebral arteries (MCA). Afterwards, ductus venosus within the fetal liver ensures the shunting of oxygenated blood from the umbilical vein directly to the heart and brain at the expense of the liver.[9,68,69] Continued deterioration of the ductus venosus Doppler flow reflects increasing cardiac failure and fetal acidosis.[69–71]

Early-onset placental dysfunction is in particular associated with morphological signs of MVM, as a consequence of insufficient trophoblast invasion and lack of spiral artery remodelling.[72] Figure 2 demonstrates the timeline of fetal distress and the corresponding Doppler flow measurements in early-onset placental dysfunction. In early-onset placental dysfunction, the fetal deterioration may occur in 4 to 6 weeks.[9]

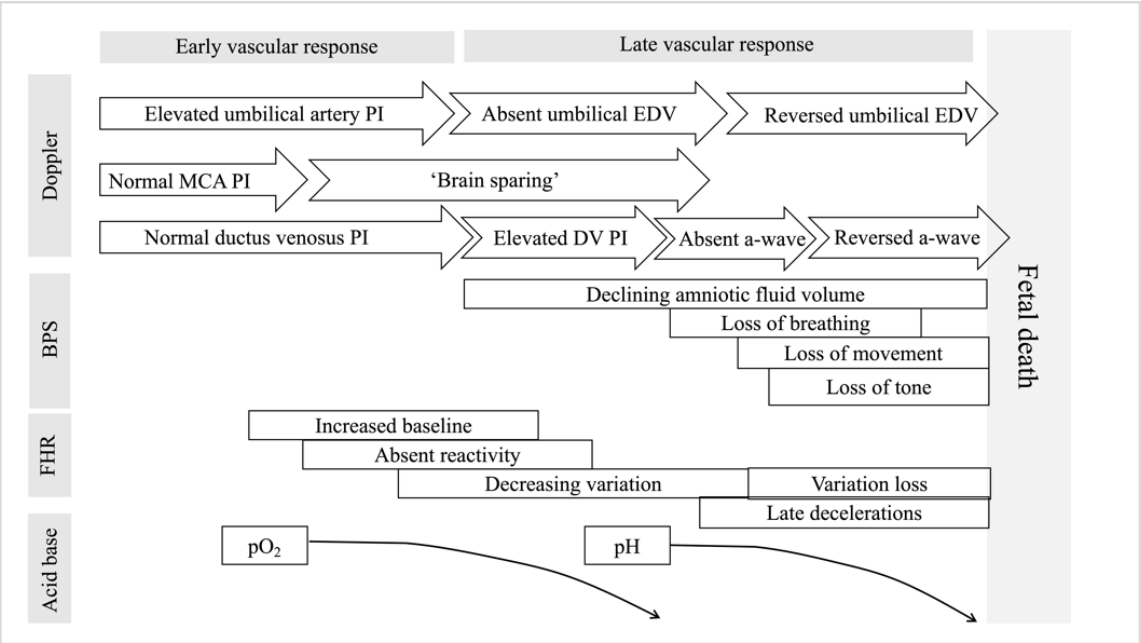

Figure 2 Fetal response to early-onset placental dysfunction.

PI, pulsatility index, EDV, end-diastolic velocity, MCA, middle cerebral artery, DV, ductus venosus, BPS, Biophysical profile score, FHR, fetal heart rate. Adapted from Baschat[9].

Late-onset placental dysfunction is a more heterogenous group. In late-onset, the pulsatility index (PI) of the umbilical artery (UA) may be elevated, however, only in cases of extensive involvement of the placenta.[13] Instead, the cerebroplacental ratio (CPR), which combines UA PI and MCA PI, decreases as a sign of hypoxia.[13,73–75] Figure 3 shows the physiological changes in late-onset placental dysfunction, the progression may occur in up to 9 weeks.[9] As demonstrated in Figures 2 and 3, Doppler flow measurements are an important tool in fetal monitoring in SGA pregnancies.

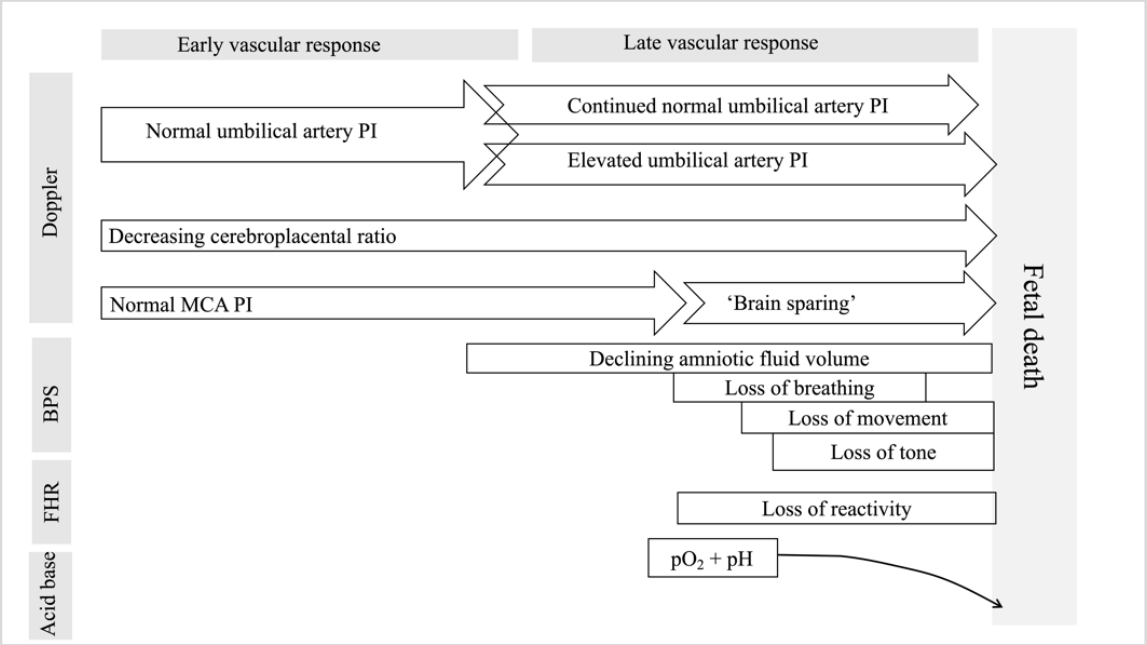

Figure 3 Fetal response to late-onset placental dysfunction.

PI, pulsatility index, MCA, middle cerebral artery, BPS, biophysical profile score, FHR, fetal heart rate. Adapted from Baschat[9]

2.4. ANTENATAL IDENTIFICATION OF PLACENTAL DYSFUNCTION

2.4.1. FETAL ASSESSMENT

2.4.1.1 Fetal size

Fetal size is the most frequently used proxy of placental function. Assessment of fetal growth by clinical examination and symphysis-fundal height measurements is mandatory in the low risk antenatal clinic.[14] Moreover, ultrasound EFW can be performed either routinely or on indication.[11–14] However, fetal size is not a perfect proxy of placental function, as intrauterine EFW are imprecise. In addition, some small fetuses are constitutionally small but not suffering from placental dysfunction. It is optimal to include more variables to identify FGR among SGA fetuses. If the fetuses are very small, if the abdominal circumference (AC) is very small, or if the fetal circulation is affected, then it is more likely that the fetus is suffering from placental dysfunction. In order to reach international consensus on the definition of early- and late-onset FGR, a Delphi procedure was performed in 2016[15], and the following definitions were agreed upon by a panel of experts. Early FGR before 32 weeks of gestation is defined by AC < 3rd centile, EFW < 3rd centile or absent end-

diastolic flow in the UA. Moreover, early FGR can be defined by AC or EFW < 10th centile in combination with uterine artery (UtA) PI > 95th centile and/or UA-PI > 95th centile. Late FGR is ≥ 32 weeks of gestation with AC < 3rd centile or EFW < 3rd centile. Moreover, late FGR can be defined by a combination of at least two of three following criteria; AC or EFW < 10th centile, AC or EFW crossing centiles > 2 quartiles on non-customized growth centiles and CPR < 5th centile or UA-PI > 95th centile.

2.4.1.2 Doppler flow measurements

To improve the identification of FGR, fetuses suffering from placental dysfunction among SGA pregnancies, Doppler flow measurements are added to fetal weight estimates for additional information on the fetal and umbilical circulation.[14,15,67,76]

Doppler flow measures of UA, MCA and ductus venosus (DV) are registered as GA-corrected PI, automatic calculated as PI = (peak systolic flow velocity - enddiastolic flow velocity)/time-averaged maximum flow velocity. PI reflects the downstream vascular resistance. The clinical use of PI is facilitated by this measure being angle independent.

Umbilical artery (UA)

Doppler flow measurements of the UA reflects vascular resistance in the placenta.[67] In early-onset FGR, abnormal UA PI provides a strong indicator of placental dysfunction; however, at later gestation, UA PI often remains normal even in cases of severe placental dysfunction.[77,78]

Middle cerebral artery (MCA)

Doppler flow measurement of the MCA reflects fetal cerebral vascular resistance, which is directly related to fetal oxygenation. When the fetus is hypoxic, the resistance is decreased, leading to increased fetal cerebral perfusion, also known as "brain sparing".[79] MCA is particularly sensitive to milder cases of placental dysfunction especially at late gestation.[76,80] The use of MCA is known to improve the prediction of adverse obstetric outcomes such as neurological outcomes and acute cesarean section due to fetal distress.[81,82]

Cerebroplacental ratio (CPR)

The CPR is defined as the ratio between MCA and UA. The CPR is more sensitive to fetal brain sparing than MCA alone, and low CPR reflects fetal hypoxia. A low CPR is associated with stillbirth, adverse neonatal outcomes and SGA.[73–75] In early gestation, the predictive value of CPR alone is not significantly better than UA and MCA alone.[73–75,83] However, in late gestation in SGA pregnancies with normal UA

PI, the CPR may improve the prediction of placenta-related complications in pregnancy.[74]

Ductus venosus (DV)

DV regulates the flow of well-oxygenated umbilical vein blood to the liver. In cases of hypoxia/acidosis, DV shunting increases the blood supply to the brain at the expense of the right liver lobe.[68,84] In cases of severe hypoxia and a continuous deterioration of the fetal condition, cardiac dysfunction is reflected in the DV blood flow as increased PI, absent or reversed a-wave.[9,85] Progression of DV Doppler abnormalities usually indicates the need for delivery.[86]

2.4.1.3 Biophysical profile

The biophysical profile includes cardiotocography, assessment of the amniotic fluid and fetal movements. Redistribution of fetal blood flow leads to reduced perfusion of the fetal kidneys and thereby oligohydramnios.[87] Reduced fetal movements and non-reassuring fetal heart rate indicates that the fetus is severely affected by hypoxia and acidemia.[88] The biophysical profile provides additional surveillance to the SGA fetus with abnormal Doppler flows in order to plan and time the delivery balancing prematurity and preventing stillbirth.[9,87]

2.4.2. PLACENTAL ASSESSMENT

Evaluation of the placenta, either perfusion, size or function, can be useful in the detection of placental dysfunction.

2.4.2.1 Placental size

Placental size can be estimated by ultrasound in the first trimester, and it has been demonstrated that low placental size at the 11 – 13 week scan is associated with FGR and preeclampsia..[89–91] At later gestation, placental volume is more difficult to obtain by ultrasound because of the large placental size. However, over the last few years several studies have provided reliable estimates of placental volume by placental MRI[92–97], and a small placenta is related to obstetric complications such as SGA[93,95,96].

2.4.2.2 Uterine artery (UtA)

Doppler flow measurement of UtA reflects the resistance of the maternal spiral arteries and maternal placental perfusion. High resistance is closely related to placental hypoxia and placental dysfunction.[98,99] UtA is a predictor of placenta-related obstetric outcomes of pregnancy such as FGR and preeclampsia when performed in the first trimester.[100,101] Although associated with impaired transformation of spiral

arteries and adverse outcome, the performance of UtA PI varies and is considered an inaccurate measure as a free-standing test of placental dysfunction.[102–106]

2.4.2.3 Serum markers

Numerous molecules are produced or expressed in the placenta, and the cellular products may enter maternal circulation. Several placental markers in maternal serum have been associated with placental dysfunction.[107,108] Pregnancy-associated Plasma Protein-A (PAPP-A) is produced in the syncytiotrophoblast, regulates the availability of insulin-like growth factor and thereby stimulates fetal growth. Placental growth factor (PlGF) is part of the vascular endothelial growth factor family, supports trophoblastic growth and has pro-angiogenic effects on feto-placental circulation. Soluble fms-like tyrosine kinase-1 (s-Flt-1) is a soluble receptor that has angiogenic properties by binding circulating PlGF. Low levels of PAPP-A measured in the 1st trimester is associated with preclampsia and preterm delivery.[109] Decreased PlGF and increased s-Flt-1 measured in any gestation is associated with SGA and preeclampsia.[110,111]

2.4.2.4 Magnetic Resonance Imaging (MRI)

MRI is based on the magnetic properties of protons within the body, with the hydrogen nucleus being the most frequent in the body. When a human is placed within the MRI system, the magnetic field aligns the hydrogen nuclei parallel (or anti-parallel). This alignment causes longitudinal magnetization. During an MRI acquisition, the aligned nuclei, and thereby the longitudinal magnetization, is tilted 90° by a radiofrequency pulse, creating the transversal magnetization. The longitudinal relaxation time (or T1) is defined as the time of 63% recovery of the longitudinal magnetization, given in milliseconds (ms). During the T1 relaxation, the transversal magnetization is reduced due to the nuclei diphase. The transverse relaxation time (or T2) is defined as the time in which there is a 37% reduction of the transversal magnetization, given in ms (Figure 4). T1 and T2 relaxation are independent, but parallel processes.

T2 relaxation*

T2* relaxation (T2*) is a combination of "true" T2 relaxation and relaxation from magnetic field inhomogeneities.[112] T2* is shorter than T2 relaxation since the magnetic field inhomogeneities cause the nuclei to dephase faster. T2* is defined as T2 relaxation, the time in which the transversal magnetization is reduced to 37% and given in ms (Figure 4). The T2* value can be obtained using the average signal, fitted as a function of the echo times (TE) using the mono-exponentially decaying function given by $M_0 \times e^{-TE/T2^*}$, with the equilibrium magnetization (M_0) and T2* as a free parameter and a non-linear least-squares fitting algorithm[113].

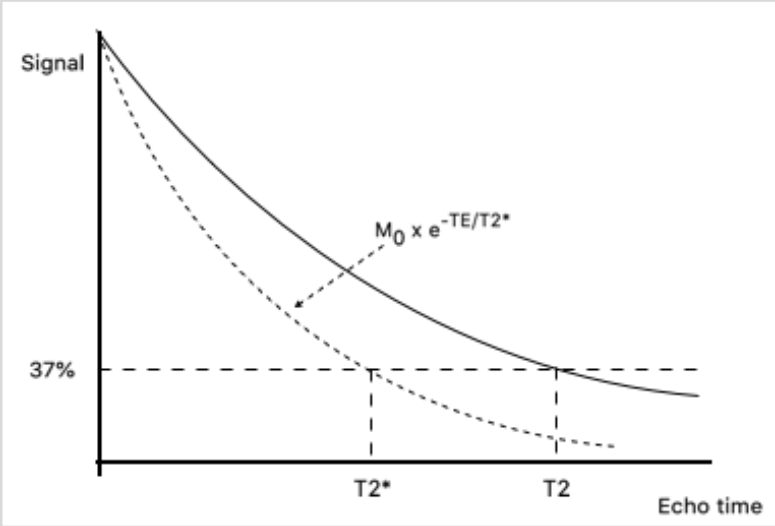

Figure 4 T2* relaxation (small dashed line)

T2*-weighted MRI utilizes the magnetic properties of hemoglobin, and the T2* value is directly related to the concentrations of deoxyhemoglobin within the tissue.[112] A decrease in tissue oxygenation will lead to an increased T2* value, caused by a higher amount of deoxyhemoglobin.[112] T2* is a robust tissue constant, and a T2*-weighted MRI scan can easily be performed within one minute. In addition to tissue oxygenation, T2* also reflects tissue morphology since T2* is affected by hemorrhages, iron deposition or calcification. Since deoxyhemoglobin works as an endogenous contrast agent, T2* weighted MRI can be accomplished without adding exogenous contrast agents. This makes it a favorable method to use during pregnancy.

T2* and the placenta

Normal reference values for placental T2* have been established, and a negative correlation with GA has been revealed (Figure 5). At 24 weeks of gestation, the T2* value was 120±17ms (mean±SD), at 32 weeks it was 84±16ms and 47±17ms at 40 weeks.[20] Increasing metabolic demands of the placenta and the fetus leads to a decreased oxygenation of the intervillous space with increasing GA.[18] Furthermore, the physiologic maturation of the placenta may change the morphology and thereby the T2* value.

