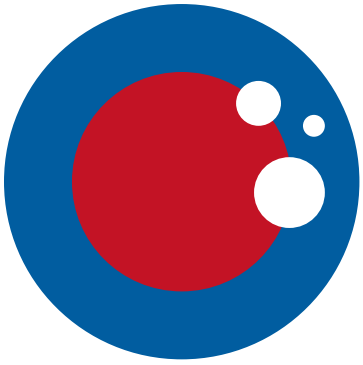
Fetal sex dependency in pregnancy; fetal and maternal outcomes

The Generation R Study



Zoe A. Broere - Brown

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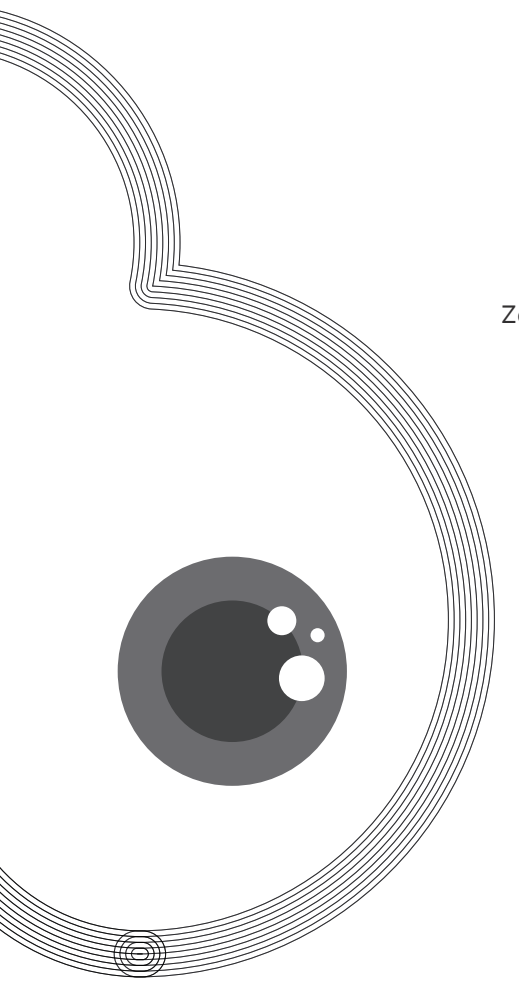

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Paranimfen	Martijn den Dekker Laura Benschop

Content

Manuscripts based on this thesis

Brown ZA, Schalekamp **-** Timmermans S, Tiemeier HW, Hofman A, Jaddoe VWV, Steegers EAP. Fetal sex specific differences in human placentation. *Placenta. 2014;35:359-64*

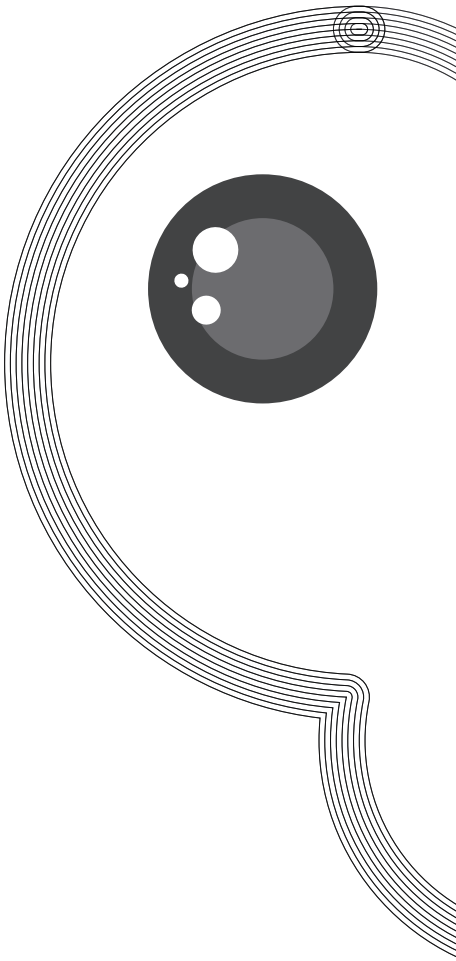
Broere - Brown ZA, Schalekamp **-** Timmermans S, Hofman A, Jaddoe VWV, Steegers EAP. Fetal sex dependency of maternal vascular adaptation to pregnancy. *BJOG. 2016;123:1087-95*

Broere - Brown ZA, Baan E, Schalekamp **-** Timmermans S, Verburg BO, Hofman A, Jaddoe VWV, Steegers EAP. Sex specific differences in fetal and infant growth patterns. *Biology of Sex Differences. 2016;7:65-73*

Broere - Brown ZA, Schalekamp **-** Timmermans S, Jaddoe VWV, Steegers EAP. Fetal growth and Placental Growth Factor umbilical cord blood levels. *Fetal Diagnosis and Therapy. 2017 in press*

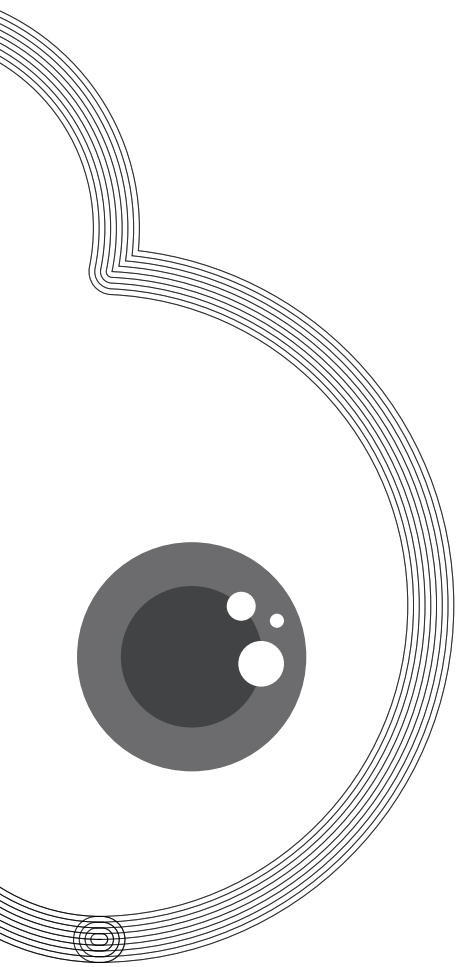
Broere - Brown ZA, Schalekamp **-** Timmermans S, Jaddoe VWV, Steegers EAP. The discrepancy between small for gestational age and fetal growth restriction on childhood outcomes. S*ubmitted*

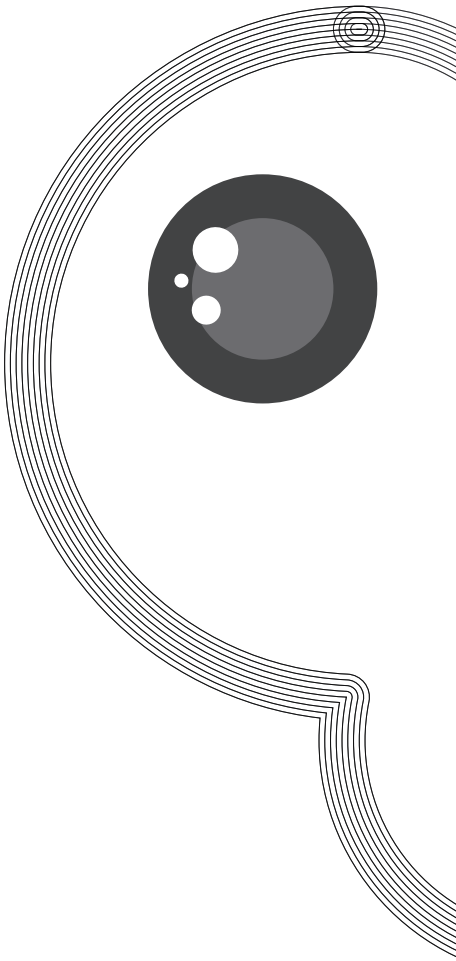
Broere - Brown ZA, Benschop L, Tielemans M, Schalekamp **-** Timmermans S, Muka T, Goncalves R, Bramer W, Schoufour J, Voortman T, Steegers EAP, Franco O. Fetal sex and maternal pregnancy outcomes; a systematic review and meta-analysis. *Submitted*



Part I

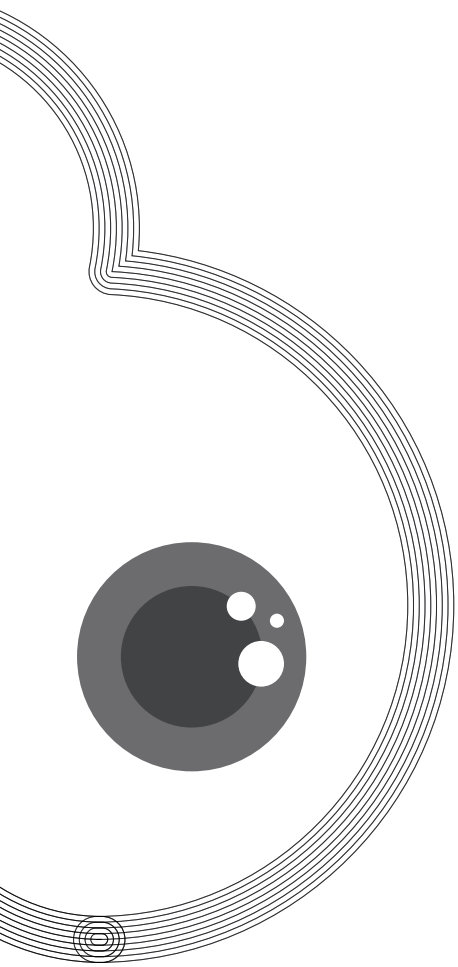
Introduction





Chapter 1

General introduction and aims of the thesis



Pregnancy course is highly important for the future health of the newborn and the mother. The Developmental Origin of Health and Disease (DOHaD) paradigm describes how during conception and pregnancy, the interplay between maternal and environmental factors induces physiological changes and thereby programs fetal and child growth and development (1). Several studies have been performed showing that born small for gestational age (SGA) is associated with adverse health in later life (2). Birth weight is unlikely to be a causal factor per se leading to cardiovascular disease in later life. Birth weight is merely an end-point of different fetal exposures and growth patterns, and the starting point of childhood growth. It is more likely that these exposures are the causal factor leading to adverse health in infancy and adult life. True growth restricted fetuses may experience a failure to reach their growth potential because of a pathological slow-down in the fetal growth pace. However, fetal growth patterns are difficult to measure as the biological growth potential of the fetus can, at best, be estimated and not directly measured. Additionally, a slow-down in the fetal growth pace i.e. fetal growth restriction (FGR) does not always have to lead to SGA.

Also for the mother pregnancy course is of importance for future health. Women with pre-eclampsia have an increased risk for hypertension, ischemic heart disease, stroke, venous thromboembolism and overall mortality after only several years (3, 4). The placenta forms the active interface between the maternal and fetal blood circulation. It regulates both maternal physiological changes during pregnancy as well as fetal nutrient supply and fetal development. Maternal cardiovascular physiological changes include an initial fall in systemic vascular tone followed by an increase in cardiac output and an expansion of plasma volume, which subsequently leads to gradual lowering of the systolic and diastolic blood pressure until mid-pregnancy and a rise from mid-pregnancy to delivery (5, 6). Normally, these adaptations result in a better placental perfusion. Impaired placentation associated with abnormal placental perfusion and placental dysfunction may be a key factor in the development of pre-eclampsia, SGA but also other pregnancy outcomes (7, 8).

Previous research on placenta-mediated pregnancy complications suggest that the interplay between the mother, placenta and fetus might be sex dependent. Since not only the fetus but also the placenta has a sex. Due to differences in placental sex and thereby placentation and placental physiology sex specific differences in placenta-mediated pregnancy complications may occur (9-30). The presence of these sex specific differences in pregnancy complications and/

or adaptation to pregnancy may thereby also have consequences for postnatal child and maternal health. Sex differences in disease incidence, presentation, diagnosis and outcome to treatment in both children and adults exist. We propose that these sexual dimorphisms could well have a fetal origin (31).

Aims of this thesis

The aims of this thesis are:

1. To assess different definitions on fetal growth restriction and their associations with childhood outcomes (Chapter 2);
2. To identify a fetal biomarker at birth to retrospectively assess fetal growth restriction (Chapter 3);
3. To evaluate fetal sex specific differences on a placental, fetal and maternal level (Chapter 4, 5, 6 and 7).