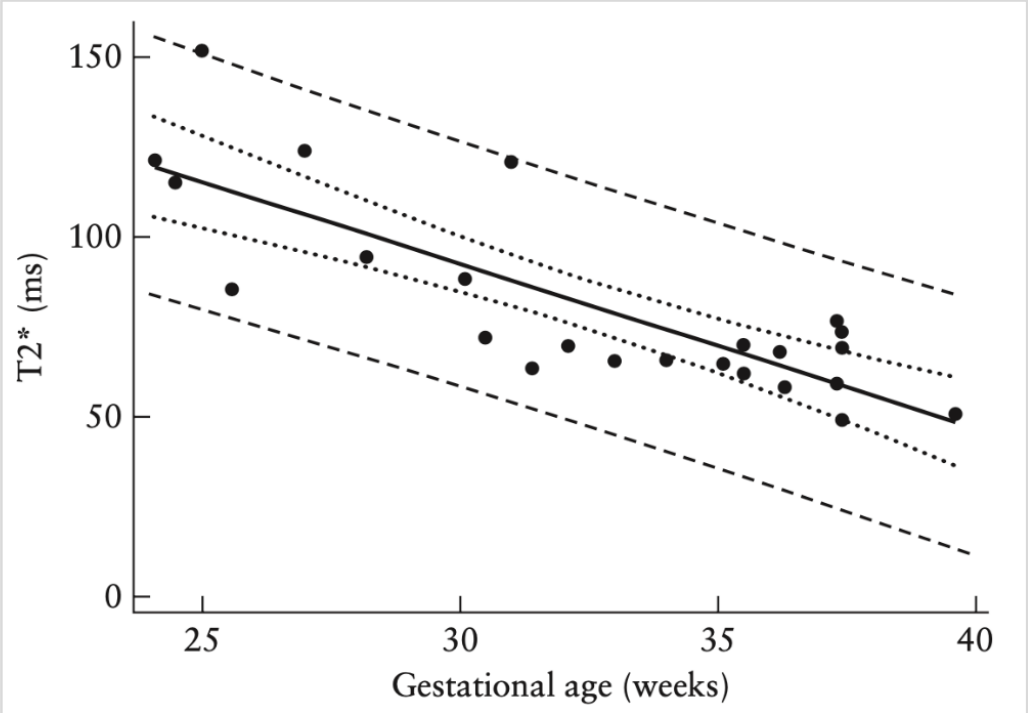

Figure 5 Placental T2* values in 24 normal pregnancies.

—— mean, ···· 95% confidence interval and --- 95% prediction interval. From Sinding et.al.[20], with permission from publisher (John Wiley and Sons).

Prior studies have demonstrated an association between low placental T2* values and placenta-related outcomes of pregnancy, such as FGR and gestational hypertensive disorders including preeclampsia.[20–23,25] The lower T2* values found in pregnancies complicated by placental dysfunction may be due to pathological lesions such as fibrosis, infarcts and necrosis in addition to placental hypoxia.[24,114]

T2* weighted placental MRI is a robust method, as the movement artifacts are limited, and the fetus and the surrounding maternal tissue have similar magnetic susceptibility[114], which also reduces MRI image artefacts. Moreover, placental T2* has a high inter-observer reproducibility, especially when the T2* value is reported as a mean of more than one placental slice.[20]

Ethical and safety considerations of MRI

MRI is a widely-used method to examine if the fetus has any structural malformation (e.g. cerebral[115]) or if invasive placental disorders[116] are suspected during ultrasound examination. If so, then an MRI with a 1.5 Tesla (T) magnetic field is performed to give additional information to the ultrasound previously done. Present data have not documented any harmful effects of MRI during pregnancy[117,118], nor have any studies

shown an association between MRI and adverse fetal outcome.[119,120] The potential harmful effects are acoustic noise, static magnetic fields and tissue heat.

Acoustic noise: The MRI scanner generates noise in the range from 80 to 120 decibel (dB).[121] The fetus is protected by the maternal abdomen and by the amniotic fluid, which reduces noise exposure by at least 30 dB.[122] No studies have shown hearing impairment of fetuses exposed to 1.5 T MRI during pregnancy.[123,124]

Static magnetic fields may interact with living tissue in various ways, such as by magnetic induction, which may create electric currents by moving electrolytes in the blood vessels.[125] No changes in heart rate or systolic/diastolic blood pressure have been demonstrated when humans were exposed to 8T for 1 hour.[126] Likewise, studies using cardiotocography have demonstrated no effects on fetal heart rate during MRI.[127,128] Another mechanism is the magneto-mechanical effect from the static magnetic field, which induces the reorientation of molecules. However, the magneto-mechanical effect is considered too small to affect human tissue in vivo since human tissue does not contain strong ferromagnetic components.[125] According to a review by the International Commission on Non-Ionizing Radiation Protection (ICNIRP), no consistent effects of static magnetic field exposure on reproduction and development have been seen in mammalian species.[129]

The *thermal effects* of ultrasound examination have been investigated, and a temperature elevation of 1.5°C is generally considered as the threshold and safe for the fetus.[130] The radiofrequency pulses used for generating the MR images may deposit heat in the tissues. Therefore, during the MRI acquisition, a specific absorption rate (SAR value, Watt/kg) is calculated in order to prevent tissue heating. The SAR value will estimate the amount of thermal energy conducted and correlates to the tissue heat deposited. According to the recommendations by the ICNIRP[131], the whole-body SAR value should be kept below 2 W/kg during a one-hour scan, equivalent to a rise in adult and fetal tissue temperature of 0.5°C and a rise of fetal temperature to less than 38°C. Within a 1.5 T MRI system, the fetal peak SAR value is approximately 50% of that generated in the mother[132], and using standard sequences at 1.5T, the SAR value does not exceed the recommended maximum value, neither for the mother nor the fetus.[132]

Clinical application of placental T2*

The need for a new non-invasive method to estimate placental function directly is critical. By the use of T2* weighted placental MRI, placental oxygenation can be assessed non-invasively. Placental dysfunction is closely related to placental hypoxia, as absent remodeling of the spiral arteries leads to MVM, which causes placental hypoxia and placental dysfunction.[17,133] Thus, it is possible that in vivo assessment of

placental oxygenation by T2* weighted placental MRI would add important knowledge regarding placental function to the current methods used.

T2* weighted placental MRI is a fast and robust method.[20,24] This, and the ability to discriminate between normal and dysfunctional placentas[20–23] , makes placental T2* a promising clinical marker of placental function. Moreover, Sinding et al.[21] found higher prediction of low BW using placental T2* when compared to UtA Doppler flow. However, the predictive performance of placental T2* in prospective clinically well-defined cohorts remains unexplored.

CHAPTER 3. AIM OF THE THESIS

The overall purpose of this project was to discuss the various definitions of low birthweight using universal versus national birthweight standard curves. Moreover, the antenatal screening of SGA was investigated in a local clinical setting. Finally, the identification of placental dysfunction was investigated using placental MRI in a cohort of SGA pregnancies.

The specific aims of the three studies of the project were

Study I:
- to generate a Danish standard birthweight curve based on the Intergrowth-21st criterion
- to compare the Danish standard birthweight curve to the universal Intergrowth-21st standard birthweight curve
- to evaluate the difference in adverse outcomes of SGA status when using the two standard birthweight curves

Study II:
- to assess the performance of the Danish screening program for small-for-gestational-age in a local clinical setting
- to investigate the obstetric consequences of false-positive and false-negative cases

Study III:
- to evaluate T2* weighted placental MRI as a biomarker of placental dysfunction, such as SGA at birth, preeclampsia, preterm delivery, or abnormal placental histolological examination in a specific cohort of SGA fetuses with normal fetal Doppler flows
- to investigate the correlation between placental T2* and fetal Doppler flows at the time of MRI

CHAPTER 4. STUDY I

The definition of normal BW varies worldwide[1–4,27], as different BW curves are used as the reference for normal weight[26,27,30–32]. Some centers claim to have universal BW curves suitable for all populations such as the International Fetal and Newborn Growth Standards for 21st Century (Intergrowth-21st)[27,134], while others use population specific BW curves. In Denmark, the Scandinavian BW curve by Maršál et al.[26] is traditionally used. Currently, there is no standard BW curve based on Danish BW data.

The objective of ***Study I*** was to generate a Danish standard BW curve based on the Intergrowth-21st criterion. Moreover, the Danish standard BW curve was compared to the universal Intergrowth-21st BW curve to challenge the idea of one universal standard BW curve. To further perspectivate the findings, the Danish standard BW curve was compared with the Danish population average and the currently used BW curve in Denmark by Maršál et.al.[26] Finally, the difference in adverse outcomes of SGA status when using the two standard BW curves were evaluated.

4.1. METHODS

4.1.1. REGISTERS

Every Danish resident is assigned a unique civil registration number (CPR-number) at the time of birth or immigration. All contacts with the health care system involve the use of this CPR-number. This enables the linkage of all Danish registries on an individual level. In addition, the Danish Health Care system is tax-funded, which ensures equal and free access to health care services for all residents.

The Danish Fetal Medicine Database[135] was established in January 2008, and combines information from each obstetric department in Denmark. All information regarding pregnancy ultrasound scans and answers on genetic examinations is collected in each department using Astraia software (gmbh, Munich, Germany).

The Danish Medical Birth Register[136] was established in 1973 and includes maternal and neonatal delivery data.

The Danish National Patient Registry[137] was established in 1977, with complete nationwide coverage in 1978. This registry includes all outpatient visits and hospitalizations in Denmark. Every visit is registered with admission and discharge

date, diagnosis codes and treatment codes. The registration in the Danish Health Care system, and thereby in the registry, follows the International Statistical Classification of Diseases and Related Health Problems (ICD)[138].

The Danish National Prescription Registry[139] was established in 1994 in som regions of Denmark, but valid on an individual basis around 1997. The register includes all claims of prescription-based medicine at Danish pharmacies and consists of date, Anatomical Therapeutic Chemical (ATC) Classification code, dosis and package size.

4.1.2. STUDY POPULATION

This study is based on prospectively collected nation-wide register-data. The study includes two study populations: a study population called "Danish population" and a subgroup called "Danish Standard cohort". Flowchart of the study populations are presented in Figure 6.

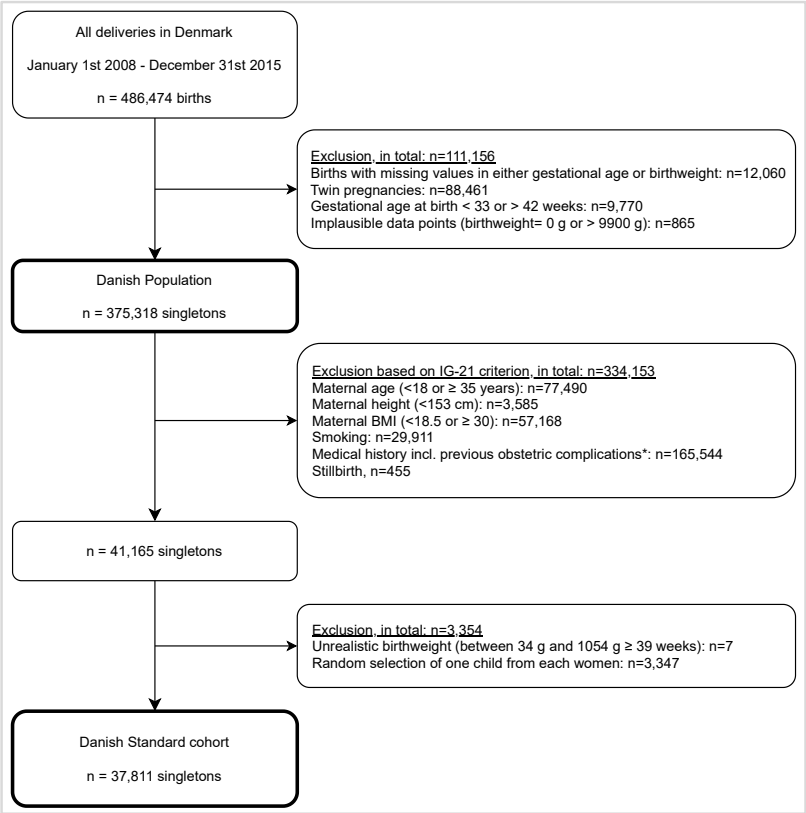

Figure 6 Flowchart of the study populations.

** Medical history including previous obstetric complications are defined as the criteria by Intergrowth-21st [27,140] (IG-21) and in this setting defined as relevant diagnosis or medication 6 months prior to or during pregnancy (9 months), a total of 15 months prior to delivery. A full list of the used diagnosis and medications codes are provided in Appendix A, Table S1 and S2.*

Danish Population

The study population "Danish Population" includes all singleton pregnancies with date of birth from January 1st 2008 to December 31st 2015 in Denmark. We excluded pregnancies with missing data in either BW or GA at birth, or pregnancies with deliveries <33 or >42 weeks of gestation, since these were the gestational weeks used in the BW curves by Intergrowth-21st.[27] Additionally, 865 pregnancies were excluded from the analysis due to implausibility (GA at birth and BW not appropriate for each other, BW = 0 g or BW > 9900 g).

GA was established based on ultrasound using either crown-rump-length measures in 1st trimester[141] or biparietal diameter in 2nd trimester[142], as this is the standard in Denmark. More than 94% of the Danish population attend a 1st trimester ultrasound scan including pregnancy dating.[135,143]

Danish Standard cohort

The subgroup "Danish Standard cohort" includes only healthy women with uncomplicated pregnancies and was retrieved from the study population "Danish Population".

We used the exclusion criteria from Intergrowth-21st, which excludes maternal age <18 and ≥35 years, body mass index <18.5 and ≥30 kg/m^2 and height <153 cm, pregnancies following fertility treatment or miscarriages in >1 of 2 consecutive pregnancies. Additionally, women with previous complicated pregnancies (including preeclampsia/eclampsia/HELLP, preterm delivery (<37 weeks), BW<2500 g or >4500 g, neonatal or fetal death, congenital malformations) were excluded. Moreover, in current pregnancy, women smoking during pregnancy or using alcohol with consequences for the infant, fetal anomaly/congenital disease in current pregnancy, anemia and sexually transmitted disease were excluded. The women may not have proteinuria or hypertension (≥ 140 mmHg (systolic) and/or ≥ 90 mmHg (diastolic) at any time during pregnancy. Moreover, the women were excluded if they had any relevant past medical history. The specific diagnosis codes from the International Classification of Diseases and Related Health Problems (ICD), 10th edition and the specific medication codes from the Anatomical Therapeutic Chemical (ATC) Classification System used for exclusion are listed in Appendix A, Table S1 and S2. Intergrowth-21st have specified relevant medical history as 6 months prior to pregnancy. To handle the massive amount of data regarding medical history, all women with relevant diagnosis and/or treatment withing 15 months prior to delivery (6 months prior to and 9 months during pregnancy) were excluded.

Intergrowth-21st[140] has requirements for occupational risks. Information regarding this is not available in the above-mentioned registers and databases. However, in

Denmark, any risk for the pregnant women is addressed at the first pregnancy examination at the general practioner. If there is any occupational risk, sick leave will be recommended.[144] Therefore, the expectation is that potential risk is minized or not present at all for Danish pregnant women in a way that complicates pregnancy above the allowed limit.

Finally, we excluded 7 unrealistic BW at term and afterwards randomly selected one pregnancy from the remaining women to avoid dependent data.

4.1.3. STATISTICAL ANALYSIS

We employed quantile regression to obtain the BW curves without parametric distribution assumptions.[144,145] In detail, a non-parametric quantile regression within a locally polynomial framework models the relation between GA and BW for both the Danish Population and the Danish Standard cohort. The quantile regressions are fitted as univariate models increasing the comparability to the Intergrowth-21st standard curves. Partially linear fitting between adjacent gestational weeks and piece-wise cubic polynomials with 3-5 knots permit feasible smooth curves.[144,146] This method is robust for the 3% and 97% quantiles, especially in the register data. The Intergrowth-21st standard BW curve is restricted to GA 33 weeks as the lower limit due to their limit set at minimum 50 observations. Therefore, 33 weeks is also the lower limit in both the Danish Population BW curve and Danish Standard BW curve. Separate models were fitted for both boys and girls.

The models were fitted using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA) updated 13.2 and R version 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria).

Several definitions are used worldvide for low BW and SGA. Both 2.3rd, 2.5th, 3rd, 5th and 10th centile are presented in the literature.[3,6,27,30,147,148] To allow for comparison with the standard BW curve by Intergrowth-21st, the 3rd, 50th and 97th centiles were chosen as the relevant centiles in this study.

Outcomes were BW, stillbirth and neonatal death (< 28 days from delivery).

4.1.4. APPROVALS

Retrospective register-based studies do not require ethical approval in Denmark.

Data handling was approved by a regional notification to the Danish Data Protection Agency, journal number: 2008-58-0028 and local reference-ID: 2017-67. All data

handling was done within the environment of Statistics Denmark in an anonymous set-up, where individuals cannot be identified, but it enables linkage between different registries and databases on the individual level.[144]

4.2. RESULTS

The Danish Population includes 375,318 singleton pregnancies, whereas the Danish Standard cohort includes 37,811 pregnancies selected in accordance with the Intergrowth-21st criterion (Figure 6). The women in the Danish Standard cohort had higher weight, height, age and parity, when compared to the Intergrowth-21st study population (Table 1).

Table 1 Maternal and neonatal characteristics

	Intergrowth-21st (n=20,486)	**Danish Standard (n=37,811)**	**Danish Population (n=375,318)**
	Mean ± SD or absolute numbers (percentages)		
Maternal age (years)	28.0 ± 4.0	29.3 ± 3.3	30.4 ± 4.9
Maternal height (cm)	161.8 ± 5.6	168.1 ± 6.2	167.9 ± 6.5
Maternal weight (kg)	61.3 ± 8.6	64.4 ± 9.2	69.0 ± 15.4
Maternal BMI (kg/m^2)	23.4 ± 2.9	22.2 ± 2.5	24.5 ± 7.8
Ethnicity			
Caucasian	-	35,127 (92.9%)	349,484 (93.1%)
Asian	-	934 (2.5%)	8,194 (2.2%)
Oriental	-	579 (1.5%)	4,741 (1.3%)
Afro Caribbean	-	326 (0.9%)	3,599 (1.0%)
Other	-	646 (1.7%)	6,557 (1.7%)
Missing	-	199 (0.5%)	2,743 (0.7%)
Non-Smoking	-	37,811 (100.0%)	326,164 (86.9%)
Nulliparous	12,996 (63.4%)	18,199 (48.5%)	171,022 (45.6%)
Spontaneous initiation of labor	13,470 (65.8%)	33,308 (88.1%)	294,130 (78.4%)
Cesarean section	7,452 (36.4%)	5,409 (14.3%)	76,349 (20.3%)
NICU admission longer than 1 day	1,184 (5.8%)	966 (2.6%)	18,237 (4.9%)
Preterm birth (<37 weeks)	1,136 (5.5%)	552 (1.5%)	15,096 (4.0%)
Term low birthweight (≥37 weeks' gestation and <2500 gram)	651 (3.2%)	204 (0.5%)	4,138 (1.1%)
All low birthweight (<2500 gram)	1,129 (5.5%)	365 (1.0%)	9,954 (2.7%)
Neonatal mortality (<28 days)	22 (0.1%)	19 (0.1%)	210 (0.06%)
Boys	10,482 (51.2%)	19,326 (51.1%)	197,477 (51.3%)
Term birthweight (≥37 weeks' gestation)	3300 g ± 500 g	3597 g ± 465 g	3521 g ± 513 g
Weight measures	Birthweights	Birthweights	Birthweights

All values are mean±SD for continuous variables and absolute numbers (percentages) for categorical variables. BMI, body mass index, NICU, neonatal intensive care unit.

The BW medians (50th centile) defined by the Danish Standard cohort were higher at all gestations than those defined by Intergrowth-21st (Figure 7). Thus, at term (GA 40+0), the median BW for males were 3700 g vs. 3380 g (difference: 320 g) and for

females 3555 g vs. 3260 g (difference: 295 g), respectively[27]. The BW medians (50th centile) based on the Danish Population were very similar to those based on the Danish Standard, at term 3680 g for males and 3536 g for females (difference to Danish Standard: 20 g (males) and 19 g (females)) (Figure 7). Moreover, the BW median based on Maršál et al[26] were in line with the Danish Standard (at term: males 3634 g (difference: 65 g) and females 3522 g (difference: 26 g)) (Figure 7).[26]

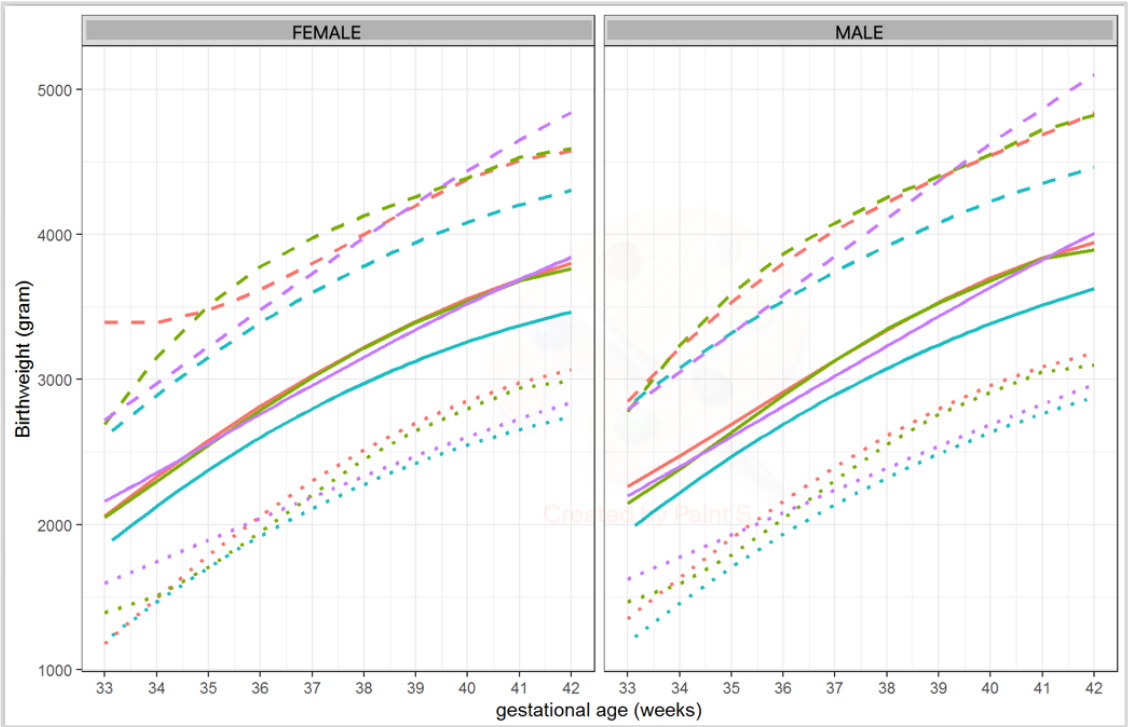

Figure 7 Birthweight curves for the Intergrowth-21st standard, Maršál et al, the Danish standard and the Danish population.

Birthweight curves by Intergrowth-21st [27](blue), the Danish standard BW curve (red), the Danish population BW curve (green) and the currently used BW curve in Denmark by Maršál et al[26](purple) for females (left) and males (right). Each centile is marked with different lines: 3rd centile (dotted), 50th centile (full) and 97th centile (dashed).

The prevalance of SGA neonates (BW<3rd centile) in the Danish Population was markedly different depending on whether the definition was based on the Intergrowth-21st standard (0.7%, n=2,640) or the Danish Standard (3.9%, n=14,698) (Table 2).

Table 2 Adverse outcomes according to SGA-definition by Intergrowth-21st and Danish Standard

	Total Danish population (n=375,318)	Intergrowth-21st		Danish Standard	
		SGA (BW<3rd centile) (n=2640)	Non-SGA (N=371,678)	SGA (BW<3rd centile) (n=14,698)	Non-SGA (n=360,620)
	Number (per 1000 pregnancies)	Number (per 1000 pregnancies)	Number (per 1000 pregnancies)	Number (per 1000 pregnancies)	Number (per 1000 pregnancies)
Stillbirth	455 (1.2)	28 (10.6)	427 (1.2)	71 (4.8)	384 (1.1)
Neonatal death < 28 days	210 (0.6)	14 (5.3)	196 (0.5)	28 (1.9)	182 (0.5)

The SGA definition of Intergrowth-21st is based on the 3rd centile given in Villar et al 2014[27]. SGA, small-for-gestational-age, BW, birthweight.

In the Danish population, stillbirth was seen in 455 pregnancies, equivalent to 1.2 in every 1000 pregnancies. Among SGA defined by the Intergrowth-21st Standard, the rate of stillbirth was higher (10.6 per 1000 pregnancies) when compared to SGA defined by the Danish Standard (4.8 per 1000 pregnancies). However, regardless of which of the two curves used, the majority of stillbirth occurred in non-SGA pregnancies (Intergrowth-21st: 93.8% (427/455), Danish Standard: 84.4% (384/455)) (Table 2).

Neonatal death (< 28 days from delivery) occurred in 210 children in the Danish population, which is equivalent to 0.6 in every 1000 pregnancies. Among SGA defined by the Intergrowth-21st Standard, the rate of neonatal death was higher (5.3 per 1000 pregnancies) when compared to SGA defined by the Danish Standard (4.8 per 1000 pregnancies). Yet, the majority of neonatal death occurred in non-SGA pregnancies regardless of the definition (Intergrowth-21st: 93.3% (196/210), Danish Standard: 86.7% (182/210)) (Table 2).

4.3. DISCUSSION

In this study, we demonstrated that the BW curves defined by a Danish Standard cohort including uncomplicated pregnancies from healthy women selected in accordance with the Intergrowth-21st criterion were markedly higher than those defined by the universal Intergrowth-21st standard. This finding does not support the idea of one universal BW curve to be used in all populations. Furthermore, the number of neonates classified as SGA (BW<3rd centile) increased from 0.7% with the use of the Intergrowth-21st standard to 3.9% with the Danish Standard. The relative risk of stillbirth and neonatal death was at least doubled among SGA defined by the Intergrowth-21st Standard, when compared to SGA defined by the Danish Standard. However, the vast majority of adverse outcomes occurred in non-SGA, regardless of which of the two standards used as SGA-definition.

It is a limitation of this study that we used data from registers instead of prospective data collection in a cohort study, as misclassification cannot be excluded, despite the high validity of Danish registers.[135–137,139] Strengths of this study are the size of the Danish Standard cohort, our strict adherence to the Intergrowth-21st criteria and the use of the same statistical methods as Intergrowth-21st standard BW curve.

It remains a matter of debate if one universal standard BW curve can be applied to all populations. Intergrowth-21st claims that their standard BW curve is universal.[27,134] Previous studies have also applied the Intergrowth-21st standard BW curve on local populations. However, none of these studies adhered strictly to the Intergrowth-21st criteria. Previous studies have used local criteria[31,149], local population references[150–153], or customized growth charts[154,155]. According to our data, the Danish standard median BW is approximately 300 g higher than median BW defined by Intergrowth-21st at term. This may partly be explained by the average Danish women being 6 cm taller than the average woman in Intergrowth-21st (Table 1). However, according to the Perinatal Institute, UK[156], this difference may only lead to a difference in BW of 50g (7.6g per cm[156]). The curves mainly differ due to other factors including ethnic and socio-economic differences between the populations. Thus, the Danish public healthcare system is characterized by high quality, free and equal access for everyone, and the Danish population is privileged by free education, unemployment benefits and free maternity leave at least four weeks before term.

The Intergrowth-21st authors[27] argue in favor of one universal standard BW curve[27,134] as only 1.9% - 3.5% of the difference in median BW between their populations can be attributed to population differences. On the other hand, the World Health Organization (WHO) considers it prudent to test the universal standard curve in each population to see if adjustments are required to meet local needs.[149] WHO argues that differences remain between ethnic groups with equal health care conditions and maternal characteristics.[149,157] Our findings support the idea of locally adapted standards. In this study, the Danish Standard curve was almost identical to the unselected Danish Population curve. This finding supports the validity of the Danish standard BW curve, as normal BW defined by a standard curve should be at least as high as normal BW defined by an unselected population curve since the population curve also includes pathological pregnancies associated with SGA.[28] The two curves being almost identical demonstrates that currently the proportion of pathology in the Danish population is rather low.

Surprisingly, the median BW of the Danish standard curve was highly in accordance with the median BW of the BW curve by Maršál et al.[26], which is the current clinically used BW curve in Denmark. The Maršál BW curve is from 1996 and is a Scandinavian BW curve including both Swedish and Danish women.[26] Although not being exactly

a standard curve, the Maršál BW curve used some selection of the population. However, not all pathology was excluded, as women smoking up to 10 cigarettes/day and diabetes in pregnancy was not excluded from the study population. The Maršál BW curve was based on serial intrauterine ultrasound measures every three to four weeks and BW from 86 singleton pregnancies, resulting in 759 fetal weights and 86 BW pooled in the curve. The curve is based on a different statistical method than Intergrowth-21st and the Danish standard, since Maršál et al[26] used a fourth-degree polynomial. The fact that the two curves (Maršál and the Danish standard) are almost identical is reassuring in a Danish clinical perspective – and it supports the continuous use of Maršál BW curve in Denmark. The curves being identical is most likely related to similar ethnicity and equally high healthcare standards in Scandinavia.

The Maršál[26] BW curve used a combination of intrauterine weight estimates (EFW) and BW. The women were excluded in case of preterm delivery before 37 weeks of gestation, accordingly only intrauterine weights are included prior to 37 weeks of gestation. Preterm BW were excluded from the curve, as neonates born preterm may suffer from placental dysfunction and therefore tends to be smaller than intrauterine fetuses at equvalent GA. The Intergrowth-21st universal standard BW curve and the Danish standard curve constructed in this study is based on BW down to 33 weeks of gestation. Thus, it is possible that the preterm BW (33-37 weeks) used to construct the standard curves in our study and Intergrowth-21st may underestimate normality.

The Danish population is a rather homogeneous low-risk population in which a national standard BW curve seems appropriate. However, in a more heterogeneous multi-ethnic population, a more customized approach may be useful to take individual risk factors, such as ethnicity into account. Customized BW curves are widely distributed in a number of countries, however, the selection of individual factors for customization remains a matter of debate.[29,155,158,159]

Even if we manage to improve the antenatal identification of low BW, the identification of the true high-risk fetuses suffering from placental dysfunction is an ongoing obstetric challenge. The fundamental problem is that low BW alone is not a good marker of placental dysfunction. This is supported by Table 2, where the vast majority of adverse outcomes occurred in normal weighted neonates. In order to improve the identification of high-risk fetuses, fetal well-being needs to be assessed by the use of ultrasound Doppler flow measurements of fetal blood flow and biophysical profile including estimates of fetal movements, fetal heartrate monitoring and the amount of amniotic fluid. In the clinic, these examinations are performed in small fetuses to discriminate between high-risk fetuses suffering from plaental dysfunction and healthy constitutionally small fetuses. Thereby, these examinations tend to reduce the FPR of the SGA screening. Another approach to improve the

antenatal identification of placental dysfunction is to use direct markers of placental function. Current direct markers of placental function include serum markers such as PAPP-A, PlGF and several others.[110,160–164] In addition, placental function may also be estimated by MRI. It has been demonstrated that T2* weighted MRI, which is sensitive to tissue hypoxia, is a reliable marker of placental dysfunction.[24,112,165] By use of these markers, it may be possible to further reduce both the number of false positive and false negative cases.

The implementation of Intergrowth-21st Standard in the Danish population would reduce the number of SGA pregnancies. This may lead to suboptimal fetal monitoring and delayed delivery in undetected SGA fetuses.[5,31] On the contrary, the number of false positive SGA pregnancies would be reduced, which may reduce the number of unnecessary obstetric interventions and improve the attention on the true SGA pregnancies. These issues will be discussed further in the overarching general discussion (Chapter 7).

In conclusion, this study does not support one universal BW curve to fit all populations, since the Danish standard BW median is markedly higher than that of the Intergrowth-21st standard. Moreover, the risk of stillbirth and neonatal death was higher in SGA defined by Intergrowth-21st, although the vast majority of adverse outcomes occurred in the group of non-SGA pregnancies.

CHAPTER 5. STUDY II

Antenatal screening of SGA is highly important, as it allows for timely delivery and obstetric interventions in labor. Unfortunately, SGA screening is challenged by low sensitivity and high FPR. In Denmark, ultrasound screening for SGA is on indication only; however, the number of women referred for ultrasound scans and the performance of SGA screening remains unexplored.

The objective of ***Study II*** was to assess the performance of the Danish screening program for SGA in a local clinical setting. Moreover, the obstetric consequences of false-positive and false-negative cases was investigated.

5.1. METHODS

5.1.1. STUDY POPULATION

We included all 3,113 women with singleton pregnancies attending a 1st trimester ultrasound scan at Aalborg University Hospital with due dates in 2015.

Due date for all pregnancies were calculated based on the crown-rump-length at their 1st trimester ultrasound scan using the reference by Robinson and Fleming[141]. EFW in gram was calculated using the formula by Hadlock et al.[34] using head circumference, abdominal circumference and femur length. EFW deviation (percent) and BW deviation (percent), both measures according to GA, were calculated using the weight curve by Maršál et al.[26], as this is the reference used in Denmark. Expected SGA was defined by an EFW deviation ≤ -15% of expected for GA (10th centile) at the last ultrasound scan before delivery. SGA at birth was defined as BW ≤ -22% of expected for GA (2.3rd centile). Expected appropriate-for-gestational-age (AGA) was defined by an EFW deviation > -15% of expected for GA, whereas AGA at birth was BW > -22% of expected for GA.

All sonographers and doctors performing ultrasound scans were certified by the Fetal Medicine Foundation.

Women delivering in hospitals outside the North Denmark Region or pregnancies resulting in abortion/miscarriage before 22 weeks of gestation were excluded from the study (n=185). In total, 2,928 were included in the further analysis.

5.1.2. DATA COLLECTION

Data regarding ultrasound scans was retrieved from the local Fetal Medicine database (Astraia software gmbh version 1.24.10, Munich, Germany). Maternal, pregnancy and neonatal characteristics and delivery information were retrieved from electronic patient records (Clinical Suite^{TM}, version 18.0.4.0; DXC Technology, Tysons, VA, USA and Application System/400, International Business Machines Corporation, Armonk, NY, USA).

5.1.3. STATISTICAL ANALYSIS

Sensitivity and FPR were used to describe the performance of the Danish screening program for SGA using EFW≤-15% (expected SGA) and BW≤ - 22% (SGA at birth) as binary outcomes.

Using logistic regression, the odds ratios (OR) of obstetric and neonatal outcomes were calculated between expected and unexpected groups of SGA and AGA neonates. OR was adjusted for GA at birth, BW deviation (%), maternal body mass index and parity (OR_{adj}). Adverse neonatal outcomes were umbilical artery < 7.1, Apgar score < 7 after 5 minutes, stillbirth or neonatal death within 28 days from delivery. All adverse outcomes were analyzed as univariate analysis and afterwards garthered in one variable, as “adverse outcome”.

The statistical software package Stata MP version 15.0 (StataCorp LP, College Station, TX, USA) was used for data analysis and p-values <0.05 were considered statistically significant.