Setting

All studies described in this thesis are embedded within the Generation R Study, an ongoing prospective population based cohort study from early pregnancy onwards in Rotterdam, the Netherlands. This study was designed to identify early environmental, biological and social determinants of growth, development and future health (32, 33). Study participants were pregnant women living in Rotterdam and expected to deliver between April 2002 and January 2006. Enrollment was aimed in the first trimester but was possible until the birth of the child. In total 9778 women were included, of which 8880 (91%) women enrolled prenatally. Detailed measurements were planned in early pregnancy (<18 weeks gestation), mid-pregnancy (18 – 25 weeks gestation) and late pregnancy (>25 weeks gestation) and included fetal ultrasound measurements, physical examinations, collection of biological samples and self-administered questionnaires. Information on perinatal and maternal pregnancy outcomes, including intra-uterine growth, placental parameters (uteroplacental vascular resistance and placental weight), birth weight, gestational age at birth, gestational hypertension and pre-eclampsia were all available. At the age of six years all children were invited to visit the Generation R Research Center together with their mothers to study their growth, development and cardiovascular health using innovative and detailed tools (34). The Generation R Study has been approved by the Medical Ethical Committee of the Erasmus MC,

University Medical Center Rotterdam, and the medical ethical review boards of all participating hospitals. All participants provided written informed consent. The Generation R Study follows the STROBE guidelines (35).

Outline of the thesis

Part I of this thesis is a general introduction describing the background and hypotheses for the studies presented in this thesis.

Part II is focused on fetal outcomes. **Chapter 2** focuses on different definitions of fetal growth restriction and the associations of these definitions with childhood outcomes. In **Chapter 3** the association between cord blood placental growth factor (PlGF) and fetal growth restriction is assessed. In **Chapter 4** sex specific differences in prenatal and postnatal growth is assessed.

Part III of this thesis addresses the interaction between fetal sex and the mother and placenta. In **Chapter 5** we examine the association between fetal sex and placental biomarkers. **Chapter 6** focuses on the association between fetal sex and maternal vascular adaptation to pregnancy and if this association is mediated by the placental biomarkers addressed in **Chapter 5**. **Chapter 7** presents a systematic review and meta-analyses on fetal sex specific differences in maternal pregnancy complications.

To resume this thesis, **Chapter 8** elaborates on main findings, underlying mechanisms and implications for the research and clinical field.

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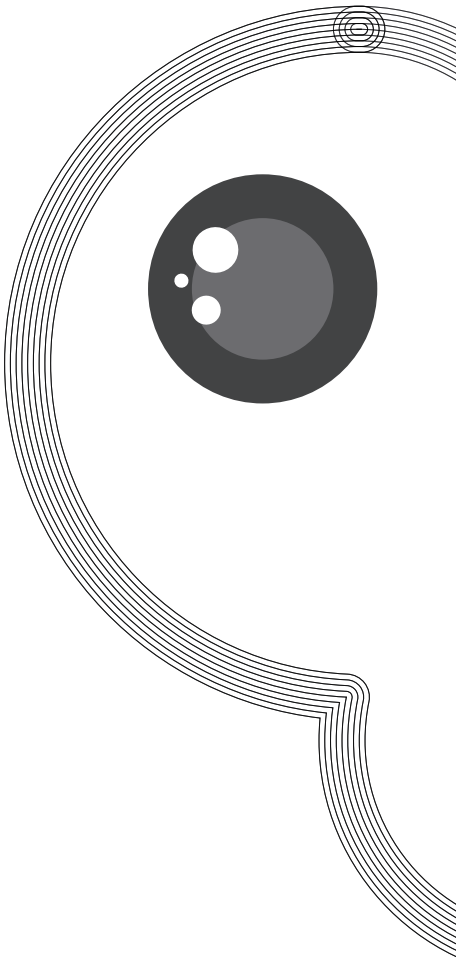
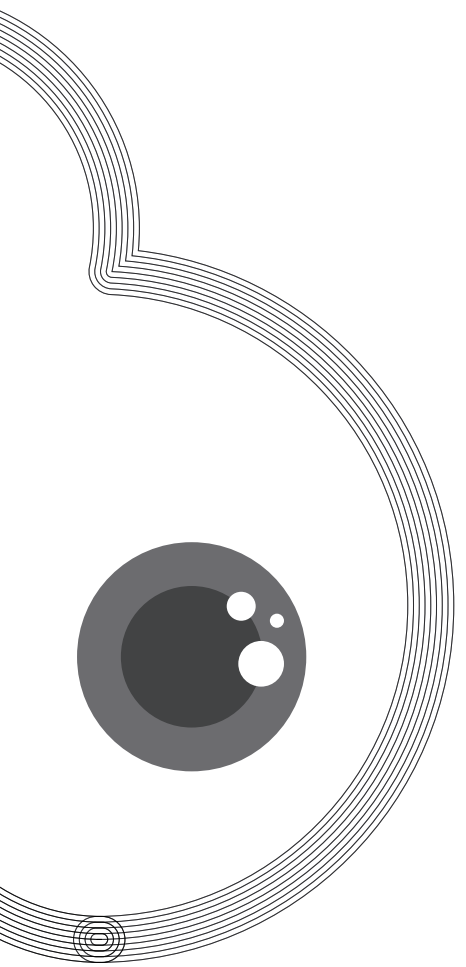
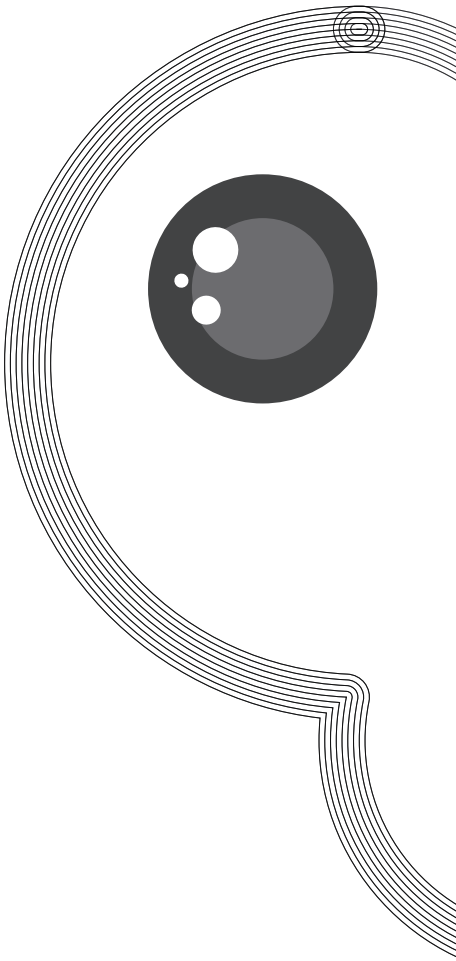


Part II

Fetal outcomes





Chapter 2

The discrepancy between small for gestational age and fetal growth restriction on childhood outcomes

Z.A. Broere - Brown
S. Schalekamp – Timmermans
V.W.V. Jaddoe
E.A.P. Steegers

Submitted

Abstract

Introduction: Small for gestational age (SGA) is frequently used to define fetal growth restriction (FGR). However, FGR describes a slowdown in fetal growth and is therefore not synonymous with SGA which may introduce misclassification. We distinguished FGR from SGA and investigated the effect of both on delivery and childhood outcomes.

Methods: From a prospective population-based cohort study we included 7959 live singleton births with data available on second trimester estimated fetal weight (EFW) and birth weight. We used a decrease in growth of >40 percentiles between second trimester EFW and birthweight to define a deviating growth curve (i.e. FGR). SGA was defined as birthweight <p5. As outcome measures we investigated several delivery outcomes, accelerated growth at two years and cardiovascular outcomes at six years.

Results: FGR occurred in 27.2% in SGA neonates and in 10.3% of neonates with an appropriate for gestational age (AGA) birthweight. Of all FGR fetuses, 90% was born AGA. SGA neonates less often experienced low APGAR, but more often were delivered by instrumental delivery or cesarean section and were more often admitted at the NICU. These associations were not found for FGR neonates. Both FGR and SGA were associated with accelerated growth at two years, a smaller aortic diameter and lower left ventricular mass at six years. The effect estimates of SGA on these outcomes were higher compared with FGR.

Conclusion: Both FGR and SGA are associated with unfavorable clinical outcomes in childhood. In addition to SGA, FGR neonates should be considered a high risk group.

Introduction

Fetal growth restriction (FGR) is considered a severe complication of pregnancy associated with substantial perinatal morbidity and mortality and contributing to disease in adulthood (1, 2). The Development and Origins of Health and Disease theory (DOHaD) states that in case of adverse fetal exposure, the unborn fetus can modify its own development such that it will be prepared for survival in an environment in which resources are likely to be short. Although these adaptations may be beneficial for short term survival, they may have adverse consequences at delivery or in later life (3). FGR is difficult to assess as the biological growth potential of the fetus can, at best, be estimated and not directly measured. Therefore, in scientific research FGR is frequently classified as a neonate born small for gestational age (SGA). However, FGR is not necessarily synonymous with SGA. FGR fetuses may experience a failure to reach their biological growth potential because of a pathological slow-down (deviating growth curve) in the fetal growth pace. On the other hand it is estimated that approximately 50-70% of the SGA fetuses are constitutionally small with normal perinatal outcomes (4, 5).

In this study we compared SGA with FGR based on the fetus individual growth curve and assessed differences in delivery outcomes, accelerated growth in infancy and cardiovascular outcomes at the age of six years.

Methods

Study design

This study was embedded in The Generation R Study, a population-based prospective cohort study from early pregnancy onwards (6). All mothers with an expected delivery date between April 2002 and January 2006 were eligible. Response at baseline was 61%. The study was approved by the Medical Ethics Committee of the Erasmus Medical Center Rotterdam, the Netherlands in 2001. Written informed consent was obtained from all mothers. For the present study we included pregnancies with a live born singleton birth with a known second trimester estimated fetal weight (EFW) and birth weight (N = 7,959) (**Figure 2.1**).

Fetal growth

To assess estimated fetal weight (EFW) ultrasound examinations were performed in the second trimester of pregnancy (median 20.5 weeks of gestation, 90%

range 18.9 – 22.9). Fetal biometry (head circumference [HC], abdominal circumference [AC] and femur length [FL]) was measured transabdominally. EFW was calculated using the formula of Hadlock with parameters AC, HC and FL (in cm): EFW = 10^ (1.326 – 0.00326*AC*FL + 0.0107*HC + 0.0438*AC + 0.158*FL (7). Ultrasound examinations were performed using Aloka® model SSD-1700 (Tokyo, Japan) or the ATL-Philips® Model HDI 5000 (Seattle, WA, USA). SGA was defined as a gestational and fetal sex adjusted birth weight under the fifth percentile (≤ 1.78 SD). Since there is no definition of how much a growth curve needs to deviate before it can be designated as a deviating growth curve we used five cut-offs to define FGR; a decrease in growth of at least 30, 35, 40, 45 or 50 percentiles respectively between the second trimester and birth. This approach has been suggested in previous studies and aims to approach a slowdown in fetal growth. We did not explore a decrease in growth of less than 30 percentiles since these deviations in growth could well be the results of measurement errors. The results using the cut-off of 40 percentiles are presented, results on the other cut-off values can be found in the Supplemental materials. Choosing cut-offs to define FGR may lead to misclassification. The initial estimated fetal weight in the second trimester of pregnancy should be above the 40th percentile, otherwise the fetus is not able to deviate from its growth curve with more than 40 percentiles. Therefore, in an additional analysis we classified all fetuses with an EFW in the second trimester under the 40th percentile and with a maximum decrease of growth (birth weight under the first percentile) as FGR (n = 85).