5.1.4. APPROVALS

Data collection were approved by the Danish Patient Safety Authority, journal number 3-3013-1673/1. Data storage and handling were approved by a regional notification to the Danish Data Protection Agency, journal number 2008-58-0028, with local reference-ID: 2016-61 and 2018-104.

5.2. RESULTS

In this local cohort of 2,928 singleton pregnancies at Aalborg University Hospital, 98 (3.3%) were SGA at birth (BW≤-22%). 1,849 (63%) had at least one ultrasound scan with EFW on clinical indication after 24 weeks gestation, and 219 (12%) was expected SGA (last EFW≤-15%) (Figure 8, Table 3).

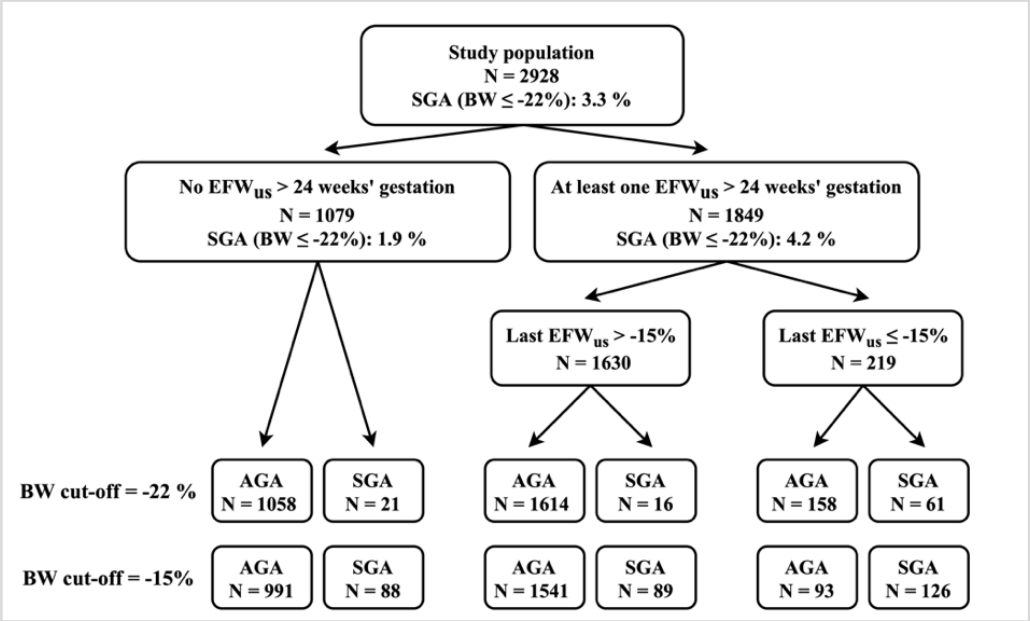

Figure 8 Flowchart of the studypopulation in Study II.

AGA, appropriate-for-gestational-age, SGA, small-for-gestationalage, EFW_{us}, estimated fetal weight by ultrasound, BW, birthweight. From Hansen et.al.[166]*, with permission from publisher (John Wiley and Sons).*

The overall sensitivity of the Danish screening program for SGA at birth was 62% at a FPR of 5.6% (Table 3). For comparison with screening programs defining SGA as BW≤ -15%, these results are added in both Figure 8 and Table 3.

Table 3 Performance of the screening program for small-for-gestational-age in Denmark

Total population, n	2928	
Women never referred to ultrasound	37% (1079/2928)	
Women referred to ultrasound	63% (1849/2928)	
Time between last ultra-sound and birth (days), median (interquartile range)	11 (2, 28)	
SGA cut-off	BW ≤ −22%	BW ≤ −15%
SGA at birth	3.3% (98/2928)	10.3% (303/2928)
Last EFW_{us} ≤ −15%	7.5% (219/2928)	7.5% (219/2928)
Sensitivity (last EFW_{us} ≤ −15% and SGA at birth)	62% (61/98)	41.6% (126/303)
False-positive rate	5.6% (158/2830)	3.5% (93/2625)

SGA, small-for-gestational-age, EFW_{us}, estimated fetal weight by ultrasound. From Hansen et.al.[166]*, with permission from publisher (John Wiley and Sons).*

The maternal and neonatal characteristics of SGA pregnancies and AGA pregnancies are presented in Table 4 and Table 5, respectively. Within SGA, the group of expected SGA were significantly smaller, born at a lower GA, had a higher number of ultrasound scans, and a shorter time interval between the last EFW_{us} and delivery when compared to the group of SGA-expected AGA (Table 4).

Table 4 Maternal and neonatal characteristics of SGA pregnancies

Characteristics	SGA			*P*-value
	Total	Expected SGA (last EFW_{us}≤-15%)	Expected AGA (last EFW_{us}>-15% or no EFW_{us})	
	n=98	n=61	n=37	
Gestational age at birth (weeks)	39.1 (37.0, 40.6)	38.3 (36.4, 39.7)	40.4 (38.6, 41.0)	0.0007*
Birthweight (gram)	2458 (2115, 2688)	2380 (2008, 2605)	2650 (2360, 2815)	0.0008*
Birthweight deviation (%)	-26.5 (-29.6, -23.3)	-27.5 (-30.4, -24.1)	-24.9 (-28.1, -22.7)	0.008*
Number of ultrasound examinations (with EFW_{us})	2 (1, 4)	3 (2, 5)	0 (0, 2)	0.00*
Gestational age at last ultrasound (weeks)	37.0 (35.1, 38.9)	37.1 (35.6, 39.1)	36.5 (34.8, 37.5)	0.13
Time between last ultrasound and birth (days)	4 (1, 11)	2 (1, 6.5)	22 (6.8, 31)	0.00*
Girls	57 (58%)	35 (57%)	22 (60%)	0.84
Maternal Body Mass Index (kg/m^2)	23.7 (21.6, 27.6)	23.7 (21.0, 28.1)	23.6 (22.0, 26.5)	0.84
Maternal age (years)	29 (25, 34)	28 (24.5, 33)	30 (26.5, 34)	0.21
Nulliparous	62 (63%)	37 (61%)	25 (68%)	0.49
Cigarette smoker	22 (22%)	16 (26%)	6 (16%)	0.25
Maternal hypertensive disorders	18 (18%)	16 (26%)	2 (5.4%)	0.01*
Maternal diabetic disorders	2 (2.0%)	1 (1.6%)	1 (2.7%)	0.72

Data are presented as median (interquartile range) or number (percent). Comparison of characteristics between groups of SGA (expected SGA and expected AGA) by Chi^2 test for categorical variable and by Mann-Whitney U test for continuous variables.
** $p<0.05$. SGA = BW≤ -22%, Expected SGA = EFW_{us} ≤ -15% at last ultrasound scan. Expected AGA = normal symphysis-fundal height measurements and/or EFW_{us}>-15% at last ultrasound scan. SGA, small-for-gestational-age, AGA, appropriate-for-gestational-age, BW, birthweight, EFW_{us}, ultrasound estimates of fetal weight.*

Within AGA, the group of expected AGA were significantly larger, had fewer ultrasound scans with a longer time interval between the last EFW_{us} and delivery when compared to the group of AGA-expected SGA (Table 5).

Table 5 Maternal and neonatal characteristics of AGA pregnancies

Characteristics	AGA			*P*-value
	Total	Expected AGA (Last EFW_{us}>-15% or no EFW_{us})	Expected SGA (Last EFW_{us}≤-15%)	
	n=2830	n= 2672	n=158	
Gestational age at birth (weeks)	40.1 (39.0, 41.0)	40.1 (39.0, 41.0)	39.9 (38.6, 41.0)	0.06
Birthweight (gram)	3585 (3250, 3900)	3620 (3291, 3929)	3045 (2750, 3273)	0.00*
Birthweight deviation (%)	-0.1 (-7.4, 8.5)	0.5 (-6.1, 9.0)	-14 (-17, -9.8)	0.00*
Number of ultrasound examinations (with EFW_{us})	1 (0, 2)	1 (0, 2)	3 (1, 4)	0.00*
Gestational age at last ultrasound (weeks)	37.1 (35.3, 39.7)	37.0 (35.3, 39.6)	38.1 (36.6, 40.1)	0.0001*
Time between last ultrasound and birth (days)	12 (3, 29)	13 (3, 30)	4 (1, 14)	0.00*
Girls	1,346 (48%)	1,261 (47%)	85 (54%)	0.11
Maternal Body Mass Index (kg/m^2)	23.9 (21.5, 27.7)	24.0 (21.6, 27.7)	22.7 (20.5, 26.0)	0.0003*
Maternal age (years)	29.5 (26, 33)	30 (26, 33)	29 (26, 33)	0.65
Nulliparous	1,299 (46%)	1,216 (46%)	83 (53%)	0.09
Cigarette smoker	233 (8.2%)	212 (7.9%)	21 (13%)	0.08
Maternal hypertensive disorders	120 (4.2%)	106 (4.0%)	14 (8.9%)	0.003*
Maternal diabetic disorders	168 (5.9%)	162 (6.1%)	6 (3.8%)	0.24

*Data are presented as median (interquartile range) or number (percent). Comparison of characteristics between groups of AGA (expected AGA and expected SGA) by Chi^2 test for categorical variable and by Mann-Whitney U test for continuous variables. * $p<0.05$. AGA = BW>-22%, Expected SGA = EFW_{us} ≤ -15% at last ultrasound scan. Expected AGA = normal symphysis-fundal height measurements and/or EFW_{us} > -15% at last ultrasound scan. SGA, small-for-gestational-age, AGA, appropriate-for-gestational-age, BW, birthweight, EFW_{us}, ultrasound estimates of fetal weight.*

The sensitivity decreased markedly with GA, and after 41 weeks of gestation, the sensitivity was 38% at a FPR of 5.6% (Table 6).

Table 6 Performance of the screening program for small-for-gestational-age in Denmark in relation to gestational age at birth

		Gestational age at birth				
	Overall	<34 weeks	34^0-36^6 weeks	37^0-39^6 weeks	40^0-40^6 weeks	≥41 weeks
Total population, n	2928	46	130	1,146	845	761
SGA at birth (BW ≤ −22%)	3.3% (98/2928)	24% (11/46)	10% (13/130)	3.4% (39/1146)	2.2% (19/845)	2.1% (16/761)
Sensitivity of screening program	62% (61/98)	73% (8/11)	85% (11/13)	72% (28/39)	42% (8/19)	38% (6/16)
SGA referred to ultrasound	79% (77/98)	82% (9/11)	92% (12/13)	90% (35/39)	68% (13/19)	50% (8/16)
Last EFW_{us} ≤ −15%	7.5% (219/2928)	26% (12/46)	15% (20/130)	8.6% (99/1146)	4.7% (40/845)	6.3% (48/761)
False-positive rate	5.6% (158/2830)	11% (4/35)	7.7% (9/117)	6.4% (71/1107)	3.9% (32/826)	5.6% (42/745)

SGA, small-for-gestational-age, BW, birthweight, EFW_{us}, estimated fetal weight by ultrasound. From Hansen et.al.[166], with permission from publisher (John Wiley and Sons).

The screening performance is highly depended on the EFW cut-off. In this cohort, an EFW cut-off of -12% leads to a sensitivity of 86% at a FPR of 17%, while an EFW cut-off of -22% results in a sensitivity of 57% at a FPR of 1.6% (Table 7).

Table 7 Screening performance at different ultrasound estimated fetal weight cut-off values

Population n=1849 SGA (BW≤-22%) n=77	Estimated fetal weight cut-off value			
	EFW_{us} -12%	**EFW_{us} -15%**	**EFW_{us} -18%**	**EFW_{us} -22%**
Last EFW_{us} ≤ -15%, n	367	219	133	73
True positive, n	66	61	54	44
Sensitivity	86% (66/77)	79% (61/77)	70% (54/77)	57% (44/77)
False positive rate	17% (301/1772)	8.9% (158/1772)	4.5% (79/1772)	1.6% (29/1772)

Included in this table are only patients referred to ultrasound scan (n=1849). SGA, small-for-gestational-age, EFW_{us}, estimated fetal weight by ultrasound scan, BW, birthweight.

Among SGA neonates, those that were falsely classified as AGA had a significantly lower risk of induction of labor (OR_{adj}=0.13, 95% CI: 0.04-0.41) and lower risk of elective cesarean section (0% vs. 27%, p<0.01), when compared to expected SGA neonates (Table 8).

Table 8 Outcome of small-for-gestational-age pregnancies

Outcome	SGA				
	Total n = 98	Expected SGA (Last EFW_{us} ≤ −15%) n = 61	Expected AGA (Last EFW_{us} > −15% or no EFW_{us}) n = 37	OR (95% CI), *P*-value	Adjusted[a] OR (95% CI), *P*-value
Cesarean delivery	36% (35/98)	47% (26/61)	24% (9/37)	0.43 (0.17-1.07), *P* = 0.07	0.71 (0.24-2.13), *P* = 0.54
Elective cesarean section among all cesarean sections	20% (7/35)	27% (7/26)	0	**	**
Intended vaginal delivery	77% (75/98)	72% (44/61)	84% (31/37)	2.31 (0.93-5.72), *P* = 0.07	1.41 (0.47-4.22), *P* = 0.54
Induction among intended vaginal deliveries	57% (44/75)	83% (34/44)	35% (10/31)	0.14 (0.05-0.39), *P* = 0.00*	0.13 (0.04-0.41), *P* = 0.00*
Vacuum among vaginal deliveries	13% (8/63)	17% (6/35)	7.1% (2/28)	0.37 (0.07-2.01), *P* = 0.25	0.41 (0.07-2.30), *P* = 0.31
Umbilical artery pH <7.1	7.0% (6/86)	7.1% (4/56)	6.7% (2/30)	0.93 (0.16-5.39), *P* = 0.93	0.54 (0.08-3.58), *P* = 0.52
Apgar score <7 after 5 min	4.2% (4/95)	5.0% (3/60)	2.9% (1/35)	0.56 (0.06-5.59), *P* = 0.62	0.50 (0.04-5.78), *P* = 0.58
Stillborn	2.0% (2/98)	1.6% (1/61)	2.7% (1/37)	1.67 (0.10-27.47), *P* = 0.72	0.66 (0.02-27.39), *P* = 0.83
Neonatal death	1.0% (1/98)	1.6% (1/61)	0	**	**
Adverse outcome[b]	11% (11/98)	13% (8/61)	8.1% (3/37)	0.58 (0.14-2.36), *P* = 0.45	0.53 (0.12-2.37), *P* = 0.41

SGA-expected SGA is used as reference group. [a] Adjusted for gestational age at birth, birthweight deviation (%), maternal body mass index and parity. [b] Umbilical artery pH <7.1, Apgar score <7 after 5 min, stillborn or neonatal death in one variable. SGA, small-for-gestational-age, EFW_{us}, estimated fetal weight by ultrasound, AGA, appropriate-for-gestational-age, OR, odds ratio, CI, confidence interval. From Hansen et.al.[166]*, with permission from publisher (John Wiley and Sons).*

The group of AGA neonates, who were falsely classified as SGA, was more likely to have induction of labor (OR_{adj}=2.51, 95% CI: 1.70-3.71), when compared to those identified as AGA (Table 9). Moreover, there was a trend towards a higher number of cesarean sections (OR_{adj}=1.44, 95% CI: 0.96-2.18).

Table 9 Outcome for appropriate-for-gestational-age pregnancies

	AGA				
Outcome	Total n = 2830	Expected AGA (Last EFW_{us} > −15% or no EFW_{us}) n = 2672	Expected SGA (Last EFW_{us} ≤ −15%) n = 158	OR (95% CI), *P*-value	Adjusted[a] OR (95% CI), *P*-value
Cesarean delivery	20% (572/2830)	20% (535/2672)	23% (37/158)	1.22 (0.84-1.79), *P* = 0.30	1.44 (0.96-2.18), *P* = 0.08
Elective cesarean section among all cesarean sections	38% (215/572)	38% (203/535)	32% (12/37)	0.79 (0.39-1.60), *P* = 0.50	1.49 (0.68-3.26), *P* = 0.32
Intended vaginal delivery	83% (2349/2830)	83% (2221/2672)	81% (128/158)	0.82 (0.56-1.20), *P* = 0.30	0.69 (0.46-1.05), *P* = 0.08
Induction among intended vaginal deliveries	29% (688/2349)	28% (631/2221)	45% (57/128)	2.02 (1.41-2.90), *P* = 0.00*	2.51 (1.70-3.71), *P* = 0.00*
Vacuum among vaginal deliveries	8.3% (187/2258)	8.4% (179/2137)	6.6% (8/121)	0.77 (0.37-1.61), *P* = 0.49	0.66 (0.31-1.44), *P* = 0.30
Umbilical artery pH <7.1	4.6% (118/2590)	4.6% (113/2443)	3.4% (5/147)	0.73 (0.29-1.81), *P* = 0.49	0.73 (0.28-1.87), *P* = 0.51
Apgar score <7 after 5 min	0.8% (22/2812)	0.8% (21/2654)	0.6% (1/158)	0.80 (0.11-5.98), *P* = 0.83	0.65 (0.08-5.22), *P* = 0.68
Stillborn	0.3% (9/2830)	0.3% (9/2672)	0	**	**
Neonatal death	0.1% (2/2830)	0.8% (2/2672)	0	**	**
Adverse outcome[b]	5.1% (144/2830)	5.2% (138/2672)	3.8% (6/158)	0.72 (0.31-1.67), *P* = 0.45	0.63 (0.27-1.50), *P* = 0.30

AGA-expected AGA is used as reference group. AGA; appropriate for gestational age, SGA; small for gestational age, EFW_{us}; estimated fetal weight by ultrasound, OR; odds ratio, CI; confidence interval. a) Adjusted for gestational age at birth, birthweight deviation (%), maternal body mass index and parity. b) Umbilical artery pH <7.1, Apgar score <7 after 5 min, stillborn or neonatal death in one variable. From Hansen et.al.[166]*, with permission from publisher (John Wiley and Sons).*

Despite this difference in obstetric interventions, we could not demonstrate a significant difference in adverse neonatal outcomes within either the SGA or AGA pregnancies (Tables 8 and 9). Though, these outcomes are also rare in Denmark.

5.3. DISCUSSION

At Aalborg University Hospital, 63% of the pregnant women were referred to ultraound EFW after 24 weeks of gestation. SGA screening showed an overall sensitivity of 62% (FPR: 5.6%), but markedly lower at term. AGA neonates falsely classified as SGA lead to an increased risk of obstetric interventions, when compared to correctly classified AGA neonates.

A strength of this study is the unselected study population from a well-defined geographic area. Thus, initiated by a free and equal access to the Danish Health Care

system and >95% of Danish pregnant women attending the 1st trimester ultrasound scan[135]. Moreover, only 5.6% of the pregnancies in our geographic area were lost to follow up. The small size of this study is a limitation, as it was not powered to investigate rare neonatal outcomes. In addition, associations between outcomes and indications for ultrasound scan were not consistently available in the patient record, and therefore not evaluated in this study.

In Denmark, the weight cut-offs used to define fetuses at risk of SGA and to diagnose SGA at birth are different. Last EFW ≤ -15% defines fetuses at risk of SGA, while BW≤ -22% defines SGA at birth. The EFW cut-off is higher than the BW cut-off, to compensate for the inaccuracy of ultrasound EFW, which is estimated to be ± 8% for Hadlock's formula[34]. As demonstrated by our data, it is possible to improve the sensitivity of the SGA screening by changing the EFW cut-off; however, it also leads to a higher FPR and thereby more obstetric interventions. The definition of SGA as BW ≤ - 22% are most often used, as the definition of growth restriction without the need of abnormal Doppler flows.[15]

The performance of the Danish SGA screening program has improved considerably, when compared to a previous Danish study from 2002.[39] This may be due to improved ultrasound diagnostics and a higher proportion of women referred to ultrasound EFW in our cohort (63%), when compared to 3.7% in the previous Danish study.[39]

In general, SGA screening can be based on either routine third trimester ultrasound EFW or ultrasound EFW on clinical indication also known as selective screening. Screening performance is highest on routine ultrasound EFW when performed multiple times or late in third trimester.[12,167,168] However, routine screening also leads to a high FPR.[12,167,168] Another approach is the selective screening of high risk pregnancies. Selective screening is more cost-effective than routine ultrasound EFW; however, in general, the sensitivity is lower than in routine screening.[12] This is documented by Sovio et al.[12] as they compared routine third trimester ultrasound EFW with selective ultrasound EFW. In the current Danish selective screening program, the performance is higher (sensitivity: 62%) than in the study on selective screening by Sovio et al.[12] (sensitivity: 32%), which more likely is the result of a higher proportion referred for ultrasound EFW (63% in our study[166]) than the 42% referred on clinical indication by Sovio et al.[12]

Despite the improvements in the Danish SGA screening program, there is still room for improvement in order to increase the sensitivitiy, especially at term when our sensitivity is decreased to 42% (FPR=3.9%). The low performance at term may partly be caused by lower accuracy of the EFW, e.g. due to a deep cephalic presentation.[169,170] The low performance at term may also be explained by a low

proportion of SGA neonates referred to ultrasound EFW at term (68%) when compared to earlier gestation, where 92% of SGA born at GA 34+0 to 36+6 weeks were referred to EFW_{us}. Introduction of a routine ultrasound EFW in late third trimester may increase the sensitivity at term. However, this would also lead to an increase in the FPR and accordingly increase the number of obstetric interventions. Another approach to improve SGA screening would be to optimize the selection of pregnancies referred for ultrasound EFW. In addition, direct markers of placental dysfunction such as maternal serum markers or placental MRI may also improve SGA screening.

This study was not designed nor powered to investigate adverse outcomes related to antenatal detection of SGA. However, inspite of the very small numbers, our data does not show any benefit of antenatal detection in regards to adverse outcome. This raises an important discussion regarding the potential harm of such screening. Previous literature is conflicting regarding this.[5,41–45] In the current study, we demonstrate that detected SGA are more severe cases which can explain why the outcome does not improve by antenatal detection, even in the adjusted analysis. However, the pathology associated with severe SGA may be difficult to fully statistically adjust for. In addition, the outcome may not be the full picture as long-term consequences also should be taken into account when to assess the true benefit of SGA screening. There is no doubt that correct antenatal detection of SGA is an advantage, but we may need to adjust the clinical management of SGA. The ultimative goal would be to identify placental dysfunction rather than SGA, in order to select the truly growth restricted fetuses among SGA.

In conclusion, the Danish antenatal screening of SGA has improved markedly over the last 20 years. However, performance remains low at term, and therefore false positive and false negative cases remain a clinical challenge.

CHAPTER 6. STUDY III

Direct markers of placental function are highly clinically relevant to identify placental dysfunction. Placental T2* estimated by MRI is related to placental hypoxia and thereby placental dysfunction, and the associations between low T2* value and pregnancy complications such as FGR and preeclampsia is well known. However, the identification of placental dysfunction using placental T2* in a well-defined SGA cohort with normal fetal Doppler flow remains to be explored.

The objective of ***Study III*** was to evaluate placental T2* as a biomarker of placental dysfunction defined by SGA at birth, preeclampsia, preterm delivery, or abnormal PHE in a cohort of SGA pregnancies with normal fetal Doppler flow. Moreover, the correlation between placental T2* and fetal Doppler flows were investigated.

6.1. METHODS

6.1.1. STUDY POPULATION

All singleton pregnant women aged ≥ 18 years at Aalborg University Hospital from February 1st 2018 to November 13th 2019 with ultrasound EFW ≤ - 22% of expected for GA[26] (2.3rd centile) and normal fetal Doppler flows were considered for inclusion. Normal fetal Doppler flows were defined as UA PI < 2 SD[171] and MCA PI > - 2 SD[171].

We excluded non-Danish speakers, severe fetal malformations, severe maternal anxiety or claustrophobia and women with any contraindications to MRI.

All participating women gave written informed consent. Data were managed using Research Electronic Data Capture "REDCap"[172] hosted at Aalborg University Hospital, North Denmark Region.

6.1.2. ULTRASOUND

Due dates were calculated based on the crown-rump-length at 1st trimester ultrasound scan using the reference by Robinson and Fleming[141] by Fetal Medicine Foundation certified sonographers and doctors using GE Voluson™ E10 (GE Healthcare, Milwaukee, WI, USA).

Ultrasound EFW in gram was calculated using the formula by Hadlock et al.[34] based on head circumference, abdominal circumference and femur length (all measures are according to the reference by Verburg et.al. 2008[142], which is the currently used reference in Denmark). EFW deviation in percent of expected for GA were calculated

using the reference curve by Maršál et al.[26], as this is the reference used in Denmark. Doppler flow measurements in the UA, MCA and UtA were registered as PI and converted into Z-scores for comparison across gestation. The reference curves used were Parra-Cordero et al.[171] for both UA and MCA and Gómez et al.[173] for UtA (mean).

6.1.3. MAGNETIC RESONANCE IMAGING – T2*

T2* weighted placental MRI were performed in a 1.5 T wide-bore 70 cm system (GE Optima™ MR450w, GE Healthcare, Milwaukee, WI, USA). Placental T2* was obtained with 16 echoes in 5 placental slices, oriented transversal of the placenta, each slice in one breath hold (12 seconds). The T2* weighted placental MRI protocol was as follows; TE_{16}: 3.0 msec to 67.5 msec in steps of 4.3 msec, TR: 71.2 msec, flip angle 30°, spacing: 20.0 mm, slice thickness: 8.0 mm, FOV: 38.0x38.0 cm, frequency: 256 and phase: 160. The total MRI examination time was approximately 30 minutes, as the T2* weighted MRI was part of a multi-sequence placental MRI research protocol. Each T2* weighted MR image was evaluated for susceptibility artefacts and uterine contractions.[174] None of the 92 T2* weighted MRI scans were excluded due to image artefacts.

To evaluate safety during placental MRI, the SAR and root mean square of the MRI effective component of the B(1) field (B_{1+RMS}) were recorded during MRI acquisition. SAR is an estimate of the absorbed energy within the tissue, when exposed to radiofrequency electromagnetic field and is patient dependent. On the contrary, B_{1+RMS} is a known value based on the specific sequence used during the MRI acquisition, which is not patient dependent. B_{1+RMS} expresses a time-averaged radiofrequency magnetic field component, and thereby also reflects radiofrequency exposure. During normal operating mode, whole body SAR should not exceed 2 W kg^{-1} as an average over 6 min. Moreover, B_{1+RMS} should be below 2.8 µT. During T2* weighted placental MRI in this study, B_{1+RMS} was 0.35 µT, whereas the maximum whole-body SAR was 0.01 W kg^{-1}.

Data analysis was performed prospectively by a single observer, who was blinded to all clinical outcomes. Regions of interest (ROI) were manually drawn in three slices covering the entire cross-section of the placenta using an in-house developed MatLab based software (MathWorks, Natick, MA, USA). The T2* value was obtained using the average signal within each ROI, fitted as a function of the echo times using mono-exponentially decaying function with the equilibrium magnetization (M_0) and T2* as a free parameter and a non-linear least-squares fitting algorithm[113] (Figure 4). The placental T2* values were calculated as a mean of three slices and converted to Z-scores by adjusting for GA at MRI according to previous published normal reference

values[20] (Figure 5). Moreover, the obstetrician and pathologist were blinded to the T2* value.

6.1.4. OUTCOMES OF PLACENTAL DYSFUNCTION

Four outcomes defining placental dysfunction were selected.

1) SGA at birth was defined as BW ≤ -22% of expected for GA according to the Scandinavian reference by Maršál et al.[26].

2) Preeclampsia was defined in accordance with the International Society for the Study of Hypertension in Pregnancy[175] as systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg developed *de novo* from 20 weeks of pregnancy. In addition, the hypertension must be accompanied by either proteinuria, maternal organ dysfunction or signs of uteroplacental dysfunction (Table 10).

Table 10 Definition of preeclampsia based on the International Society for the Study of Hypertension in Pregnancy[175]

Preeclampsia definition		
Mandatory		
Gestational hypertension	≥ 140 / ≥ 90 mmHg, developed de novo ≥ 20 weeks of pregnancy	Repeated measures at least 4 hours apart or two consecutive visits.
Accompanied by one or more of the following manifestations		
Proteinuria	24-h urinary protein ≥ 300 mg/day or Albumin/Creatinine Ratio ≥ 30 mg/mmol ~ 265,2 mg/g (DK reference interval)	Urine dipstick as a first screen tool, if positive, then further analysis.
Maternal organ dysfunction, represented by either: - Acute kidney injury* - Liver involvement - Neurological complications - Hematological complications	 Creatinine: ≥ 90 µmol/L Alanine aminotransferase: > 40 IU/L with/without right upper quadrant or epigastric abdominal pain e.g. eclampsia, altered mental status, blindness, stroke, clonus, severe headaches, persistent visual scotomata. Thrombocytopenia with platelet count < 150,000/µL ~ 150 x 10^9/L (DK reference interval), Disseminated Intravascular Coagulation or hemolysis.	
Uteroplacental dysfunction	Fetal growth restriction (EFW<10^{th} centile, in particular: EFW<3^{rd} centile and/or abnormal UA Doppler) or abnormal umbilical artery Doppler (PI>95^{th} centile, absent end-diastolic flow or reversed end-diastolic flow) or stillbirth	
In case of chronic hypertension – superimposed preeclampsia occurs when		
Development of maternal organ dysfunction (as above) or rise in blood pressure and new-onset proteinuria (if not pre-existing)	Same cut-off values as above	
HELLP syndrome		
A serious manifestation of preeclampsia, not a separate disorder	Hemolysis, Elevated Liver enzymes and Low Platelet count	

** Serum uric acid: not a diagnostic criterion, but elevated levels are associated with worse maternal and fetal outcomes. EFW, estimated fetal weight, HELLP, Hemolysis Elevated liver enzymes Low platelet.*

3) Preterm delivery was defined as birth < 37 weeks of gestation. A detailed description of each of the preterm cases including the corresponding T2* value is showed in Table 11.

Table 11 Cases with preterm delivery

Case	Cause / indication	Clinical information	SGA	PE	Placental histology	T2* z-score
1	SGA	Last estimated fetal weight: -31,6%. Normal fetal and umbilical Doppler flow measures. Cesarean section, GA 36+5, birthweight: -23,9%.	Yes	No	Abnormal MVM	0.250 MRI GA 35+2
2	Suspected placental abruption (+PPROM)	PPROM in gestational age 28+3. Suspected placental abruption due to vaginal bleeding. Acute cesarean section, GA 33+3, birthweight: -22,7%.	Yes	No	Abnormal FVM and MVM	-2.993 MRI GA 31+3
3	Asphyxia and suspected intestinal ischemia	Reduced fetal movements, CTG with reduced variability and without any accelerations. Ultrasound scan: no fetal movements and intraperitoneal fluid within the fetus. Acute cesarean section, GA 34+1. Birthweight: -7,2%, Operation post partum with removal of 20 cm intestine.	No	No	No placental histology.	-0,435 MRI GA 26+5
4	PPROM	Several contacts due to reduced fetal movements. Last EFW: -27,8%. Abnormal fetal and umbilical Doppler flow measures before delivery. PPROM GA 36+3. Stimulation of contractions. Vaginal delivery, GA 36+3, birthweight -33,8%.	Yes	No	Abnormal MVM	-2,126 MRI GA 35+1
5	Preeclampsia	Preeclampsia with hypertension, proteinuria and symptoms. Last estimated fetal weight: -26,8%. Normal fetal and umbilical Doppler flow. Stimulation of contractions, vaginal delivery. GA 36+2, birthweight -23,4%.	Yes	Yes	Abnormal MVM	-2,642 MRI GA 33+0
6	SGA	IVF with egg donation (prophylactic Acetylsalicylic acid). Last estimated fetal weight: -31,2%. Normal fetal and umbilical Doppler flow measures. Cesarean section, GA 36+6, birthweight = -20,2%.	No	No	Normal	-1,058 MRI GA 33+4
7	PPROM	Previous stroke, prophylactic low molecular weight heparin. PPROM GA 28+1. Last estimated fetal weight: -18,5%. Normal fetal and umbilical Doppler flow measures. Vaginal delivery, GA 34+0, birthweight -15%.	No	No	Abnormal MVM	-2,487 MRI GA 33+2
8	Preeclampsia	Smoker. Hypertension from GA 28. Preeclampsia from GA 30 due to proteinuria. Last estimated fetal weight: -33,7%. Normal fetal and umbilical Doppler flow measures. Elective c-section (indication: preeclampsia and FGR), GA 34+0, birthweight: -36,8%.	Yes	Yes	Abnormal MVM	-2,596 MRI GA 30+0
9	PPROM	Smoker. PPROM GA 34+5. Last estimated fetal weight: -25,0%. Normal fetal and umbilical Doppler flow measures. Vaginal delivery, GA 34+5, birthweight: -21,5 %.	No	No	Normal	-1,387 MRI GA 30+3
10	FGR	Single umbilical artery. Last estimated fetal weight: -53,4%. Abnormal fetal and umbilical Doppler flow measures before delivery. Emergency cesarean section, category 2, GA 28+3, birthweight: -51,9%.	Yes	No	Abnormal FVM and MVM	-4,266 MRI GA 27+0
11	FGR + preeclampsia	Smoker. Preeclampsia. Abnormal uterine artery Doppler flow measure. Last estimated fetal weight: -22,5%. Abnormal fetal, but normal umbilical Doppler flow measure before delivery. Acute c-section (signs of asphyxia on CTG, preeclampsia and IUGR), GA 35+6, birthweight: -35,2%.	Yes	Yes	Abnormal MVM	-2,925 MRI GA 31+1
12	FGR	Several large uterine fibroids. Last estimated fetal weight: -38,7%. Abnormal fetal and umbilical Doppler flow measures before delivery. Emergency cesarean section, category 2, GA 27+6, birthweight: -41,2%.	Yes	No	Abnormal FVM	-2,127 MRI GA 27+3

SGA, small-for-gestational-age, PE, preeclampsia, GA, gestational age, PPROM, preterm premature rupture of membranes, MVM, maternal vascular malperfusion, FVM, fetal vascular malperfusion, FGR, fetal growth restriction, MRI, magnetic resonance imaging, EFW, estimated fetal weight, CTG, cardiotocography

4) Abnormal PHE was defined in this study as vascular malperfusion; either maternal (MVM) or fetal (FVM) vascular malperfusion according to Amsterdam Consensus Statement[63]. Placental findings indicating MVM include placental hypoplasia (weight below 10th centile and/or thin umbilical cord (<8 mm at term or below 10th centile), infarctions, retroplacental hemorrhage, decidual arteriopathy, accelerated villous maturation and distal villous hypoplasia. FVM is due to obstruction to fetal blood flow (e.g. umbilical cord lesions, hypercoagulability, cardiac dysfunction) and include thrombosis and/or obliteration of fetal vessels, fibrous avascular villi and villous karyorrhexis. The pathologist was blinded to the MRI findings.