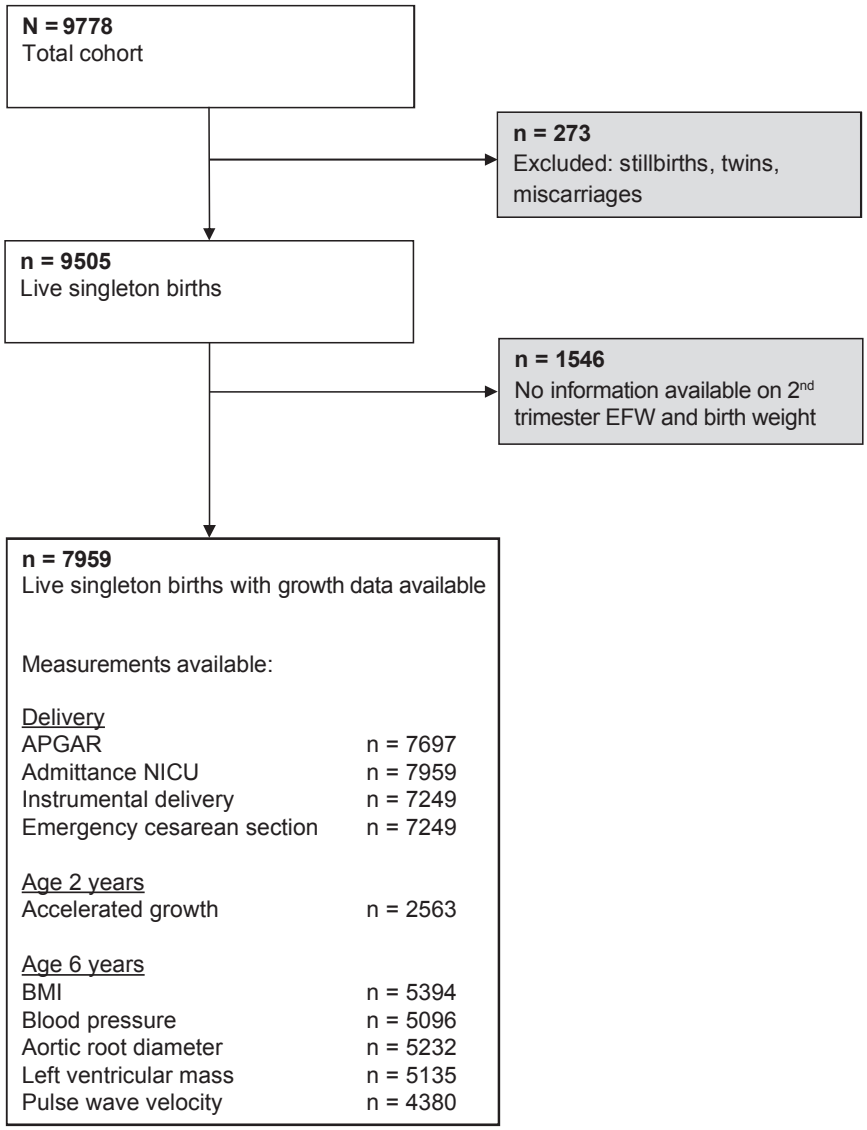
Delivery outcomes

Gestational age at birth and birth weight were obtained from midwives and hospital registries. APGAR score at five minutes and delivery mode (spontaneous versus instrumental or emergency cesarean section) were obtained from standardized delivery registrations of midwives and obstetricians. An APGAR score below seven after five minutes was considered low (8, 9). Information concerning admittance to the neonatal intensive care unit (NICU) was obtained using hospital and national registries.

Infant growth

Well-trained staff in the Community Health Centers obtained postnatal growth characteristics according to standard schedule and procedures at the age of 24 months. Standard deviation scores for childhood weight were obtained with Dutch growth references charts (Growth Analyzer 3.0; Dutch Growth Research Foundation, Rotterdam, the Netherlands). Postnatal accelerated growth was

Figure 2.1 Flowchart



Flowchart showing our in- and exclusion criteria. The total study population consists out of 7959 pregnancies. Abbreviations: EFW: estimated fetal weight. NICU: neonatal intensive care unit. BMI: body mass index.

defined as an increase between birth and two years of age in their position on the age-specific weight distribution by at least 0.67 SDS, representing the width of each percentile band on a standard growth chart (10, 11).

Childhood cardiovascular outcomes

We invited all children to a dedicated research facility in the Erasmus University Medical Center, Sophia Children's Hospital for detailed measurements at the age of six years (mean 6.2 ± 0.5) (6). We measured height and weight and calculated body mass index (BMI). Systolic (SBP) and diastolic blood pressure (DBP) were measured in the right brachial artery by using the validated automatic sphygmomanometer Datascope Accutor Plus (Paramus, NJ, USA) (12). We selected a cuff with a width approximately 40% of the arm circumference and long enough to cover 90% of the arm circumference.

Carotid-femoral pulse wave velocity (PWV) was assessed by using the automatic Complior SP device (Artech Medical, Pantin, France) with participants in supine position. The distance between the recording sites at the carotid (proximal) and femoral (distal) artery was measured over the surface of the body to the nearest centimeter. Through piezoelectric sensors placed on the skin, the device collected signals to assess the time delay between the upstroke of carotid and femoral waveforms. Carotid-femoral pulse wave velocity was calculated as the ratio of the distance traveled by the pulse wave and the time delay between the waveforms, as expressed in meters per second (13, 14). To cover a complete respiratory cycle, the mean of at least 10 consecutive pressure waveforms was used in the analyses. PWV can be measured reliably with good reproducibility in large pediatric population-based cohorts (14, 15).

Two-dimensional M-mode echocardiographic measurements were performed using the ATL-Philips Model HDI 5000 or the Logiq E9 (GE Medical Systems, Wauwatosa, Wisconsin, USA) devises. Echocardiography was used to measure the aortic root diameter (AOD), interventricular septum thickness in diastole (IVSTD), left ventricular internal diameter in diastole (LVIDD) and the left ventricular posterior wall thickness in diastole (LVPWTD) using methods recommended by the American Society of Echocardiography (16). Left ventricular mass (LVM) was calculated using the formula derived by Devereux et al: $LVM = 0.80 \times 1.04 ((IVSTD + LVIDD + LVPWTD)^3 - (LVIDD)^3) + 0.6$ (17, 18).

Covariates

We obtained information on maternal age, ethnicity, educational level, folic acid use and smoking in pregnancy by questionnaire at enrollment (6). We measured first trimester maternal blood pressure with the validated oscillometric sphygmomanometer (OMRON Healthcare Europe BV, Hoofddorp, the Netherlands).

Statistical analyses

We examined the associations of FGR and SGA with delivery outcomes and accelerated growth after two years using logistic regression models. For the analyses on cardiovascular outcome measurements we constructed standard deviation score values ([observed value – mean] / SD) for the childhood cardiovascular outcome measures to enable comparison of effect estimates for the different outcomes. We did not create age adjusted standard deviation scores as the childhood outcomes were measured in a small age range and age of the child was included as a covariate in all models. We used three different linear and logistic regression models to examine the associations of FGR and SGA with infant growth and childhood cardiovascular outcomes. The basic model was adjusted for child's sex, age and ethnicity. The confounder model was additionally adjusted for maternal age, maternal educational level, smoking in pregnancy and folic acid use. We selected these confounders on the basis of their associations with both the exposure and the outcome of interest and / or a change in effect estimate of more than 10%. We considered the confounder model to be the main model. For the analyses on delivery outcomes we additionally adjusted for gestational age at birth and birthweight i.e. the neonatal model. For analyses on accelerated growth and cardiovascular outcomes, we additionally adjusted for current BMI i.e. childhood model. Since the group of FGR fetuses is a mixed population with both AGA and SGA fetuses, we wanted to exclude the possibility that the effect of FGR depends on being SGA. Therefore effect modification was tested on the multiplicative scale. If $p < 0.10$ was fulfilled regression analyses concerning FGR were performed in strata; SGA fetuses and AGA fetuses.

For all analyses, the percentages of missing values of covariates were lower than 20%. We imputed missing data of the covariates by using multiple imputations (19). Ten datasets were created and analyzed together. Statistical analyses were performed using the Statistical Package of Social Sciences version 21.0 for Windows (IBM Corp., Armonk, NY, USA).

Results

SGA

Baseline characteristics are shown in **Table 2.1**. SGA fetuses had a mean birth weight of 2484 ± 402 grams (1.7th percentile).

SGA was associated with a lower risk of a low APGAR score after 5 min (OR 0.37 [95% CI 0.16;0.83]) but increased risks of an instrumental delivery (OR 1.47 [95% CI 1.05;2.07]), an emergency cesarean section (OR 1.93 [95% CI 1.29;2.88]) and NICU admittance (OR 4.21 [95% CI 3.12;5.67]) compared to AGA neonates (**Figure 2.2**). Children born SGA had an increased risk of accelerated growth at the age of 2 years (OR 7.93 [95% CI 4.63;13.60] **Figure 2.3**). SGA neonates had a lower BMI (-0.28 SD [95% CI-0.16;-0.40]), a smaller aortic root diameter (-0.39 SD [95% CI -0.51;-0.27]) and lower left ventricular mass (-0.36 SD [95% CI -0.48;-0.24]) at the age of six years compared to AGA neonates (**Table 2.2**).

FGR

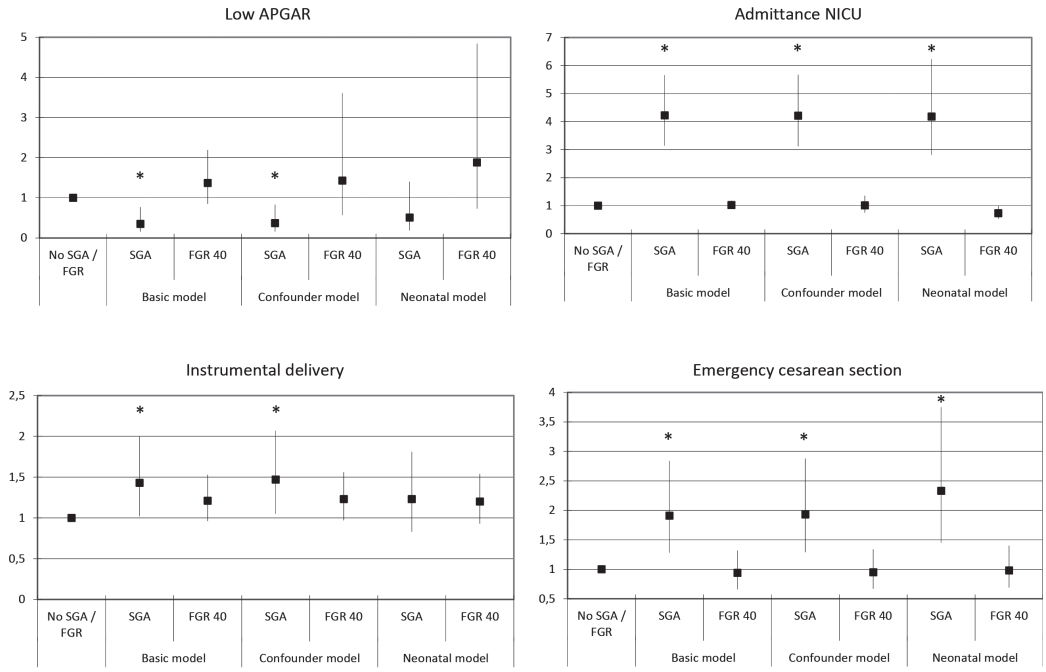
Baseline characteristics are shown in **Table 2.1**. FGR fetuses had a mean birth weight of 2992 ± 383 grams (21.5th percentile). Using the FGR definition of a decrease in fetal growth of at least 40 percentiles, of all SGA fetuses 96 (23.5%) were growth restricted. Of the AGA fetuses, 862 (11.4%) experienced FGR. Of all FGR fetuses, 90% was born AGA.

No associations were found between FGR and delivery outcomes (**Figure 2.2**). FGR was associated with an increased risk of accelerated growth at the age of two years (OR 2.86 [95% CI 2.17-3.76] compared to non FGR fetuses (**Figure 2.3**). FGR neonates had a lower BMI (-0.16 SD [95% CI-0.24;-0.08]), a smaller aortic root diameter (-0.14 SD [95% CI -0.22;-0.06]) and lower left ventricular mass (-0.17 SD [95% CI -0.25;-0.09]) at the age of six years compared to non FGR fetuses (**Table 2.2**). Results on delivery outcomes, accelerated growth and cardiovascular outcomes using the other cut-offs for defining FGR are depicted in **Supplemental Figure S.2.1, S.2.2** and **S.2.3** respectively. **Supplemental Table S.2.1** describes a crosstab showing possible overlap between SGA and FGR.

For all outcomes no effect modification was found between FGR and SGA. Our additional analyses in which we classified all fetuses with an EFW in the 2nd trimester under the 40th percentile and with a maximum decrease of growth (birth weight under the 1st percentile) as FGR, showed that all the effect

estimates remained the same. We concluded that potential misclassification did not affect out results. We decided to continue with the original definition i.e. a decrease of growth of at least 40 percentiles.

Figure 2.2
Associations between FGR and SGA on delivery outcomes



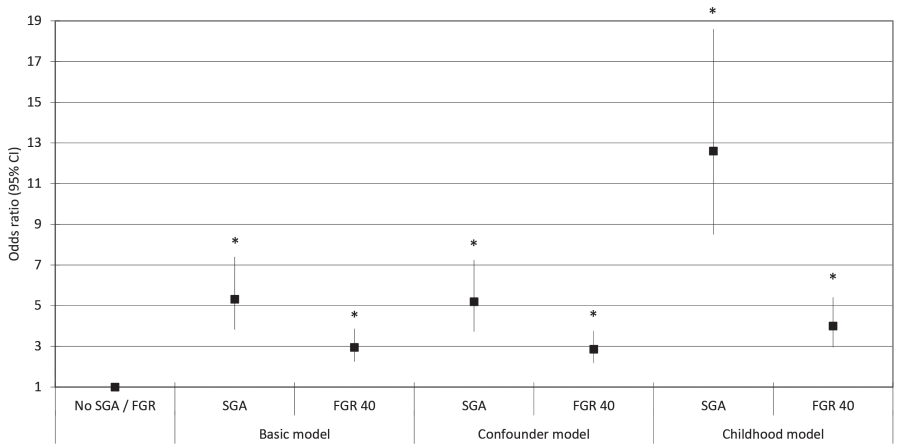
Values represent odds ratio's with the 95% confidence interval and are based on logistic regression models. Basic model: adjusted for child's age, sex and ethnicity. Confounder model: additionally adjusted for maternal age, educational level, smoking, and folic acid use and diastolic blood pressure at intake. Neonatal model: additionally adjusted for gestational age at birth and birth weight. * p < 0,05 Abbreviations: NICU: neonatal intensive care unit. SGA: small for gestational age. FGR: fetal growth restriction.

Table 2.1 Baseline characteristics

	Total study population	FGR	SGA
	n = 7959	n = 958	n = 408
Maternal age	29.7 (5.2)	28.9 (5.6)	29.1 (5.7)
Anthropometrics			
Height (cm)	167.2 (7.4)	165.8 (7.1)	164.0 (7.0)
Weight (kg)	69.3 (13.3)	68.0 (13.0)	63.8 (12.5)
BMI (kg/m2)	23.8 (19.3 - 33.6)	23.7 (19.0 - 34.1)	22.7 (18.4 - 31.6)
Ethnicity			
Western	4434 (58.3%)	484 (53.4%)	180 (46.6%)
Non-Western	3171 (41.7%)	423 (46.6%)	206 (53.4%)
Educational level			
Low	971 (12.2%)	133 (13.9%)	55 (13.5%)
Middle	3698 (46.5%)	497 (51.9%)	215 (52.7%)
High	3290 (41.3%)	328 (34.2%)	138 (33.8%)
Smoking habits			
No	5784 (72.7%)	635 (66.3%)	247 (60.5%)
Yes - stopped	693 (8.7%)	86 (9.0%)	31 (7.6%)
Yes - continued	1482 (18.6%)	237 (24.7%)	130 (31.9%)
Folic acid use			
No	2382 (29.9%)	340 (35.5%)	151 (37.0%)
Before 10 weeks	2484 (31.2%)	308 (32.2%)	135 (33.1%)
Preconception start	3093 (38.9%)	310 (32.4%)	122 (29.9%)
Nulliparous (%)	4470 (56.2%)	610 (63.7%)	289 (70.8%)
Gestational age at birth (wks)	40.1 (37.0 - 42.0)	39.7 (36.6 - 41.6)	39.9 (35.9 - 41.9)
Preterm birth <37 wks (%)	297 (3.7%)	37 (3.9%)	11 (2.7%)
Birthweight (gr)	3412 (560)	2992 (383)	2484 (402)
Birthweight (percentile)	47.1 (28.7)	21.5 (14.8)	1.72 (1.14)
Placenta weight (gr)	620 (415 - 900)	555 (380 - 769)	480 (300 - 678)
Ratio placenta / birhtweight	0.19 (0.04)	0.19 (0.04)	0.20 (0.04)
Pre-eclampsia (%)	157 (2.1%)	36 (4.2%)	32 (8.7%)
Sex (% male)	4005 (50.3%)	477 (49.8%)	222 (54.4%)

Data are represented as n (%) or as the mean (SD) or as the median (90% range). FGR was defined as a decrease in growth of at least 40 percentiles between the second trimester and birth. Abbreviations: BMI: body mass index. FGR: fetal growth restriction. SGA: small for gestational age.

Figure 2.3 Associations between fetal growth restriction, small for gestational age and cardiovascular outcome and accelerated growth at the age of two years



Values represent odds ratio's with the 95% confidence interval and are based on logistic regression models. Basic model: adjusted for child's sex, visit interval and child's ethnicity. Confounder model: basic model and additionally adjusted for maternal age, educational level, smoking, folic acid use and diastolic blood pressure at intake. Childhood model: confounder model and additionally adjusted for child's current body mass index. * $p < 0.05$. Abbreviations: SGA: small for gestational age. FGR: fetal growth restriction.

Discussion

Main findings

This study shows that both SGA as well as FGR are associated with accelerated growth at the age of two years and altered cardiovascular measurements at the age of six. The effect estimates of the observed associations were larger for SGA than for FGR. Interestingly, despite the fact that 90% of the FGR neonates are born AGA, substantial associations were found between FGR and accelerated growth at the age of two years and cardiovascular outcomes at the age of six years.

Strengths and limitations

The main strength of this study was the extensive prospective data collection on fetal growth, childhood health and environmental influences. This enabled us to adjust for multiple confounding factors and investigate the effects of fetal growth on three different time points (i.e. at delivery and at the age of two and six years) in a large sample of 7959 participants.

Table 2.2 Associations between fetal growth restriction and cardiovascular outcomes

	Basic model		Confounder model		Childhood pathway	
	β (95% CI)	p-value	β (95% CI)	p-value	β (95% CI)	p-value
BMI (SD)						
No FGR / SGA	reference		reference		NA	
FGR 40	-0.13 (-0.21;-0.05)	0.002	-0.16 (-0.24;-0.08)	<0.001		
SGA	-0.23 (-0.35;-0.11)	<0.001	-0.28 (-0.16;-0.40)	<0.001		
Systolic blood pressure (SD)						
No FGR / SGA	reference		reference		reference	
FGR 40	0.01 (-0.07;0.10)	0.77	-0.00 (-0.09;0.08)	0.93	0.03 (-0.06;0.11)	0.56
SGA	0.11 (-0.02;0.24)	0.11	0.07 (-0.05;0.20)	0.26	0.13 (0.00;0.26)	0.04
Diastolic blood pressure (SD)						
No FGR / SGA	reference		reference		reference	
FGR 40	0.07 (-0.02;0.15)	0.13	0.05 (-0.03;0.14)	0.24	0.06 (-0.03;0.14)	0.18
SGA	0.15 (0.02;0.28)	0.02	0.12 (-0.01;0.25)	0.06	0.14 (0.01;0.26)	0.04
Aortic root diameter (SD)						
No FGR / SGA	reference		reference		reference	
FGR 40	-0.14 (-0.22;-0.07)	0.001	-0.14 (-0.22;-0.06)	0.001	-0.10 (-0.18;-0.03)	0.01
SGA	-0.39 (-0.50;-0.27)	<0.001	-0.39 (-0.51;-0.27)	<0.001	-0.33 (-0.44;-0.21)	<0.001
Left ventricular mass (SD)						
No FGR / SGA	reference		reference		reference	
FGR 40	-0.17 (-0.25;-0.09)	<0.001	-0.17 (-0.25;-0.09)	<0.001	-0.11 (-0.19;-0.04)	0.004
SGA	-0.35 (-0.23;-0.47)	<0.001	-0.36 (-0.48;-0.24)	<0.001	-0.27 (-0.38;-0.16)	<0.001
Pulse wave velocity (SD)						
No FGR / SGA	reference		reference		reference	
FGR 40	0.00 (-0.09;0.10)	0.93	0.00 (-0.09;0.09)	0.97	-0.01 (-0.10;0.09)	0.89
SGA	-0.05 (-0.19;0.09)	0.48	-0.05 (-0.19;0.09)	0.47	-0.07 (-0.21;0.07)	0.35

Values are regression coefficient with the 95% CI and are based on linear regression models. Basic model: Adjusted child's sex, visit interval and child's ethnicity. Confounder model: Basic model and additionally adjusted for maternal age, educational level, smoking, folic acid intake and diastolic blood pressure at intake. Childhood pathway model: Confounder model and additionally adjusted for child's current body mass index. Abbreviations: CI: confidence interval. NA: not applicable. FGR: fetal growth restrction. SD: standard deviation. SGA: small for gestational age.

Follow-up data at six years was available in 65% of our study population. The non-response at baseline and after six years would lead to biased effect estimates if the associations would be different between those included and not included in the analyses. However, given the prospective nature of the study, this seems unlikely. Although, it might have led to a selection of a more healthy population and might affect the generalizability of our results.

Estimation of fetal weight with ultrasound and the Hadlock formula has a mean absolute error of 8-13% dependent of the size of the fetus (20). However, there is a risk of overestimation in pregnancies with suspected large for gestational age (LGA) fetuses and an underestimation in pregnancies with suspected SGA (21). Since the definition of FGR used in this study is based on the percentiles of EFW this may have led to misclassification. In that case the associations between FGR and our outcomes would have been biased towards the null. Hence, our associations might reflect underestimations.

Interpretation

One of the key findings of this study is that FGR neonates have in an increased risk of accelerated growth and altered cardiovascular outcomes at the age of six years despite the fact that 90% of FGR neonates are born AGA. In this study, we defined FGR independent of birth weight or other measurements during pregnancy such as a fetal abdominal circumference under the 5th percentile, the pulsatility index of the umbilical or the middle cerebral artery or biomarkers. Since there is no consensus on how much a growth curve needs to deviate before it can be designated as a deviating growth curve several cut-offs were used based on a decrease in growth expressed in percentiles. A disadvantage of this approach is that the change in weight per percentile is not constant but increases towards the more sparsely populated extremes of a distribution. Hence, a fetus initially at the 90th percentile of a weight distribution and ending at the 50th percentile is, expressed in estimated fetal weight, more growth restricted compared with a fetus initially at the 70th percentile and ending at the 30th percentile. Only a portion of the neonates born small for gestational age were growth restricted, ranging from 15.9% to 32.8% depending on the used cut-off. The fact that the group of SGA fetuses is a heterogeneous group consisting out of fetuses which are constitutionally small and FGR fetuses is well known and accepted. In previous research on SGA different attempts were made in trying to stratify these two groups by calculating the ponderal index, use the birth weight of a

sibling as a reference, or the usage of customized charts or prediction models (22-28). However, in all these studies attention was solely focused on SGA neonates in which FGR is merely a form of SGA. Neonates born AGA are not subject of investigation whereas this study shows that 88.9% to 90.7% of the FGR neonates were born AGA and that from all AGA born fetuses 6.9% to 17.4% is in fact growth restricted. Despite these high percentages substantial associations between FGR and accelerated growth and altered cardiovascular measurements during childhood were found. Already during pregnancy FGR fetuses have a higher pulsatility index of the umbilical artery compared with fetuses without a growth restriction ($p < 0.01$). A sensitivity analyses was performed repeating the analysis in only FGR fetuses born AGA which gave the same results (data not shown). This indicates that FGR neonates born AGA should be considered as a high risk group with more emphasize needed in future research.

The effect estimates of the associations on accelerated growth at the age of two and cardiovascular measurements at the age of six were higher for SGA children compared with those with FGR. One might hypothesize that this implies that birth weight, the endpoint of a growth pattern, is more important than the growth pattern itself. However, it could also be that the effects are not measurable yet in case the fetus did not reach a certain lower limit of birth weight (i.e. a threshold effect). The difference in birth weight between SGA neonates and FGR neonates could explain why associations were found with delivery outcomes for SGA neonates but not for FGR neonates. SGA neonates were more often delivered by an emergency cesarean section or an instrumental delivery compared with FGR neonates. Due to their low birth weight SGA neonates are more prone to experience fetal distress with as consequence an increased risk of seizures, respiratory diseases, hypoglycemia and hyperbilirubinemia with admittance at the NICU compared with their AGA counterparts (29). This foresight could have influenced the practicing physician by lowering the threshold when to perform an emergency cesarean section. This would also explain why preterm birth before 37 weeks of gestation occurs more often in SGA fetuses compared with FGR fetuses (3.9% vs 2.7%, Table 1). Partly this will be iatrogenic due to the knowledge that the fetus is SGA. This tendency to intervene earlier might be an explanation why SGA neonates more often are delivered by vaginal instrumental delivery or cesarean section, in the presence but perhaps also absence of non-reassuring fetal heart rate monitoring, but less often have an APGAR score below seven after 5 minutes. This is not the case for FGR fetuses since the majority of these fetuses were born AGA.