6.1.5. STATISTICAL ANALYSIS

Logistic regression and receiver operating characteristics (ROC) curves were used to investigate the predictive performance between placental T2* and the four outcomes of placental dysfunction. The results are presented as area under the ROC curve (AUC).

Placental T2* was compared between uncomplicated pregnancies and pregnancies complicated by subgroups of placental dysfunction using student t-test.

Each woman may have more than one outcome. If so, for the analysis of each outcome separately, the women were included in each of the outcome groups. When garthering the outcomes in either clinical manifestations (SGA at birth, preeclampsia and/or preterm birth) and/or abnormal PHE, the women were included in the relevant groups as they met the criteria for the outcome groups.

The correlation between placental T2* Z-score and each of the three Doppler flows; UA PI Z-score, MCA PI Z-score and mean UtA PI Z-score was investigated using linear regression analysis and Pearson's correlations coefficients.

For the outcome preterm delivery, only pregnancies with placental MRI performed before 37 weeks of gestation were included in the analysis (n=76). Moreover, only pregnancies with a PHE (n=81) were used in the analysis of abnormal PHE as outcome.

Statistical analyses were performed using Stata®, version 15.1 (Stata Corp, College Station, TX, USA). P-values < 0.05 were considered statistically significant.

6.1.6. APPROVALS

The study was approved by the North Denmark Region Committee on Health Research Ethics, local reference ID: N-20170052.

Data collection and handling was approved by a regional notification to the Danish Data Protection Agency, local reference-ID: 2017-148.

6.2. RESULTS

During the study period, 227 pregnancies fulfilled the inclusion criteria (EFW≤-22% and normal fetal Doppler flows). We excluded 43 women due to non-Danish speaking, fetal malformation or maternal claustrophobia/anxiety. Of the 184 women available for recruitment, 92 accepted inclusion (Figure 9).

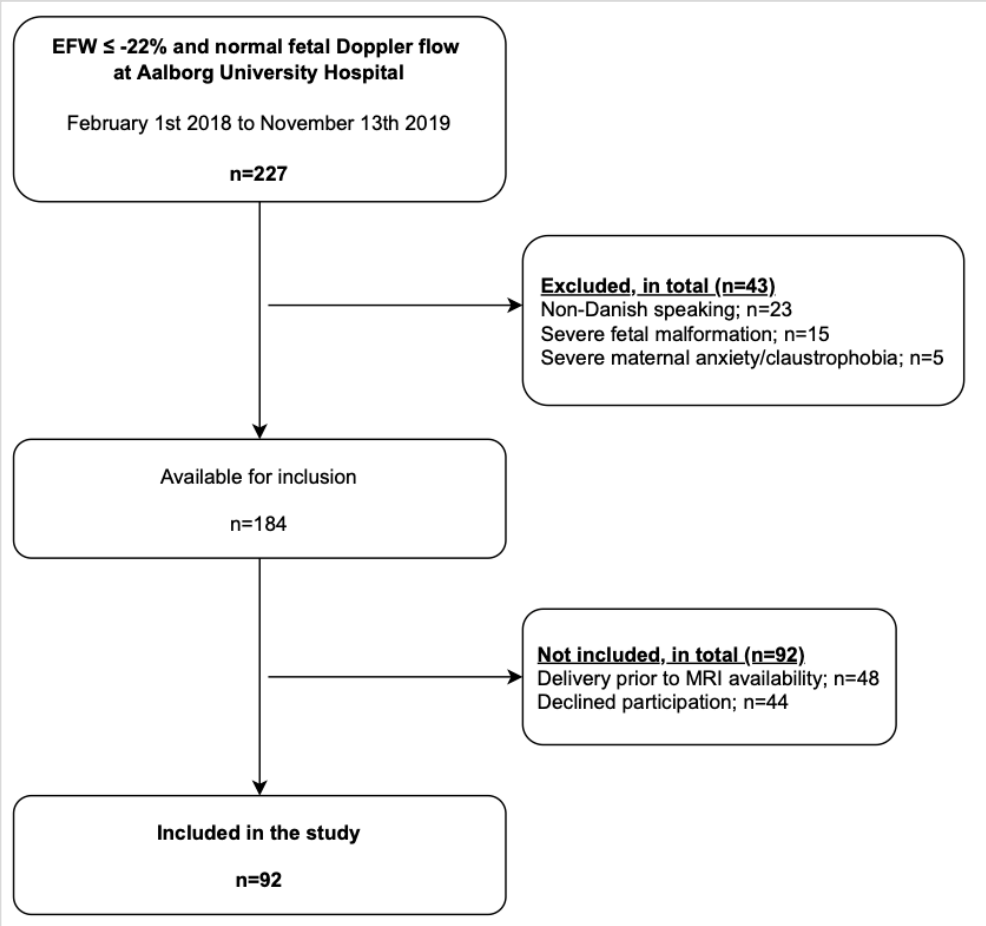

Figure 9 Flowchart of the study population in Study III.

EFW, estimated fetal weight, MRI, magnetic resonance imaging.

Among the pregnancies that were available for inclusion, the recruited and non-recruited women were quite similar (Table 12). At birth, those recruited had a median BW 60 g higher than those non-recruited. Moreover, at the last ultrasound scan before delivery, the proportion of abnormal fetal Doppler flows measured in the UA and the MCA was slightly lower among the recruited (UA: 6.5% vs. 7.3% and MCA: 4.8% cs. 6.3%, respectively).

Table 12 Maternal and neonatal characteristics of the study population and the non-recruited patients

	Study population n=92	Non-recruited n=127
Median (interquartile range) or number (percent)		
Maternal age (years)	29 (26.5, 34)	30 (26, 34)
Pregestational maternal BMI (kg/m^2)	23.1 (20.6, 26.6)	21.3 (19.2, 25.3)
Smoking (at 1st trimester scan)	16 / 92 (17.4%)	17 / 127 (13.4%)
Nullipara	42 / 92 (45.7%)	69 / 127 (54.3%)
Gestational age at inclusion ultrasound (weeks)	30.3 (27.9, 33.2)	30.7 (28.0, 36.9)
EFW deviation at inclusion ultrasound (%) [a]	-24.8 (-27.0, -23.2)	-23.9 (-26.2, -22.7)
Gestational age at birth (weeks)	39.0 (37.8, 40.1)	38.6 (37.0, 40.3)
Preterm birth (delivery < 37 weeks)	12 / 92 (13.0%)	28 / 127 (21.9%)
Birthweight (gram)	2730 (2440, 2975)	2670 (2350, 2960)
Birthweight deviation [a] (%)	-20.0 (-22.5, -14.2)	-19.4 (-25.0, -13.7)
Low birthweight (≤ - 22% [a])	27 / 92 (29.3%)	45 / 127 (35.2%)
Extreme low birthweight (≤ -33% [a])	8 / 92 (8.7%)	11 / 127 (8.6%)
Abnormal fetal Doppler flow (last ultrasound before delivery) - Umbilical artery PI [b] *(z-score ≥2.000)* - Middle cerebral artery PI [b] *(z-score ≤-2.000)*	 6 / 92 (6.5%) 4 / 83 (4.8%)	 7 / 124 (7.3%) 7 / 112 (6.3%)
Boys	33 / 92 (35.9%)	51 / 127 (40.2%)
Preeclampsia	6 / 92 (6.5%)	12 / 127 (9.4%)
Maternal diabetes	8 / 92 (8.7%)	15 / 127 (11.8%)
Vaginal delivery	66 / 92 (71.7%)	83 / 127 (65.4%)
Induction of delivery	38 / 92 (41.3%)	53 / 127 (41.7%)

Of the 135 patients not included, 8 patients were lost to follow-up. Measurements were adjusted for gestational age (percent or Z-score) using the following references: [a] Maršál et al 1996[26]*, [b] Parra-Cordero et al 2007*[171]*. BMI, body mass index, EFW, estimated fetal weight, PI, pulsatility index.*

The clinical indication for the referral to ultrasound EFW was recorded for all included women (Table 13). Only one indication is recorded for each woman. The most frequent referral indication in the total cohort was follow-up based on the 20 weeks ultrasound scan either due to small biometries or suspected fetal malformations (22%). The women may have more than one indication, however, only one indication was registered as the primary indication. The indication noted was what first led to an ultrasound scan > 20 weeks of gestation.

Table 13 Clinical indications for ultrasound scan with estimated fetal weight of -22% or lower

	Total cohort N=92	SGA N=27	Preeclampsia N=6	Preterm delivery N=12	Abnormal placental histological examination N=40
Pregestational maternal medical history	**N=15**				
Previous abuse of drugs or alcohol	3 (3%)	1 (4%)	-	-	2 (5%)
Essential hypertension	1 (1%)	1 (4%)	-	-	1 (3%)
Other cardiovascular disease	2 (2%)	-	-	-	-
Inflammatory bowel disease	1 (1%)	-	-	-	-
Autoimmune disorders (Systemic lupus erythematosus and ulcerative colitis)	1 (1%)	-	-	-	-
Psychiatric disorders	4 (4%)	1 (4%)	-	-	2 (5%)
Other (ovarian cyst, low maternal BMI and age)	3 (3%)	1 (4%)	-	-	-
Previous obstetric history	**N=12**				
Previous SGA/FGR	3 (3%)	-	-	-	1 (3%)
Previous preeclampsia / HELLP	2 (2%)	1 (4%)	-	-	1 (3%)
Previous cesarean section	2 (2%)	-	-	-	-
Previous missed abortion or recurrent miscarriage	2 (2%)	-	-	-	2 (5%)
Previous preterm delivery	2 (2%)	-	-	-	1 (3%)
Other (previous child with gall bladder problems)	1 (1%)	-	-	-	-
Current pregnancy	**N=65**				
20 weeks ultrasound with small ultrasound biometries / suspected fetal malformations	20 (22%)	5 (19%)	2 (33%)	3 (25%)	11 (28%)
Small symphysis-fundal height measure	14 (15%)	5 (19%)	-	3 (25%)	7 (18%)
Threatened preterm labor (reduced cervical length / uterine contractions)	9 (10%)	4 (15%)	-	1 (8%)	3 (8%)
Single umbilical artery	5 (5%)	3 (11%)	1 (17%)	1 (8%)	2 (5%)
Gestational diabetes	6 (7%)	1 (4%)	1 (17%)	-	3 (8%)
Gestational hypertension / preeclampsia suspicion	2 (2%)	1 (4%)	1 (17%)	1 (8%)	1 (3%)
Reduced fetal movements	2 (2%)	1 (4%)	-	-	-
PPROM	1 (1%)	1 (4%)	-	1 (8%)	1 (3%)
Breech position	1 (1%)	-	-	-	-
Conception (egg donation)	1 (1%)	-	-	1 (8%)	-
Other (general discomfort, low PAPP-A, sadness, unspecific abdominal pain)	4 (4%)	1 (4%)	1 (17%)	1 (8%)	2 (5%)

SGA, small-for-gestational-age, BMI, body mass index, FGR, fetal growth restriction, HELLP, hemolysis elevated liver enzymes low platelet, PPROM, preterm prelabor rupture of membranes, PAPP-A, pregnancy-associated plasma protein-A.

Maternal and pregnancy characteristics are presented in Table 14. Placental MRI was performed at gestational week 26^{+5} to 39^{+6}. The median time interval between MRI and birth was 4.6 weeks (interquartile range: 2.7-7.8 weeks) (Table 14).

Table 14 Characteristics of the study population

	Total cohort N=92
Maternal characteristics	
Maternal age (years)	29 (26.5, 34)
Pregestational maternal BMI (kg/m^2)	23.1 (20.6, 26.6)
Smoking at 1st trimester scan	16 / 92 (17.4%)
Nullipara	42 / 92 (45.7%)
Maternal diabetes	8 / 92 (8.7%)
Pregnancy at time of inclusion	
EFW deviation at time of inclusion (%) [a]	-24.8 (-27.0, -23.2)
Umbilical artery PI z-score [b] at inclusion	0.103 (-0.569, 1.116)
Middle cerebral artery PI z-score [b] at inclusion	-0.179 (-0.821, 0.454)
Gestational age at inclusion (weeks)	30.3 (27.9, 33.2)
Pregnancy at time of MRI	
EFW deviation at time of MRI (%) [a]	-22.9 (-27.1, -19.0)
Abnormal uterine artery Doppler flow [c] *(mean PI Z-score>2.000) at time of MRI*	13 / 85 (15.3%)
Gestational age at MRI (weeks)	33.5 (30.6, 36.0)
Time between MRI and birth (weeks)	4.6 (2.7, 7.8)
Delivery characteristics	
Gestational age at birth (weeks)	39.0 (37.8, 40.1)
Birthweight (gram)	2730 (2440, 2975)
Birthweight deviation [a] (%)	-20.0 (-22.5, -14.2)
Extreme small-for-gestational-age (≤ -33%)	8 / 92 (8.7%)
Boys	33 / 92 (35.9%)
Vaginal delivery	66 / 92 (71.7%)
Induction of delivery	38 / 92 (41.3%)
Acute cesarean section	8 / 92 (8.7%)

Data are presented as median (interquartile range) or number (percent). Measurements were adjusted for gestational age (percent or Z-scores) using the following references: [a] Maršál et al 1996[26]*, [b] Parra-Cordero et al 2007*[171]*, [c] Gómez et al 2008*[173]*. BMI, body mass index, GA, gestational age, EFW, estimated fetal weight, PI, pulsatility index, MRI, magnetic resonance imaging.*

Placental dysfunction was revealed in 55% (51/92) of suspected SGA pregnancies with normal fetal Doppler flow. At birth, 27 (29%) neonates were SGA, 6 (7%) pregnancies were complicated by preeclampsia, 12 (13%) were delivered preterm, and 40 (49%) of the placentas that underwent PHE were abnormal. None of the 12 preterm deliveries were spontaneous or caused by placental insufficiency (Table 11). The maternal and neonatal characteristics of the four outcome groups are presented in Table 15.

Table 15 Characteristics of the study population subdivided into placenta-related outcomes of interest

	Small-for-gestational age (Birthweight ≤-22%) N=27	**Preeclampsia** N=6	**Preterm delivery** (Delivery <37.0 weeks) N=12	**Abnormal placental histological examination** (Fetal or maternal vascular malformation) N=40
	Median (interquartile range) or number (percent)			
Maternal characteristics				
Maternal age (years)	27 (23, 30)	22.5 (21, 27)	27.5 (23.5, 34)	28.5 (24, 34)
Pregestational maternal BMI (kg/m^2)	22.9 (20.6, 26.6)	25.8 (19.8, 33.1)	25.7 (22.4, 30.7)	23.4 (20.6, 26.6)
Smoking at 1st trimester scan	4 / 27 (14.8%)	2 / 6 (33.3%)	4 / 12 (33.3%)	8 / 40 (20.0%)
Nullipara	19 / 27 (70.4%)	4 / 6 (66.7%)	10 / 12 (83.3%)	22 / 40 (55.0%)
Maternal diabetes	1 / 27 (3.7%)	1 / 6 (16.7%)	0 / 12	3 / 40 (7.5%)
Pregnancy at time of inclusion				
EFW deviation at time of inclusion (%) [a]	-27.5 (-32.3, -24.9)	-25.0 (-25.8, -23.1)	-28.3 (-32.4, -24.2)	-24.9 (-29.6, -23.2)
Umbilical artery PI z-score [b]	0.075 (-0.559, 1.303)	0.297 (-0.507, 0.716)	-0.264 (-0.634, 0.578)	0.365 (-0.533, 1.262)
Middle cerebral artery PI z-score [b]	0.111 (-0.309, 0.634)	0.047 (-0.347, 0.374)	-0.568 (-0.979, 0.060)	-0.250 (-0.832, 0.378)
Gestational age at inclusion	29.9 (27.3, 34.1)	29.8 (28.3, 33.0)	29.8 (27.1, 31.0)	30.9 (28.0, 34.1)
Pregnancy at time of MRI				
EFW deviation at time of MRI (%) [a]	-28.3 (-33.3, -22.0)	-22.0 (-28.5, -19.0)	-26.9 (-32.1, -21.7)	-22.9 (-28.4, -19.6)
Abnormal uterine artery Doppler flow [c] *(mean PI Z-score>2.000) at time of MRI*	8 / 25 (32.0%)	4 / 5 (80.0%)	6 / 10 (60.0%)	10 / 37 (27.0%)
Gestational age at MRI (weeks)	32.9 (30.0, 36.4)	32.1 (30.0, 35.7)	31.3 (28.7, 33.5)	34.7 (30.2, 36.7)
Time between MRI and birth (weeks)	3.1 (1.4, 7.4)	3.7 (2.7, 4.8)	2.7 (1.4, 4.2)	3.0 (2.2, 5.5)
Delivery characteristics				
Gestational age at birth (weeks)	37.9 (36.4, 39.3)	37.0 (35.9, 37.7)	34.4 (33.7, 36.4)	38.5 (37.1, 39.9)
Birthweight (gram)	2250 (1935, 2650)	2300 (1770, 2550)	1943 (1628, 2188)	2570 (2180, 2875)
Birthweight deviation[a] (percent)	-27.7 (-33.8, -23.4)	-22.3 (-35.2, -20.6)	-23.7 (-36.0, -20.9)	-21.3 (-28.2, -17.3)
Boys	10 / 27 (37.0%)	0 / 6	6 / 12 (50.0%)	28 / 40 (70.0%)
Vaginal delivery	16 / 27 (59.3%)	4 / 6 (66.7%)	4 / 12 (33.3%)	28 / 40 (68.3%)
Induction of delivery	11 / 27 (40.7%)	5 / 6 (83.3%)	3 / 12 (25.0%)	19 / 40 (47.5%)
Acute cesarean section	3 / 27 (11.1%)	1 / 6 (16.7%)	4 / 12 (33.3%)	3 / 40 (7.5%)

Data are presented as median (interquartile range) or number (percent). Measurements were adjusted for gestational age (percent or Z-scores) using the following references: [a] Maršál et al 1996[26], [b] Parra-Cordero et al 2007[171], [c] Gómez et al 2008[173]. BMI, body mass index, GA, gestational age, EFW, estimated fetal weight, PI, pulsatility index, MRI, magnetic resonance imaging

Many pregnancies suffering from placental dysfunction showed a combination of more than one clinical manifestation and abnormal PHE. The relation between the manifestations of placental dysfunction is presented in Table 16. The proportion of abnormal PHE in pregnancies with clinical manifestations of placental dysfunction was 73.1% in SGA at birth, 81.8% in preterm deliveries, and 100% in pregnancies complicated by preeclampsia.