Both FGR as well as SGA were associated with an increased risk of accelerated growth in the first two years of life. Accelerated growth is associated with obesity in later life giving rise to impaired cardiovascular health (30). Especially in those born with a low birth weight (31, 32). This is in line with our study in which the SGA neonates, who had a higher risk of accelerated growth compared with the FGR neonates, also had poorer cardiovascular outcomes. One could also explain the associations between SGA and cardiovascular outcomes by body size since children born SGA often have a lower BMI compared with their peers. Though after adjustment for current BMI in the childhood pathway model the associations persisted. Indicating that the effect is not solely explained by current BMI and that other pathways may be involved.

Fetal growth restriction is not limited to mid and late pregnancy only. Embryonic growth and development during the first trimester of pregnancy is essential for organogenesis of the fetal cardiovascular system. Impaired early growth has also been shown to be associated with an adverse cardiovascular risk profile in children at the age of six years (33, 34). If FGR occurs in early pregnancy, gestational age is often adjusted according to the crown-rump-length. After adjustment fetal growth may seem appropriate although the neonate should have been classified as being SGA with the long term sequalae as shown in this article. Therefore more attention is needed for FGR throughout gestation and not only during the second half of pregnancy.

Conclusion

In this study in which we explored differences in outcome between SGA and FGR fetuses using an alternative approach to define FGR, we observed that despite the fact that the vast majority of fetuses with a deviating growth curve are born AGA, these fetuses constitute a vulnerable group with increased risks of accelerated growth and altered cardiovascular outcomes in childhood and in their future lives. Future research should be focused on this particular group since these newborns are now, using a birthweight < p10 to define FGR, not classified as such and are not subject to any follow up in future life.

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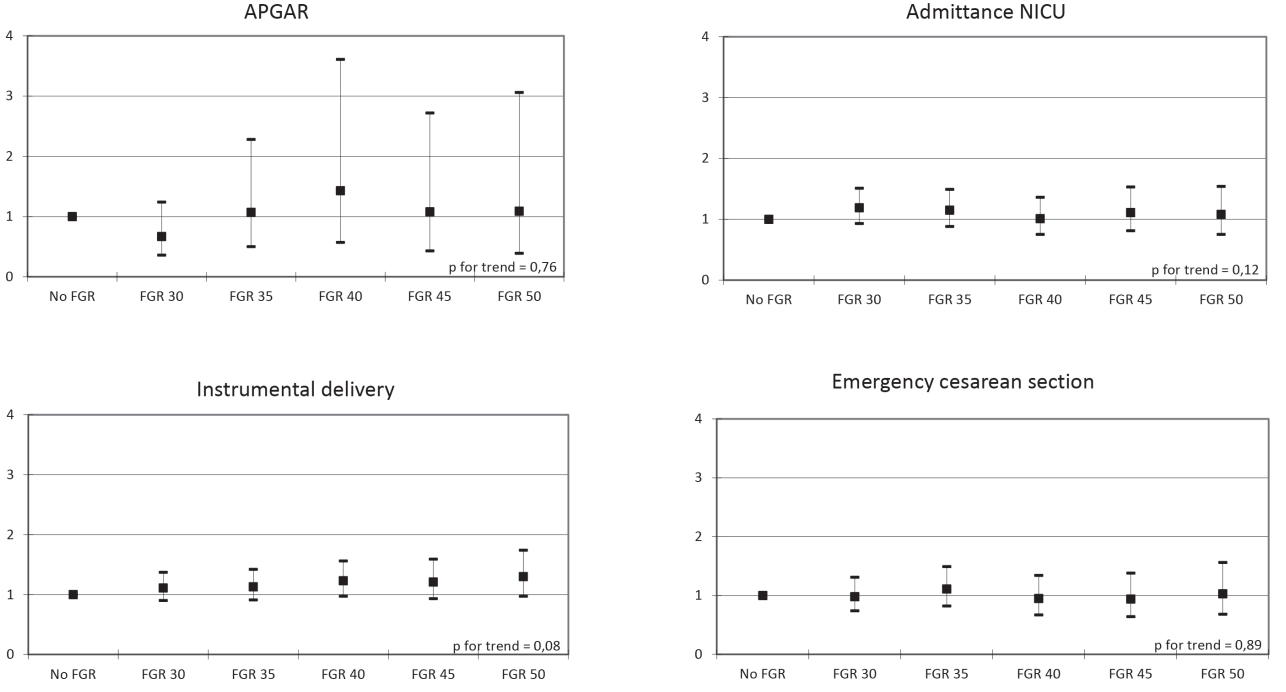
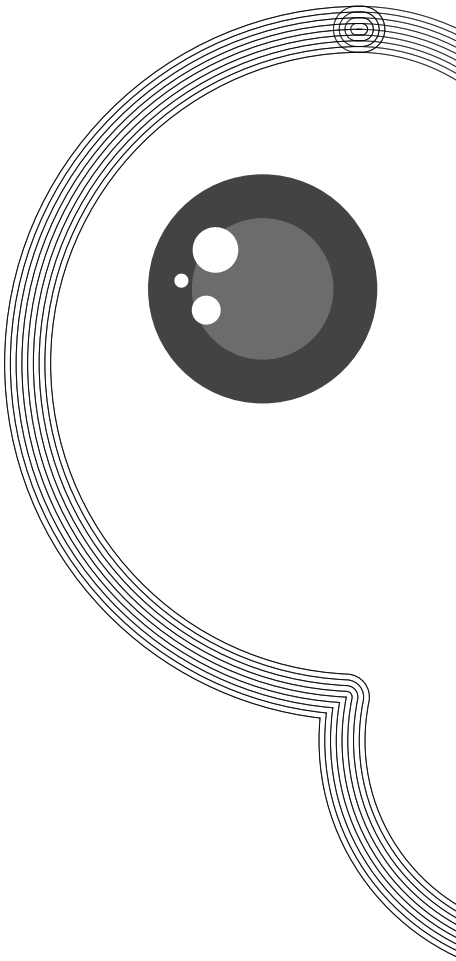
Figure S.2.1 Associations between fetal growth restriction and delivery outcomes



Values represent odds ratios with the 95% confidence interval of the confounder model that reflects the difference in delivery outcomes between fetal growth restriction (FGR) as compared to the reference group (no FGR). Models were adjusted for child's age, sex, ethnicity, maternal age, educational level, smoking, folic acid use and diastolic blood pressure at intake. Abbreviations: NICU = neonatal intensive care unit. FGR: fetal growth restriction.

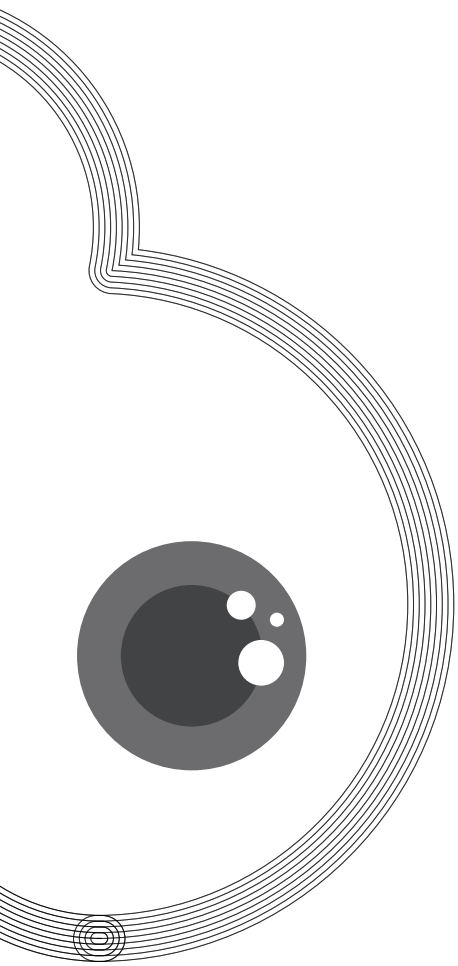
Table S.2.1 Crosstabs of fetal growth restriction and fetuses born small for gestational age

	SGA < p5		% SGA fetuses which are growth restricted	% Fetuses with normal birth weight which are growth restricted
	No	Yes		
FGR 30				
No	6237	274	32.8%	17.4%
Yes	1314	134		
FGR 35				
No	6484	293	28.2%	14.1%
Yes	1067	115		
FGR 40				
No	6689	312	23.5%	11.4%
Yes	862	96		
FGR 45				
No	6891	327	19.9%	8.7%
Yes	660	81		
FGR 50				
No	7028	343	15.9%	6.9%
Yes	523	65		



Chapter 3

Fetal growth and placental growth factor umbilical cord blood levels



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Fetal Diagnosis and Therapy, in press

Abstract

Introduction: We assessed whether umbilical cord blood placental growth factor (PlGF) levels at delivery are associated with fetal growth.

Methods: From a prospective population-based cohort study we included 3461 live singleton births. Fetal growth was assessed by birth weight, fetal growth pattern and fetal growth restriction FGR (decrease in growth between second trimester and birth of ≥40 percentiles). PlGF was assessed in the umbilical cord immediately after birth. In all analyses the highest PlGF multiple of the mean (MoM) quintile was used as a reference category.

Results: Umbilical cord PlGF was neither correlated with maternal second trimester PlGF (p=0.08) nor placental weight (p=0.18) suggesting that PlGF from umbilical cord blood was of fetal origin. Lower PlGF was associated with a lower birth weight (lowest quintile -0.60 SD [95% CI -0.71;-0.48], p for trend <0.001) and a different fetal growth pattern (p<0.001). Finally, lower PlGF was associated with FGR (lowest quintile OR 2.00 [95% CI 1.25;3.21], p for trend <0.001).

Conclusion: Lower umbilical cord PlGF levels are associated with lower birth weight, deviating fetal growth patterns and a higher odds of FGR. Hence, cord blood PlGF might be a promising biomarker to determine deviations in fetal growth and FGR retrospectively enabling follow-up of these neonates.

Introduction

Placental growth factor (PlGF) is a proangiogenic factor produced by the placenta during pregnancy (1). Extra-placental tissues associated with a high grade of vascularization, such as the heart and lungs, also produce PlGF (2). Hence, PlGF is also detectable in non-pregnant women, men and even in cord blood. To date, the majority of studies have focused predominantly on PlGF in the maternal circulation rather than the fetal circulation (3). Low levels of maternal PlGF are well known to be associated with placental development and function and subsequently with placenta mediated pregnancy processes and complications such as pre-eclampsia and fetal growth demonstrated by a lower birth weight and an increased risk for a neonate to be born small for gestational age (SGA) (4-8). Interestingly fetal PlGF levels have been associated with fetal growth. Within our cohort lower levels of umbilical cord PlGF were associated with neonates born SGA (9). However, SGA fetuses constitute a heterogeneous group consisting out of fetuses which are constitutionally small and fetuses with a fetal growth restriction (FGR) resulting in a low birth weight (10-12). Moreover neonates could have experienced FGR despite their normal birth weight. Neonates who experienced FGR may constitute a vulnerable group with increased risks of accelerated growth and altered cardiovascular outcomes in childhood, irrespective of their birth weight (13, 14). Identification of FGR neonates is therefore vital.

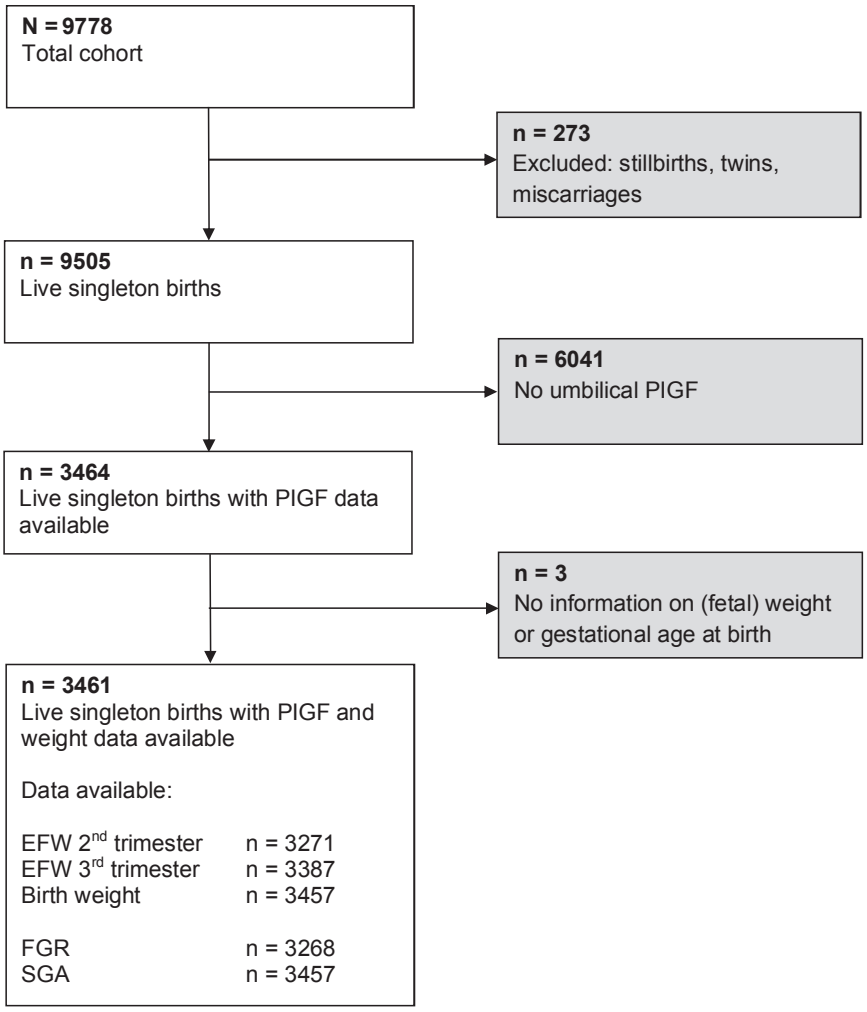
In this study we aim to assess the association between umbilical cord blood PlGF and fetal growth measured by birth weight, fetal growth pattern and FGR.

Materials and methods

Study design and ethical approval

This study was embedded in The Generation R Study, a population-based prospective cohort study from early pregnancy onwards (15). The study was approved by the Medical Ethics Committee of the Erasmus Medical Center Rotterdam, the Netherlands. Written informed consent was obtained from all participants. For the present study we included women with a live born singleton with data available concerning fetal growth and cord blood PlGF levels (**Figure 3.1**).

Figure 3.1. Flowchart



Pregnancy dating

Dating by early ultrasonography is vital to ensure accurate pregnancy dating, especially when assessing fetal growth and FGR. Dating of pregnancy was performed in early pregnancy using the first ultrasound measurement of either the crown rump length (CRL) (gestational age until 12 weeks and five days of gestation, and CRL measurement smaller than 65 mm), or biparietal diameter (BPD) (gestational age from 12 weeks and five days of gestation onwards, and BPD larger than 23 mm) (16).

Umbilical cord blood PlGF

Umbilical venous cord blood was sampled immediately after birth and transported to the regional laboratory for processing and storage at -80°C. Measurements were performed between 2008 and 2010 and all measurements factors have been shown to be stable during long-term storage (17). Serum levels of PlGF were analyzed using an immunoelectron chemiluminescence assay on the Architect System (Abbott Diagnostics BV). The between-run coefficients of variation for PlGF were 4.7% at 24 pg./mL and 3.8% at 113 pg./mL (8). Umbilical cord blood PlGF is highly dependent of gestational age at birth. Therefore multiple of the median (MoM) were created for each gestational week separately.

To investigate whether umbilical cord PlGF has a maternal origin, Pearson correlation tests were performed on umbilical cord PlGF levels with first and second trimester maternal PlGF levels and placental weight. The Pearson correlation coefficient for umbilical cord PlGF and placental weight was 0.025 ($p = 0.18$). For maternal PlGF the Pearson correlation coefficients were -0.01 for first trimester PlGF ($p = 0.69$) and 0.03 for second trimester PlGF ($p = 0.08$) suggesting that fetal PlGF levels were measured in umbilical blood instead of maternal PlGF levels.

Fetal growth

To assess estimated fetal weight (EFW) ultrasound examinations were performed in the second (median 20.5 weeks of gestation, 90% range 18.9 – 22.8) and third trimester (median 30.4 weeks of gestation, 90% range 28.8 – 32.4) of pregnancy. Fetal biometry (head circumference [HC], abdominal circumference [AC] and femur length [FL]) was measured trans abdominally. EFW was calculated using the formula of Hadlock with parameters AC, HC and FL (in cm): EFW = 10^ (1.326 – 0.00326*AC*FL + 0.0107*HC + 0.0438*AC + 0.158*FL (18). Ultrasound examinations were performed using Aloka® model SSD-1700 (Tokyo, Japan) or the ATL-Philips® Model HDI 5000 (Seattle, WA, USA). Gestational age at birth and birth weight were obtained from midwives and hospital registries. SGA was defined as a gestational and fetal sex adjusted birth weight under the fifth percentile based on charts derived from our cohort (16). Five definitions were used to define FGR: a decrease in growth of at least 30, 35, 40, 45 or 50 percentiles respectively between the second trimester and birth. Choosing cut-offs to define FGR may lead to misclassification. For a fetus to be able to deviate from its growth curve with 40 percentiles, the initial estimated fetal weight in the second trimester of pregnancy should by definition have been above the

40th percentile. Hereby fetuses with an estimated fetal weight under the 40th percentile cannot be classified as FGR and are thus classified as not growth restricted. Therefore, in an additional analyses we classified all fetuses with an EFW in the 2nd trimester under the 40th percentile and with a maximum decrease of growth (birth weight under the 1st percentile) as FGR without any differences in results.

Covariates

We obtained information on maternal age, ethnicity, educational level, parity, smoking during pregnancy and folic acid intake by questionnaire at enrollment. At intake height (cm) and weight (kg) were measured without shoes and heavy clothing and body mass index (BMI [kg/m^2]) was calculated. Pre-eclampsia was defined as the development of a systolic blood pressure (SBP) ≥ 140 mmHg and/or a diastolic blood pressure (DBP) ≥ 90 mmHg after 20 weeks of gestation plus the presence of proteinuria (0.3 g or greater in a 24-hour urine specimen or 2+ or greater [1 g/L] on a voiced specimen, or 1+ or greater [0.3 g/L] on a catheterized specimen) in previously normotensive women according to the former International Society for the Study of Hypertension in Pregnancy criteria (19).

Statistical analyses

To explore non-linearity PlGF MoM was categorized into quintiles: first quintile (PlGF MoM >1.27), second quintile (PlGF MoM <1.27 and >1.08), third quintile (PlGF MoM <1.08 and >0.93), fourth quintile (PlGF MoM <0.93 and >0.78) and fifth quintile (PlGF MoM <0.78).

To test the differences in baseline characteristics between the five PlGF MoM quintiles, ANOVA, Mann-Whitney *U* and Chi-square tests were performed. Then linear regression analyses were performed to relate cord blood PlGF MoM's to birth weight. To explore growth trajectories between different PlGF MoM categories, unbalanced repeated measurements regression models with an unstructured covariance structure were performed. The mixed model procedure was used with (estimated) weight expressed in standard deviation as a repeated outcome measure. These regression models take the correlation between repeated measurements of the same subject into account. Moreover, they have an optimal use of available measurements by allowing for incomplete outcome data. Then to analyze the associations of umbilical cord blood PlGF MoM's with FGR logistic regression models were used.

Basic models were adjusted for fetal sex and ethnicity. Confounder models were additionally adjusted for maternal age, educational level, parity, smoking during pregnancy and folic acid intake. We selected these confounders on the basis of their associations with both the exposure and the outcomes of interest or a change in effect estimate of more than 10%. We considered the confounder model to be the main model. Since angiogenic levels are disturbed in case of maternal pre-eclampsia a third model was used in which we additionally adjusted for the presence of pre-eclampsia i.e. pre-eclampsia model.

Effect modification on the multiplicative scale was tested to exclude the possibility that the effect of low cord blood PlGF MoM on fetal growth depends on fetal sex, smoking, parity, maternal BMI at intake, and SGA. If $p < 0.10$ was fulfilled regression analyses were performed in strata of that specific variable.
For all analyses, the percentages of missing values of covariates were lower than 20%. We imputed missing data of the covariates by using multiple imputations (20). Ten datasets were created and analyzed together. Statistical analyses were performed using the Statistical Package of Social Sciences version 21.0 for Windows (SPSS Inc, Chicago, IL, USA) or the Statistical Analysis System version 9.3 (SAS, Institute Inc Gary NC, USA).

Results

Baseline characteristics of the total study population are presented in **Table 3.1**. Stratification between the different PlGF MoM quintiles showed that mothers of neonates with lower PlGF MoM's were more often nulliparous ($p < 0.001$) and more often had pre-eclampsia ($p = 0.007$). The neonates themselves had a lower birth weight ($p < 0.001$).

Results on birth weight (SDS) are depicted in **Table 3.2**. All quintiles, with exception of the second quintile, were associated with lower birth weights with a p for trend <0.001 in all models compared to the first quintile.

Results from repeated measurements showed a different growth pattern in the lowest PlGF MoM quintile compared to the highest quintile (**Figure 3.2,** $p < 0.001$). This resulted in a lower estimated fetal weight and birth weight. All other quintiles had lower estimated fetal weights and lower birth weights however with a similar growth pattern compared with the highest PlGF MoM quintile.

Table 3.1. Baseline characteristics

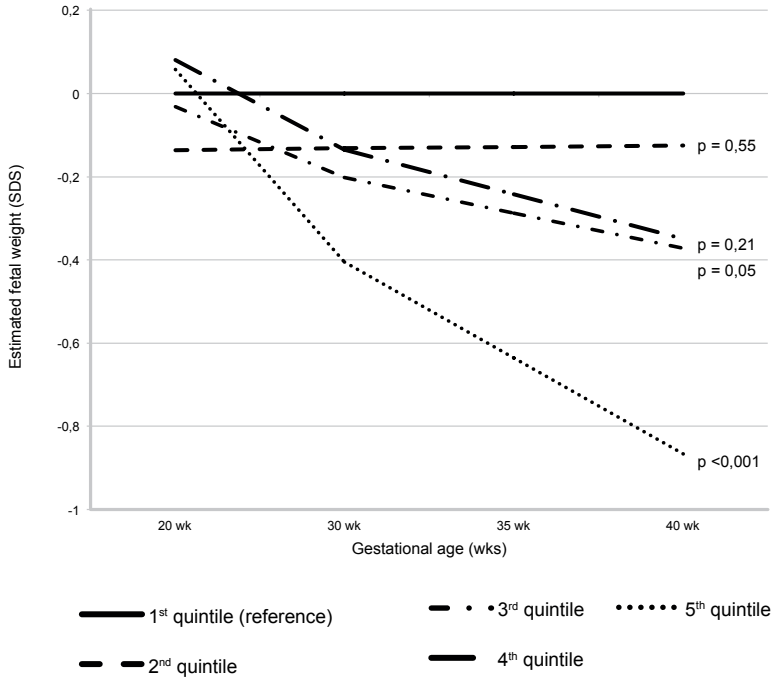
	Study population	Highest quintile	Second quintile	Third quintile	Fourth quintile	Lowest quintile	p-value
	n = 3461	n = 696	n = 695	n = 697	n = 678	n = 695	
Maternal age		29,6 ± 5,1	29,5 ± 5,3	29,6 ± 5,1	29,5 ± 5,2	29,7 ± 5,4	0,96
Anthropometrics							
Height (cm)	167,7 ± 7,4	168,0 ± 7,6	167,9 ± 7,1	167,8 ± 7,1	167,6 ± 7,7	167,0 ± 7,3	0,10
Weight (kg)	69,4 ± 12,9	69,4 ± 12,8	69,8 ± 13,0	68,9 ± 13,0	69,7 ± 13,1	69,0 ± 12,7	0,60
BMI (kg/m^2)	23,8 (19,3-33,2)	23,8 (19,3-33,2)	23,9 (19,5-33,3)	23,4 (19,0-33,1)	23,8 (19,5-33,4)	24,0 (19,4-33,4)	0,31
Ethnicity							0,94
Western	2047 (61,6%)	413 (61,9%)	414 (62,3%)	418 (62,3%)	398 (61,1%)	403 (60,3%)	
Non-Western	1276 (38,4%)	254 (39,1%)	251 (37,7%)	253 (37,7%)	253 (39,9%)	265 (39,7%)	
Educational level							0,08
Low	391 (11,3%)	77 (11,1%)	88 (12,7%)	79 (11,3%)	83 (12,2%)	65 (9,4%)	
Middle	1589 (46,1%)	326 (46,8%)	306 (44,0%)	309 (44,3%)	311 (45,9%)	346 (49,8%)	
High	1468 (42,6%)	293 (42,1%)	301 (43,3%)	309 (44,3%)	284 (41,9%)	284 (40,9%)	
Smoking habits							0,46
No	2470 (71,6%)	499 (71,7%)	506 (72,8%)	486 (69,7%)	484 (71,4%)	500 (71,9%)	
Yes - stopped	291 (8,4%)	50 (7,2%)	58 (8,3%)	64 (9,2%)	68 (10,0%)	55 (7,9%)	
Yes - continued	688 (19,9%)	147 (21,1%)	131 (18,8%)	147 (21,1%)	126 (18,6%)	140 (20,1%)	
Folic acid use - Yes (%)							0,18
No	990 (28,7%)	191 (27,4%)	201 (28,9%)	194 (27,8%)	205 (30,2%)	192 (27,6%)	
Before 10 weeks	1099 (31,8%)	218 (31,3%)	229 (32,9%)	219 (31,4%)	221 (32,6%)	229 (32,9%)	
Preconception start	1360 (39,4%)	287 (41,2%)	265 (38,1%)	284 (40,7%)	252 (37,2%)	274 (39,4%)	
Nulliparous (%)	1942 (56,3%)	320 (46,0%)	318 (45,8%)	399 (57,2%)	421 (62,1%)	490 (70,5%)	<0,001
Pre-eclampsia - Yes (n;%)	42 (1,3%)	5 (0,7%)	5 (0,7%)	5 (0,7%)	10 (1,5%)	17 (2,4%)	0,007