Table 16 The relation between outcomes of placental dysfunction

Outcome of interest	**Small-for-gestational age** (Birthweight ≤-22% [a]) N=27	**Preeclampsia** N=6	**Preterm delivery** (Delivery <37.0 weeks) N=12	**Abnormal placental histological examination** (Fetal and/or maternal vascular malformation) N=40
SGA[a] at birth (percent)		3 / 6 (50.0%)	8 / 12 (66.7%)	19 / 40 (47.5%)
Preeclampsia	3 / 27 (11.1%)		3 / 12 (25.0%)	6 / 40 (15.0%)
Preterm delivery	8 / 27 (29.6%)	3 / 6 (50.0%)		9 / 40 (22.5%)
Abnormal PHE	19 / 26 (73.1%)	6 / 6 (100%)	9 / 11 (81.8%)	

[a] Maršál et al 1996[26]. SGA, small-for-gestational-age, PHE, placental histological examination.

Placental T2* Z-score was a significant predictor of SGA at birth (AUC=0.63, p=0.030), preeclampsia (AUC=0.88, p=0.005), preterm delivery (AUC=0.81, p=0.001), and abnormal PHE (AUC=0.73, p=0.001) (Figure 10). In addition, placental T2* was a significant predictor of clinical manifestations and/or abnormal PHE of placental dysfunction (AUC=0.72, p=0.002) (Figure 10).

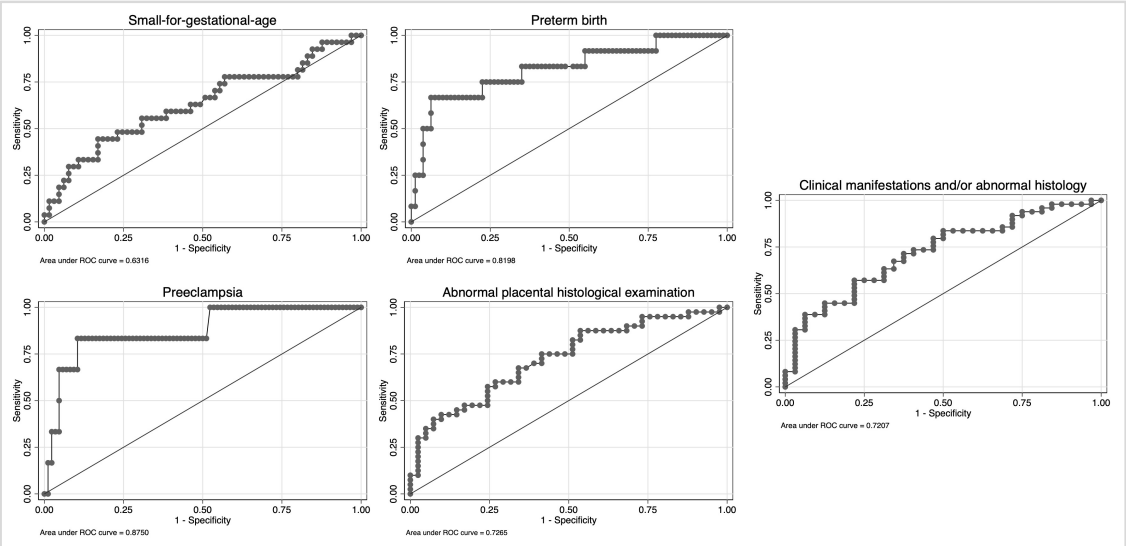

Figure 10 Receiver operating characteristics curves for placental T2* and outcomes of placental dysfunction.

Clinical manifestations and/or abnormal placental histology include clinical manifestations such as either small-for-gestational-age at birth, and/or preeclampsia, and/or preterm birth < 37 weeks of gestation, and/or abnormal placental histological examination.

Figure 11 represents the distribution of outcomes of placental dysfunction in the 81 pregnancies, where the placenta underwent PHE post partum.

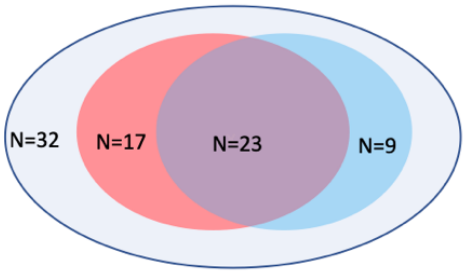

Figure 11 The distribution of placenta-related outcomes in the population with placental histological examination (PHE) (n=81).

Grey area: Normal PHE and no clinical manifestations (n=32). Red area: Abnormal PHE (n=17). Blue area: Clinical manifestations of placental dysfunction (n=9). Purple area: Abnormal PHE and clinical manifestations of placental dysfunction (n=23). Clinical manifestations of placental dysfunction include SGA at birth, preeclampsia, and/or preterm delivery.

Pregnancies complicated by placental dysfunction represented by clinical manifestations and/or abnormal PHE had significantly lower placental T2* (mean T2* Z-score = -1.096, p=0.0006), when compared to uncomplicated pregnancies (mean T2* Z-score = -0.142) (Table 17). The lowest mean placental T2* was found in the group of pregnancies with a combination of clinical manifestations of placental dysfunction and abnormal PHE (mean T2* Z-score= -1.523). Moreover, pregnancies with isolated abnormal PHE had significantly lower placental T2* (mean T2* Z-score=-0.791, p=0.045), while the placental T2* remained within normal in pregnancies with isolated clinical manifestations (mean T2* Z-score=-0.578, p=0.287).

Table 17 Comparison of placental T2* between uncomplicated pregnancies and pregnancies with placental dysfunction.

Area	N = 81	Placenta T2* z-score Mean (SD)	p-value
Purple: Abnormal PHE and clinical manifestations	23	-1.523 (1.35)	0.0001
Red: Isolated abnormal PHE	17	-0.791 (0.97)	0.045
Blue: Isolated clinical manifestations	9	-0.578 (1.01)	0.287
Red+Blue+Purple: Clinical manifestations and/or abnormal PHE	49	-1.096 (1.22)	0.0006
Grey: Normal PHE and no clinical manifestations	32	-0.142 (1.09)	Reference
No histology	N = 11		
No clinical manifestations	9	-0.678 (1.33)	0.220
Clinical manifestations	2	-1.443 (1.43)	0.114

The colours of areas refer to subgroup colours presented in Figure 11. Clinical manifestations of placental dysfunction include SGA at birth, preeclampsia and/or preterm delivery. SGA, small-for-gestational-age, PHE, placental histological examination, SD, standard deviation.

Placental T2* showed a negative linear correlation with UtA PI Z-score (r= -0.24, p=0.016), while placental T2* was positively correlated to MCA PI Z-score (r=0.29, p=0.017). There was no significant correlation between placental T2* Z-scores and UA PI Z-scores (r=0.18, p=0.17) (Figure 12).

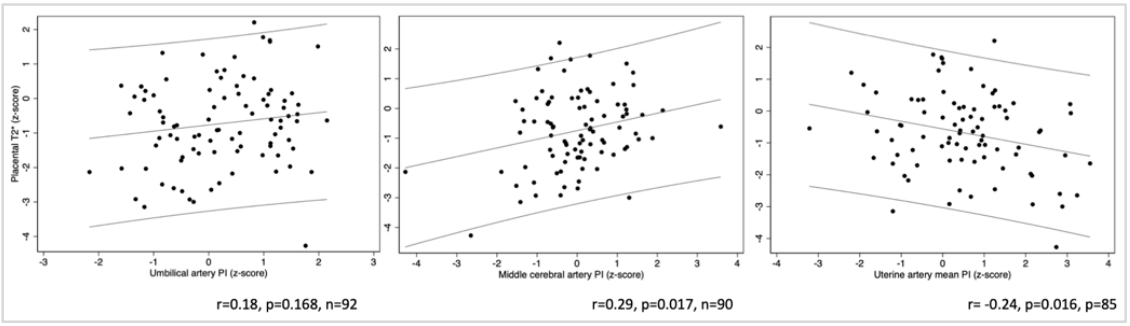

Figure 12 Correlation between placental T2* and umbilical artery PI (left), middle cerebral artery PI (middle) and uterine artery PI (right) measured at time of MRI.

The linear relation between placental T2 Z-score and umbilical artery PI Z-score (left), middle cerebral artery PI Z-score (middle) and uterine artery mean PI Z-score (right) and 95% prediction interval. r = Pearson correlation coefficient. Z-scores are adjusted for gestational age using the following references; Placental T2*: Sinding et al[20], umibilical artery PI and middle cerebral artery PI: Parra-Cordero et al[171] and uterine artery mean PI: Gómez et al[173]. PI, pulsatility index, MRI, magnetic resonance imaging.*

6.3. DISCUSSION

In this cohort of SGA fetuses with normal fetal Doppler flow, placental dysfunction was found in more than half of the included pregnancies. In this study, placental T2* was a predictor of SGA at birth, preeclampsia, preterm delivery and placental vascular malperfusion identified by PHE. The group of pregnancies complicated by placental dysfunction and the group of pregnancies with isolated abnormal PHE both showed significantly lower placental T2*, when compared to uncomplicated pregnancies. Therefore, our results suggest, that T2* weighted placental MRI can identify placental dysfunction even in cases with no clinical manifestations and prior to abnormal Dopper flows. Accordingly, placental dysfunction may be more frequent than previously presumed. Thus, placental T2* have the potential to improve the antenatal care in suspected SGA fetuses by complementing Doppler flow measurements in the identification of placental dysfunction.

It is a strength of the study that the study design was prospective and calculating placental T2* while blinded to all clinical outcomes. The placental T2* protocol used in this study has been evaluated thoroughly.[20,165] A single trained pathologist performed all PHE while using the Amsterdam criteria[63] while blinded to the placental T2* values. The PHE in this study allows identification of subclinical placental dysfunction, which is a major strength. We included 92 of the 227 women eligible for

inclusion during the study period, and no significant differences were revealed between recruited and non-recruited.

Limitations of the study are that placental MRI was performed at a wide range of gestational ages, as the predictive performance of placental T2* may vary over gestation. Moreover, our study was not powered to investigate early-onset placental dysfunction separately, since 46% of the MRIs were conducted after 34 weeks of gestation. Additionally, the pathologist in accordance with the clinical routine at the department was not blinded to the obstetric outcome. The results of the PHE were dichotomized being either normal or abnormal, and would have been further divided into degrees of pathology in a larger setting.

In this study, placental dysfunction was more frequent (55%) than expected. Abnormal PHE was the most frequent outcome, and the proportion of abnormal PHE was higher in pregnancies with clinical manifestations of placental dysfunction. However, abnormal PHE was also seen in pregnancies without clinical manifestations of placental dysfunction. Even in this group, placental T2* was significantly reduced. This finding underlines abnormal PHE as a sign of placental dysfunction, even in uneventful pregnancies including fetal weight within normal range. Placental T2* may have the ability to identify these cases of "subclinical" placental dysfunction. However, the short- and long-term consequences of this group need further evaluation. In addition, subclinical placental dysfunction needs to be considered in future evaluation of biomarkers of placental dysfunction.

We found placental T2* value to be a significant predictor of all four placenta-related obstetric outcomes. This is in accordance with previous literature including cases of low BW[20,24,25,176] and preeclampsia or gestational hypertension[22,23].

The predictive performance of placental T2* in relation to SGA at birth in our study is markedly lower (AUC=0.63) than previously reported by Sinding et al.[21] (AUC=0.92). In our SGA cohort, the inclusion criteria were well-defined as EFW ≤ -2.0 Z-score and normal fetal Doppler flows, and thereby the study population in this study is rather homogenous and only mildly affected by placental dysfunction. This is in contrast to the previous study by Sinding et al.[21], which included both healthy pregnancies and pregnancies complicated by severe placental dysfunction. The different study populations may explain the difference in the predictive performance of T2*.

This is the first study to investigate placental T2* values in pregnancies complicated by preterm delivery. All preterm deliveries in this cohort are presented in Table 11. The vast majority had abnormal PHE (9/11, one case had no PHE). Moreover, 33%

had PPROM, which has been associated with vascular lesions, e.g. accelerated villous maturation (within MVM[177,178]) in cases without infections. None of the preterm deliveries were spontaneous or caused by cervical insufficiency. This justifies that preterm delivery can be regarded as a proxy of placental dysfunction.

In this cohort, placental T2* was a significant predictor of preeclampsia, which is in line with previous results[22,23]. All 6 cases of preeclampsia in this study had abnormal PHE, indicating the strong association between the two outcomes. In this current study, cases of preeclampsia showed the strongest correlation with placental T2*, which underlines the high degree of placental dysfunction in these complicated pregnancies.

Placental T2* Z-score was significantly correlated with both MCA PI Z-score and UtA PI Z-score, and thereby they may reflect some of the same placental pathology. Current knowledge suggests that a low T2* value reflects altered tissue morphology and tissue hypoxia[24,112,114], whereas UtA PI is mainly related to the resistance of the spiral arteries[179], and MCA PI reflects redistribution of fetal blood during fetal hypoxia – also known as brain sparing[79]. Fetal hypoxia may not affect milder cases of placental dysfunction, as this is a late manifestation. Being a more direct marker of placental dysfunction, placental T2* may be more sensitive than Doppler flow measurements in milder cases of placental dysfunction, as seen in the group of pregnancies with isolated abnormal PHE. Moreover, in other clinical cohorts such as diabetes in pregnancy[180–182] and late-onset placental dysfunction[98,183], the predictive performance of Doppler flows is rather low. Therefore, the benefit of placental T2* may be even higher in these clinical subgroups than the performance observed in this current study.

In this study, we defined SGA as BW ≤ - 2.0 Z-score of expected for GA according to the reference by Maršál et al.[26]. This cut-off is equivalent to 2.3rd centile in the Danish population, and the standard SGA cut-off used in national Danish guidelines.[184] This cut-off is also in line with international consensus based on a Delphi procedure[15], where EFW <3rd centile was selected as a solitary parameter to indicate FGR. Another approach would have been to choose EFW ≤ - 15% of the expected for GA, which is equivalent to 10th centile in the Danish population. Using such cut-off would lead to a larger number of eligible women with a markedly lower proportion of placental pathology.

Currently, MRI scans are restricted by high cost and limited availability. However, the clinical importance and the promising field of placental T2* should not be limited by these practical aspects, that may change in near future.

In conclusion, placental T2* is a sensitive biomarker of placental dysfunction in SGA pregnancies, even when fetal Doppler flows are normal and in absence of clinical manifestations. Our study indicates that placental dysfunction is more frequent than previously assumed and highlights the importance of focusing directly on placental function.

CHAPTER 7. GENERAL DISCUSSION AND PERPECTIVES

The studies in this thesis explores different aspects of low BW such as definition, antenatal detection and prediction. Neonates that are born with low BW may be constitutionally SGA or they are suffering from FGR due to placental dysfunction. It is generally accepted, that placental dysfunction is associated with an increased risk of adverse neonatal and obstetric outcomes. Therefore, pregnancies complicated by low BW are considered high risk pregnancies.

Study I explores the different definitions of low BW. This study does not support one universal weight curve to be used in all populations, as the Danish standard BW curve differ markedly from the universal standard BW curve from Intergrowth-21st Project. Low BW is associated with an increased risk of stillbirth and neonatal death. However, the vast majority of adverse outcomes occurred in the group of non-SGA, regardless of which curve was used. ***Study II*** investigates the antenatal identification of SGA in a local setting. Despite having a thorough screening setup in the second and third trimester with predefined clinical examinations by midwifes and general practitioners every three to four weeks and obstetric controls including ultrasound estimates of fetal weight on indication, the identification of low BW, particularly at term, is low. ***Study III*** is a clinical prospective study using placental T2* as a method to identify placental dysfunction among SGA fetuses with normal fetal Doppler flows. In this study, placental dysfunction was defined by clinical manifestations and/or vascular malperfusion at the postnatal placental histological examination. Placental dysfunction was revealed in a large proportion of SGA pregnancies, and placental T2* was a sensitive predictor of this condition, regardless of the clinical manifestations.