Table 3.1. Continued

Gestational age at sonography (wks)							
Second trimester	20,5 (18,9-22,8)	20,5 (19,0-22,9)	20,5 (18,8-22,7)	20,5 (18,9-22,8)	20,5 (18,8-22,8)	20,5 (18,9-22,9)	0,65
Third trimester	30,4 (28,8 - 32,3)	30,4 (28,8-32,3)	30,4 (28,8-32,3)	30,3 (28,9-32,3)	30,3 (28,7-32,2)	30,4 (28,6-32,6)	0,72
Gestational age at birth (wks)	40,1 (37,4 - 42,0)	40,1 (37,1-42,1)	40,1 (37,6-42,0)	40,0 (37,4-42,0)	40,1 (37,4-42,0)	40,3 (37,3-42,2)	0,28
Estimated fetal weight 2nd trimester (gr)	382 ± 94	384 ± 91	381 ± 95	385 ± 98	380 ± 95	383 ± 93	0,87
Estimated fetal weight 3rd trimester (gr)	1614 ± 255	1619 ± 241	1627 ± 274	1611 ± 238	1603 ± 253	1612 ± 265	0,52
Birthweight (gr)	3458 ± 500	3556 ± 535	3551 ± 463	3451 ± 471	3431 ± 475	3298 ± 509	<0,001
PlGF MoM	1,00 (0,58 - 1,75)	1,47 (1,29-5,09)	1,16 (1,08-1,26)	1,00 (0,93-1,07)	0,86 (0,79-0,92)	0,64 (0,41-0,77)	<0,001

Data are represented as n (%) or as the mean (SD) or as the median with the 90% range. Differences in baseline characteristics were tested using ANOVA, Mann-Whitney U and Chi-square tests.

Figure 3.2. Associations between PlGF and fetal growth pattern



Results are based on repeated measurements regression models and reflect the differences in gestational age adjusted SD scores at 20, 30, 35 and 40 weeks of gestation. The highest quintile of PlGF is the reference category and presented as the zero-line in the graph. The model was adjusted for fetal sex, ethnicity, maternal age, educational level, parity, smoking during pregnancy and folic acid intake.

Results on the clinical outcome FGR are depicted in **Table 3.3**. Again similar results towards lower PlGF MoM's versus decreased fetal growth was observed with odds ratio's up to 3.45 (95% CI 1.83-6.53) for the fifth quintile for FGR with a decrease of growth of >50 percentiles.

Interaction analyses showed no effect modification of fetal sex, smoking, parity, maternal BMI at intake, and SGA on all outcomes.

Table 3.2. Associations between cord blood placental growth factor and birth weight

	Basic model		Confounder model		Pre-eclampsia model	
	β (95% CI)	p-value	β (95% CI)	p-value	β (95% CI)	p-value
Birth weight (SDS)						
PlGF MoM 1st quintile	reference		reference		reference	
PlGF MoM 2nd quintile	-0,02 (-0,12 - 0,09)	0,74	-0,02 (-0,12 - 0,08)	0,70	-0,02 (-0,12 - 0,08)	0,72
PlGF MoM 3rd quintile	-0,22 (-0,33 - -0,12)	<0,001	-0,19 (-0,29 - -0,08)	<0,001	-0,19 (-0,29 - -0,08)	<0,001
PlGF MoM 4th quintile	-0,29 (-0,39 - -0,18)	<0,001	-0,24 (-0,34 - -0,13)	<0,001	-0,23 (-0,33 - -0,13)	<0,001
PlGF MoM 5th quintile	-0,60 (-0,71 - -0,48)	<0,001	-0,50 (-0,61 - -0,38)	<0,001	-0,48 (-0,60 - -0,37)	<0,001
	p for trend	*<0,001*	*p for trend*	*<0,001*	*p for trend*	*<0,001*

Values are regression coefficient with the 95% CI and are based on linear regression models. Basic model: adjusted for fetal sex and ethnicity. Confounder model: basic model and additionally adjusted for maternal age, educational level, parity, smoking and folic acid intake. Pre-eclamptic model: confounder model and additionally adjusted for maternal pre-eclampsia. Abbreviations: PlGF: placental growth factor. MoM: multiple of the median. SD: standard deviation score. CI: confidence interval.

Table 3.3. Associations between cord blood placental growth factor and fetal growth restriction

	Basic model		Confounder model		Pre-eclampsia	
	β (95% CI)	p-value	β (95% CI)	p-value	β (95% CI)	p-value
FGR 30						
PlGF MoM 1st quintile	reference		reference		reference	
PlGF MoM 2nd quintile	1,05 (0,70 - 1,56)	0,82	1,03 (0,69 - 1,54)	0,90	1,03 (0,69 - 1,54)	0,90
PlGF MoM 3rd quintile	1,31 (0,89 - 1,94)	0,17	1,20 (0,81 - 1,79)	0,37	1,20 (0,80 - 1,78)	0,38
PlGF MoM 4th quintile	1,73 (1,19 - 2,52)	0,004	1,57 (1,07 - 2,31)	0,02	1,56 (1,06 - 2,29)	0,02
PlGF MoM 5th quintile	2,38 (1,67 - 3,39)	<0,001	2,01 (1,38 - 2,93)	<0,001	1,99 (1,36 - 2,90)	<0,001
	p for trend	*<0,001*	*p for trend*	*<0,001*	*p for trend*	*<0,001*
FGR 35						
PlGF MoM 1st quintile	reference		reference		reference	
PlGF MoM 2nd quintile	1,11 (0,71 - 1,73)	0,64	1,09 (0,69 - 1,71)	0,72	1,09 (0,69 - 1,70)	0,72
PlGF MoM 3rd quintile	1,50 (0,98 - 2,30)	0,07	1,39 (0,90 - 2,15)	0,14	1,39 (0,90 - 2,15)	0,14
PlGF MoM 4th quintile	1,84 (1,21 - 2,78)	0,004	1,69 (1,10 - 2,59)	0,016	1,68 (1,09 - 2,58)	0,018
PlGF MoM 5th quintile	2,35 (1,58 - 3,50)	<0,001	1,92 (1,26 - 2,93)	0,002	1,88 (1,24 - 2,87)	0,003
	p for trend	*<0,001*	*p for trend*	*<0,001*	*p for trend*	*<0,001*
FGR 40						
PlGF MoM 1st quintile	reference		reference		reference	
PlGF MoM 2nd quintile	1,04 (0,62 - 1,73)	0,89	1,02 (0,61 - 1,71)	0,95	1,02 (0,61 - 1,70)	0,96
PlGF MoM 3rd quintile	1,62 (1,01 - 2,62)	0,047	1,56 (0,96 - 2,53)	0,07	1,55 (0,95 - 2,52)	0,08
PlGF MoM 4th quintile	2,05 (1,29 - 3,26)	0,002	1,89 (1,18 - 3,04)	0,009	1,87 (1,16 - 3,01)	0,01
PlGF MoM 5th quintile	2,30 (1,47 - 3,61)	<0,001	2,00 (1,25 - 3,21)	0,004	1,97 (1,23 - 3,17)	0,005
	p for trend	*<0,001*	*p for trend*	*<0,001*	*p for trend*	*<0,001*

Table 3.3. Continued

FGR 45						
PlGF MoM 1st quintile	reference		reference		reference	
PlGF MoM 2nd quintile	1,34 (0,73 - 2,47)	0,35	1,29 (0,70 - 2,39)	0,41	1,29 (0,70 - 2,38)	0,42
PlGF MoM 3rd quintile	1,79 (0,99 - 3,22)	0,053	1,70 (0,93 - 3,09)	0,08	1,70 (0,93 - 3,09)	0,08
PlGF MoM 4th quintile	2,81 (1,62 - 4,89)	<0,001	2,68 (1,52 - 4,71)	0,001	2,64 (1,50 - 4,65)	0,001
PlGF MoM 5th quintile	3,27 (1,91 - 5,60)	<0,001	2,92 (1,66 - 5,12)	<0,001	2,88 (1,64 - 5,07)	<0,001
	p for trend	*<0,001*	*p for trend*	*<0,001*	*p for trend*	*<0,0001*
FGR 50						
PlGF MoM 1st quintile	reference		reference		reference	
PlGF MoM 2nd quintile	1,39 (0,69 - 2,80)	0,36	1,32 (0,65 - 2,69)	0,44	1,32 (0,65 - 2,68)	0,44
PlGF MoM 3rd quintile	1,50 (0,74 - 3,05)	0,26	1,41 (0,69 - 2,89)	0,34	1,42 (0,69 - 2,90)	0,34
PlGF MoM 4th quintile	2,94 (1,56 - 5,55)	0,001	2,84 (1,49 - 5,43)	0,002	2,80 (1,46 - 5,36)	0,002
PlGF MoM 5th quintile	3,76 (2,04 - 6,93)	<0,001	3,45 (1,83 - 6,53)	<0,001	3,43 (1,81 - 5,50)	<0,001
	p for trend	*<0,001*	*p for trend*	*<0,001*	*p for trend*	*<0,001*

Values are regression coefficients with the 95% CI and are based on linear regression models. Basic model: adjusted for fetal sex and ethnicity. Confounder model: basic model and additionally adjusted for maternal age, educational level, parity, smoking and folic acid intake. Pre-eclampsia model: confounder model and additionally adjusted for pre-eclampsia. Abbreviations: PlGF: placental growth factor. MoM: multiple of the median. CI: confidence interval. FGR: fetal growth restriction.

Discussion

Main findings

This study shows that umbilical cord blood PlGF is associated with fetal growth with consistent results for birth weight, growth pattern and the clinical outcome FGR. The lower PlGF levels the stronger the observed effect estimates.

Strengths and limitations

The main strength of this study was the extensive prospective data collection on fetal growth and environmental influences. This enabled us to adjust for multiple confounding factors and investigate the effects of fetal growth in a large sample of 3461 participants.

The response rate at baseline for participation in the Generation R Study was 61%. Selective participations in this study did occur since pregnant women who participated were generally higher educated, more healthy, and more frequently of Western origin than those who did not participate. Data on umbilical cord blood PlGF was available in 36.4% of our total live singleton births. The non-response at baseline would lead to biased effect estimates if the associations would be different between those included and not included in the analyses. However, given the prospective nature of the study, this seems unlikely. Nevertheless it might have led to a selection of a more healthy population which affects the generalizability of our results.