This thesis demonstrates that the definition and antenatal identification of low BW is challenging. The selection of appropriate BW curves and rational cut-offs are of major importance. Changing the curve or cut-off used to identify SGA affects the sensitivity and the FPR. This was demonstrated in ***Study I***, in which the figures were significantly different when using the Intergrowth-21st and the Danish standard curve. Whilst improving the sensitivity, the number of false-positive cases will increase. The optimal sensitivity and FPR depend on the risk associated with non-detected SGA and false-positive SGA. It is well-described that undetected SGA is associated with an increased risk of adverse outcome (OR 4.1) as compared to those identified as SGA during pregnancy[5]. On the contrary, false positive SGA may lead to more obstetrical interventions and psycological parental distress. As demonstrated in ***Study II***, false positive SGA was associated with induction of labor (OR 2.5). Likewise, a previous study by Gabbay-Benziv et al[43] also demonstrated higher rates of labor induction, cesarean sections and short-term adverse neonatal outcomes after false SGA diagnosis.[43] Preterm delivery due to antenatal identification of SGA may reduce

stillbirth, but may also be associated with adverse outcomes during childhood such as respiratory and gastrointestinal disease when compared to SGA delivered at term.[41] These associations need further investigation as they may be explained by the preterm delivery being more severely affected by placental dysfunction.

Even when the presumably right curve and cut-off is implemented in the antenatal care, the antenatal identification of SGA remains low. This may be related to inaccurate ultrasound estimates and inappropriate referral for ultrasound weight scan in the second and third trimester. The currently used ultrasound weight formula uses head circumference, abdominal circumference and femur length in the calculation of fetal weight[34]. This formula has a known standard deviation of 8%[34], however, this may be even higher if the fetal proportions are not standard, which is not the case in very small or large fetuses.[185] Moreover, limited scan quality by e.g., fetal positioning or maternal obesity may also contribute to the challenge of ultrasound estimates of fetal weight in the identification of SGA.[186] Ultrasound weight scans on indication is highly dependent on the referral pattern. Symphysis-fundal height measurements complements the clinical estimate of fetal size, however, the sensitivity varies from 27 to 76%[10]. Introducing routine scans for all pregnancies[12] and methods to improve the precision of ultrasound weight scans such as 3D ultrasound[187] or dedicated fetal MRI[188] may improve the identification of SGA. But as previously discussed in ***Study II***, the timing of such examination remains a matter of debate. In general, detection rates of SGA was higher if ultrasound was performed a few weeks prior to delivery, and lower when performed earlier in the 3rd trimester.[167,168,189]

This thesis demonstrates that antenatal detection of SGA is highly challenging. But even if we manage to perfectly succeed with the antenatal detection of SGA, one must acknowledge that the majority of adverse outcomes such as stillbirth and neonatal death is found in non-SGA pregnancies. As demonstrated in ***Study I***, approximately 90% of stillbirth occurred in pregnancies with normal BW. This finding is in line with a previous study by Poon et al[31], which demonstrated that in approximately two thirds of stillbirths occurring at term, the neonate had a normal BW[31]. It is also well-described that normal size fetuses may present with fetal cerebral and placental blood flow redistribution indicative of fetal hypoxemia and placental dysfunction[190]. Moreover, among stillbirth regardless of BW, the majority of cases had evidence of placental abnormality e.g. maternal vascular malperfusion.[191] In addition, several studies have demonstrated abnormal placental histological findings in normal BW pregnancies[177,192]. To overcome this challenge, a Delphi procedure has been completed for the definition of FGR[15] including less severe weight deviation (<10th centile) accompanied by abnormal flow in umbilical or uterine artery or measures crossing centiles to the FGR diagnosis. However, in cases of normal weight, signs of placental dysfunction are less pronounced and thereby difficult to identify clinically using current methods. Thus, low BW alone may not be a perfect proxy of placental dysfunction.

This is supported by ***Study III*** which demonstrates that placental dysfunction was found in pregnanices without clinical manifestations. This finding suggests that placental dysfunction has a wide spectrum of manifestations, and low BW is only a fraction of these manifestations. Since the outcome of interest is placental dysfunction, placental markers need to be included in the antenatal assessment – rather than focusing only on the fetus. As demonstrated in ***Study III***, the inclusion of placental histology demonstrates the broad spectrum of placental dysfunction, which can be detected antenatally by placental T2*. Placental T2* is correlated to placental dysfunction regardless of the clinical manifestations such as fetal size.

During the last decades, several placental markers have been investigated in order to identify placental dysfunction such as uterine artery pulsatility index[100] and serum markers[108,193]. This is described in detail in the Background section. The majority of these studies use low BW as a proxy for placental dysfunction, which may have confused the analysis and reduced the performance of these markers.[102,103,110,162] According to ***Study III***, the predictive performance of placental T2* was higher when using placental histology as an outcome, when compared to low BW. Re-investigation of these markers using another outcome directly related to placental function such as placental histology or placental T2* is needed. In a clinical setting, the use of serum markers and uterine artery PI is more attractive as the availability and cost of MRI, in most centres, may be a limiting factor of placental T2*.

Lastly, such methods reflecting placental function directly such as placental T2* may have the potential to detect placental dysfunction before clinical manifestations. This would enable monitoring the fetuses at risk of adverse outcomes and exploring potential treatments. At this moment, treatment with Aspirin before 16 weeks of gestation have proved to reduce the risk of preeclampsia and FGR in high-risk pregnancies.[194] However, new treatments may be developed in near future and direct placental markers are needed to evaluate the effect of treatment.[195]

These three studies do not elucidate how to manage placental dysfunction, neither do they clarify whether delivery reduces the risk of placental dysfunction, or if it is permanent damage. However, placental T2* has the potential to identify placental dysfunction regardless of the clinical manifestations. Proper identification of this condition is the first step to understand the placental pathology, to investigate possible treatments, and to understand short- and long-term consequences of the whole spectrum of placental dysfunction.

CHAPTER 8. CONCLUSION

The conclusions of each of the three studies are:

Study I:
The universal Intergrowth-21st standard median BW was lower than the Danish standard median BW. The prevalence of SGA was reduced using the Intergrowth-21st standard and the risk of adverse outcomes associated with SGA was higher. This finding does not support the idea of one universal standard BW curve to fit all populations.

Study II:
The overall sensitivity of the Danish national screening program for SGA has improved considerably over the last 20 years. However, the performance is markedly lower post-term. Among AGA neonates, false classification of SGA increased the number of obstetric interventions, when compared to correctly classified AGA.

Study III:
In this SGA cohort with normal fetal Doppler flows, histological evidence of placental dysfunction was frequent and showed a broad spectrum of manifestations. T2* weighted placental MRI was a sensitive antenatal biomarker of placental dysfunction regardless of clinical manifestations such as low BW. This finding questions low BW as a perfect marker of placental dysfunction.

The overall conclusion of the thesis is that low BW is difficult to define and identify and may not be a perfect marker of placental dysfunction. Moreover, placental dysfunction has varied clinical expressions and the presence is far more widespread than previously anticipated. Thus, there is a need for a paradigm shift in the conception of placental dysfunction.

CHAPTER 9. FUTURE WORK

In order to change the conception of placental dysfunction and support the pregnant women in the most optimal way, more research is needed.
Direct markers of placental function may reflect the full spectrum of placental dysfunction and thereby predict milder cases of this condition. However, the clinical importance of such findings remains unknown. In order to elucidate this, further investigation is needed of short- and long-term risk associated with the different severities of placental dysfunction. In addition, the risk associated with premature delivery needs to be held against the potential risk by continuing pregnancy. Most importantly, we need to evaluate if delivery reduces the fetal risk in milder cases. Maybe the negative fetal consequences of mild placental dysfunction will not improve by delivery.
In order to improve the antenatal prediction of placental dysfunction, further studies are needed to explore the timeline of changes in placental T2* in relation to changes in ultrasound Doppler flow measurements and placental histology. Such knowledge could improve surveillance of fetuses suffering from placental dysfunction. As different modalities of MRI reflect different aspects of tissues and function[196], it would be interesting to combine placental T2* with other modalities of MRI in order to improve the detection of placental dysfunction and increase the knowledge. The combination of direct placental markers such as serum markers and functional MRI with maternal characteristics, previous obstetric history and serial ultrasound findings may further improve the predictive performance. The benefit of placental T2* may be even higher in other groups of pregnancies, such as diabetes and post term pregnancies, where the clinical value of fetal Doppler flows is limited. In addition, the predictive value of placental T2* in early pregnancy needs to be explored in order to allow for treatment and thereby potential prevention of placental dysfunction. The association between placental markers and specific placental lesions may add important information, as placental dysfunction covers many different lesions with different treatment potential.

In understanding the pathology associated with placental dysfunction, further investigation of the etiology of the condition should be included. It remains unexplored if placental dysfunction is related to genetic predisposition or if placental dysfunction is a result of epigenetic changes due to a suboptimal intrauterine environment.

Placental dysfunction remains an obstetric challenge. However, the first step to a greater understanding and more knowledge is acknowledging that placental dysfunction is more than just low BW.

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APPENDICES

Appendix A. Supporting tables for Study I

Table S1 Diagnosis or procedure codes used in the selection of the Danish Standard cohort, based on Intergrowth 21st criteria[140] in Study I.

International Classification of Diseases (ICD)-10	Description
Relevant past medical history	
DA00-DB99	Certain infectious and parasitic diseases
DC00-96	Cancer
DD50-89	Diseases in blood and blood forming organs and certain diseases involving the immune system
DE00-90	Endocrinological, nutritional and metabolic diseases
DF00-99	Psychiatric illnesses and behavioral disorders
DG00-99	Diseases in the nerve system
DI00-99	Cardiac and vascular diseases
DJ00-99	Diseases in respiratory organs
DM00-99	Diseases in bones, muscles and connective tissue
DZ980	Previous Volume reducing surgery on the stomach
KJDF	Volume reducing surgery on the stomach
BWHA1 and BWHA2	Chemotherapy (basis or complex)
Complications during pregnancy or in previous pregnancies	
DO11, 14 and 15	Preeclampsia, eclampsia or HELLP
DO10, 12, 13 and 16	Other hypertensive disorders in pregnancy
DO23, 25, 264, 265, 266, 98 and 994	Other disorders complicating pregnancy
DO24	Diabetes in pregnancy, including both pre-existing and gestational diabetes
DO262, DZ352, DZ358A and DZ358B	Previous obstetrics complications including recurrent pregnancy loss, pregnancy after perinatal or neonatal death, pregnancy after previous preterm delivery or IUGR
DO42	PPROM
DO35	Fetal malformation in current pregnancy
DO360 and DO361	Alloantibodies in current pregnancy
DP043	Maternal use of alcohol with consequences for the infant
DP044	Maternal use of drugs with consequences for the infant
KMAJ00, KMAJ00A, KMAJ00B, KMAJ10, KMAJ10A and KMAJ10B	Fetal reduction (if more than 1 fetus)
BKHG	Medical treatment of threatened miscarriage, "Atosiban" (if premature contractions)
BBHF32	Betamethasone

The Danish version of the diagnosis codes from the World Health Organization International Classification of Diseases and Health Related Problems 10th revision (ICD-10) has a "D" in front. Moreover, the Danish register classifies all treatments including non-surgical procedures, care and prophylaxis with the abovementioned procedures codes (codes with "B" og "K" in front).

Table S2 Anatomical Therapeutic Chemical (ATC) Classification System codes used in the selection of the Danish Standard cohort, based on the Intergrowth-21st criteria

Anatomical Therapeutic Chemical (ATC) Classification System code	**Description**
A10	Drugs used in diabetes: Insulin/insulin analogues and oral antidiabetics
H03	Thyroid therapy
C02CA, CO2AB, C07, C08, C09	Antihypertensives, betablocking agents, calcium channel blockers and agents acting on the renin-angiotensin system
C01AA, CO1B, CO1CA and CO1D	Cardiac therapy
R03	Drugs for obstructive airway diseases: Asthma and Chronic Obstructive Pulmonary Disease
H02AA	Mineralocorticoids
H02AB	Glucocorticoids
A05AA02	Ursodeoxycholic acid
N03	Antiepileptics
N04	Anti-parkinson drugs
N05	Psycholeptics
N06A	Antidepressants
N06B	Psychostimulants, agents used for ADHD and nootropics
N07	Other nervous system drugs
B01AB	Antithrombotic agents – Heparin group
B01AC	Antithrombotic agents – Platelet aggregation inhibitors
B01AD	Antithrombotic agents – Enzymes, fibrinolytics
B05AA	Blood substitutes and plasma protein fractions
H01, L02 and G03	Pituitary and hypothalamic hormones and analogues, endocrine therapy and sex hormones and modulators of the genital system
C03	Diuretics
V03	All other therapeutic products including electrolytes
A11CC03	Alfacalcidol
L04	Immunosuppressants
J05AE, J05AF, J05AR and J05AX	Protease inhibitors, nucleoside and nucleotide reverse transcriptase inhibitors, antivirals for treatment of HIV infections (combinations) and other antivirals
P01B	Antimalarials
J04A	Drugs for treatment of tuberculosis
A03 and A04	Drugs for functional gastrointestinal disorders and Antiemetics and antinauseants
J01	Antibacterials for systemic use
D06	Antibiotics and chemotherapeutics for dermatological use
G01	Gynecological antiinfectives and antiseptics

Appendix B. Co-author statements

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Co-author statement in connection with submission of PhD thesis [Ditte Nymark Hansen]
With reference to Ministerial Order no. 1039 of 27th of August 2013 regarding the PhD Degree § 12, article 4, statements from each author about the author's part in the shared work must be included in case the thesis is based on already published or submitted papers.

Paper title: Construction of a Danish Birthweight Standard curve and the comparison with the Intergrowth Newborn Standard: A nationwide register-based cohort study.

Published ☐ Accepted ☐ Submitted **X** In preparation ☐

Place of publication: Submitted to British Journal of Obstetrics and Gynaecology (BJOG)

Has the article/manuscript been used in other PhD or doctoral dissertations?
No **X** Yes ☐ If yes, please specify:

List of authors: D. Hansen (DH) (phd-student), H. Kahr (HK), C. Torp-Pedersen (CTP), J. Feifel (JF), N. Uldbjerg (NU), M. Sinding (MS) and A. Sørensen (AS).

Contribution - who contributed and amount of contribution for the phd-student as follows:

a. **No or little contribution (0-5%)**
b. **Has contributed (5-30 %)**
c. **Has contributed considerably (40-60 %)**
d. **Has done most of the work (70-90 %)**
e. **Has essentially done all the work (> 90%)**

Example (minimum requirements)
(follows the Vancouver nomenclature)

Element	***Who contributed***	***Amount of contribution DH***
Conception/design of work	*DH, HK, CTP, MS, AS*	b
Acquisition of data	*DH, JF, HK*	c
Analysis of data	*DH, JF*	b
Interpretation of data	*DH, HK, NU, MS, AS*	c
Drafting the work	*DH, HK, MS, AS*	d
Critical revision	*DH, NU, MS, AS, HK, CTP*	c
Finalization and submission of the manuscript	*DH, HK, CTP, JF, NU, MS, AS*	d

DH contributed by writing the protocol, acquired data to support the registers that were available and assisted the data analysis. DH took part in the interpretation before drafting the manuscript. DH contributed to the critical revision and submitted the manuscript.

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Paper title: Screening for small-for-gestational-age fetuses

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Place of publication: Acta Obstetricia et Gynecologica Scandinavica, 2020 Apr; 99(4): 503-509, doi: 10.1111/aogs.13764

Has the article/manuscript been used in other PhD or doctoral dissertations?
No X Yes ☐ If yes, please specify:

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Example (minimum requirements)
(follows the Vancouver nomenclature)

Element	***Who contributed***	***Amount of contribution DH***
Conception/design of work	*DH, MS, AS*	b
Acquisition of data	*DH, HO*	d
Analysis of data	*DH, HO*	d
Interpretation of data	*DH, HO, NU, MS, AS*	c
Drafting the work	*DH, MS, AS*	d
Critical revision	*DH, NU, MS, AS*	c
Finalization and submission of the manuscript	*DH, HO, NU, MS, AS*	c

DH contributed by writing the protocol, collecting the main part of data, analyzing the main part of data, interpreting the results before writing the manuscript. Moreover, DH took part in the critical revision and submitted the manuscript.

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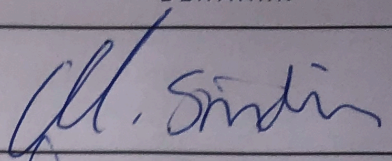
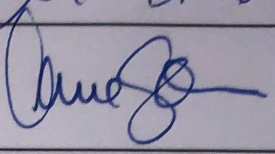
Marianne Sinding	Department of Obstetrics and Gynecology Aalborg University Hospital Reberbansgade 15, 9000 Aalborg, Denmark	
Anne Sørensen	Department of Obstetrics and Gynecology Aalborg University Hospital Reberbansgade 15, 9000 Aalborg, Denmark	

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Co-author statement in connection with submission of PhD thesis [Ditte Nymark Hansen]
With reference to Ministerial Order no. 1039 of 27th of August 2013 regarding the PhD Degree § 12, article 4, statements from each author about the author's part in the shared work must be included in case the thesis is based on already published or submitted papers.

Paper title: T2* weighted placental MRI: A predictor of placenta-related outcomes in small-for-gestational-age pregnancies

Published ☐ Accepted ☐ Submitted ☐ In preparation X

Place of publication: American Journal of Obstetrics & Gynecology (AJOG) Maternal Fetal Medicine (MFM)

Has the article/manuscript been used in other PhD or doctoral dissertations?
No **X** Yes ☐ If yes, please specify:

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Conception/design of work	*DH, DP, MS, AS*	c
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