For the purpose of this study we included only live singleton births. Therefore intra uterine fetal deaths (IUFD) with or without FGR were excluded. As a consequence the included pregnancies constitute a relatively healthy and homogenous study population.

In this study, we focused on a true deviation of fetal growth rather than on other parameters such as perinatal outcomes, Doppler parameters and placental function. SGA was defined as a gestational and fetal sex adjusted birth weight under the fifth percentile based on charts derived from our cohort. This cut-off was chosen since the fifth percentile of our cohort is more comparable with the cut off of the tenth percentile used in general clinical, compared with the tenth percentile of our cohort.

Interpretation

One of the key findings of this study is that not only the lowest quintile of cord blood PlGF is associated with fetal growth, but that also associations were found for higher quintiles and that clear trends were visible. Although this is an observational study and causality can therefore not be proven, one of the most important criteria for causation according to the Bradford Hill criteria is a biological gradient (21). In our study it is clear that a clear trend was visible in the association between neonatal PlGF levels and fetal growth parameters. Moreover the effect sizes are substantial and consistent even after adjustment for multiple confounding factors.

Fetal cord blood may contain PlGF of fetal and maternal or placental origin since PlGF crosses the placenta. However, very low correlations were found between umbilical cord blood PlGF levels on the one hand and maternal PlGF levels and placental weight on the other. Furthermore there were hardly any differences in effect estimates of the confounder models and the pre-eclampsia models. This implies that the vast majority of PlGF measured in umbilical cord blood is of fetal origin. Unfortunately no maternal PlGF levels at the end of pregnancy were available within our cohort. During pregnancy PlGF reaches its peak around 31 weeks of gestation after which it declines (7, 22). Around the time of delivery levels of maternal PlGF are comparable with levels around 19 weeks of gestation. Correlation coefficients may therefore not be drastically different even if term maternal PlGF at term had been available. This is confirmed by one study in which there was a 10-fold difference for PlGF in the maternal and fetal circulation during delivery and without a significant correlation between the two (23). It is suggested that the umbilical artery PlGF would be a better representation of the fetal circulation compared to umbilical vein PlGF which we used in our study. Some studies investigated PlGF in both the umbilical vein as well as arteries in SGA neonates compared with AGA neonates and considered the umbilical vein as a representation of the placental compartment (24). They observed differences in PlGF levels between SGA and AGA neonates in the umbilical vein but not in the umbilical artery and concluded that fetal PlGF was not altered in SGA. However, if the umbilical vein indeed is a representation of the placental compartment a significant correlation would be expected between umbilical vein PlGF and maternal PlGF or measures of placental function like placental weight. In our study these associations were not present from which we conclude that also the umbilical vein is representative for the fetal circulation.

In current literature, the clinical outcome FGR is often defined as SGA (25). However, FGR is not necessarily synonymous with SGA. FGR fetuses may experience a failure to reach their biological growth potential because of a pathological slow-down in the fetal growth pace. The Delphi study also established a definition for FGR by expert consensus, acknowledging the significant difference between FGR and SGA (25). The definitions of both early and late FGR were based on measurements taken at one single point in time. Although crossing centiles for AC or EFW were allowed in the definition for late FGR, it must be combined with another criterium. We on the other hand primarily and solely focused on deviating growth patterns. Since there is no international consensus on how much a fetal growth curve needs to deviate before it can be designated as FGR we used several cut-offs of a decrease in growth between the second trimester of pregnancy and birth. In previous research FGR fetuses without SGA are not subject of research. Nevertheless this might be a vulnerable group with increased risks of adverse health in later life. The current study shows an association between fetal PlGF and the clinical outcome FGR. These associations were not only found for the severe growth restricted fetuses, i.e. decrease of ≥50 percentiles, but also for the mild growth restricted fetuses, i.e. decrease of ≥30 percentiles. Future research has to focus on the potential of fetal PlGF to identify those neonates at risk for adverse childhood outcomes.

Since little research has been performed on neonatal PlGF not much is known about the exact origin of neonatal PlGF. PlGF is not only expressed by the placenta but also by tumor cells, endothelial cells and inflammatory cells (26). Especially endothelial cells could be a potential source of PlGF within the neonate. PlGF exerts a strong effect upon blood vessel growth and maturation and has direct proangiogenic effects on endothelial cells (27). Within cardiovascular disease endothelial function is crucial and often precedes clinical manifestations (28, 29). This is of importance when taking the Development and Origins of Health and Disease paradigm into consideration. According to this paradigm the unborn fetus can modify its own development such that it will be prepared for survival in an environment in which recourses are likely to be short. Although these adaptations may be beneficial for short term survival, they may have adverse consequences at delivery or in later life (30). Since FGR fetuses have increased risks of developing cardiac diseases in later life, low cord blood levels of PlGF could well be one of the first signs of this increased risk. Nevertheless we should consider the possibility that small fetuses produce less PlGF. In that case the underlying mechanisms of the fetuses being small cause the potential adverse outcomes in later life associated with FGR rather than PlGF itself. However, this

seems unlikely considering the incidence of pre-eclampsia as shown in Table 1 which differs considerably across the different PlGF quintiles.

Conclusion

This study demonstrates that umbilical cord blood PlGF is associated with birth weight, fetal growth pattern and FGR. Fetuses born with FGR are often born with a normal birth weight but nevertheless constitute a vulnerable group with increased risks for cardiovascular diseases in later life. Identification of this vulnerable group enables follow-up in the postnatal period preventing possible adverse events in later life. Future research is needed to investigate whether fetal PlGF can be used to identify those fetuses at risk for adverse childhood outcomes.

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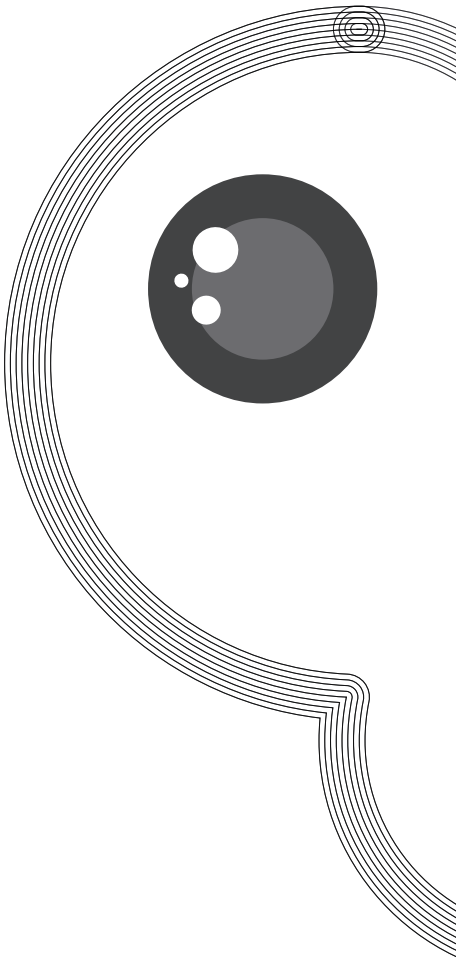
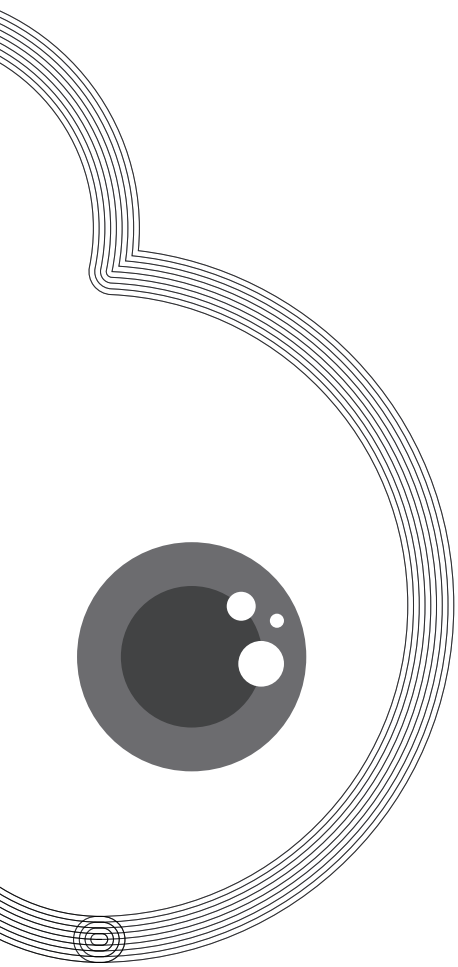


Chapter 4

Sex specific differences in fetal and infant growth patterns



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Abstract

Background: The objective of this study was to assess whether sex specific differences in fetal and infant growth exist.

Methods: This study was embedded in the Generation R Study, a population-based prospective birth cohort. In total 8556 live singleton births were included. Fetal growth was assessed by ultrasound. During the first trimester crown-rump-length (CRL) was measured. In the second and third trimester of pregnancy head circumference (HC), abdominal circumference (AC) and femur length (FL) were assessed. Information on infant growth during the first two years of life was obtained from Community Health Centers and included HC, body weight and length.

Results: In the first trimester male CRL was larger than female CRL (0.12 SD [95% CI 0.03;0.22]). From the second trimester onwards HC and AC were larger in males than in females (0.30 SD [95% CI 0.26;0.34], and 0.09 SD [95% CI 0.05;0.014] respectively). However, FL in males was smaller as compared to female fetuses (0.21 SD [95% CI 0.17,0.26]). Repeated measurement analyses showed a different prenatal as well as postnatal HC growth pattern between males and females. A different pattern in body weight was observed with a higher body weight in males until the age of 12 months where after females have a higher weight.

Conclusion: Sex affects both fetal as well as infant growth. Besides body size also body proportions differ between males and females with different growth patterns. This sexual dimorphism might arise from differences in fetal programming with sex specific health differences as a consequence in later life.

Introduction

Embryonic and fetal growth are dependent on many factors, including adequate placental development and function. This can be reflected by several placental biomarkers in maternal serum such as the pro-angiogenic placental growth factor (PlGF) and the anti-angiogenic soluble fms-like tyrosine kinase 1 (s-Flt1) (1, 2). Previous studies have shown associations between placental biomarkers and fetal growth (3-5).

Recently, fetal sex specific differences in placental biomarkers were observed with higher first trimester levels of s-Flt1, PlGF in women carrying a female fetus. This may suggest that placentation processes differ according to fetal sex (6). This difference in placental function might influence fetal growth and / or fetal programming in a sex specific manner. Indeed previous research has shown fetal sex specific differences in biometrical indices and growth patterns and fetal sex specific growth curves were created (7). However, these growth curves were based on cross-sectional data and serial measurements of the same fetus were not available. Moreover, it is of interest to investigate whether sex specific differences in fetal growth persist into infancy since the Development and Origins of Health and Disease (DOHaD) theory states that deviations in early growth are associated with adverse health in later life.

With this study we investigate whether there are fetal sex specific differences in fetal and infant growth in a large study population. We repeatedly assessed fetal growth during pregnancy by measuring crown-rump-length CRL in the first trimester and several biometrical indices (head circumference [HC], abdominal circumference [AC] and femur length [FL]) in the second and third trimester of pregnancy. After pregnancy until the age of two years growth was assessed at several timepoints by assessing HC, body weight and length. In addition we explore the effect of the presence or absence of the placental syndrome on these differences.

Materials and methods

Study design

This study was embedded in the Generation R Study, a population-based prospective cohort study from early pregnancy onwards in Rotterdam, the Netherlands (8). The study is designed to identify early environmental causes of normal and abnormal growth, development and health from fetal life until young adulthood. Eligible mothers were those who were resident in the study area at their delivery date and had a delivery date from April 2002 until January 2006. We aimed to enroll mothers in early pregnancy (before 18 weeks of gestation In total 9778 mothers were enrolled. For the present study, women with a live singleton birth with at least one prenatally assessed biometric measurement were included in this study (**Figure 4.1**). The study has been approved by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam, the Netherlands. Written informed consent was obtained from all participants.

Pregnancy dating

Precise initial dating by early ultrasonography is vital to ensure accurate pregnancy dating, especially when assessing fetal growth. Dating of pregnancy was performed using the first ultrasound measurement of either the CRL (if the gestational age was below 12 weeks and 5 days and CRL measurement was smaller than 65 mm), or the biparietal diameter (BPD) (from a gestational age from 12 weeks and 5 days onwards and with a BPD larger than 23 mm).

Fetal growth

First trimester

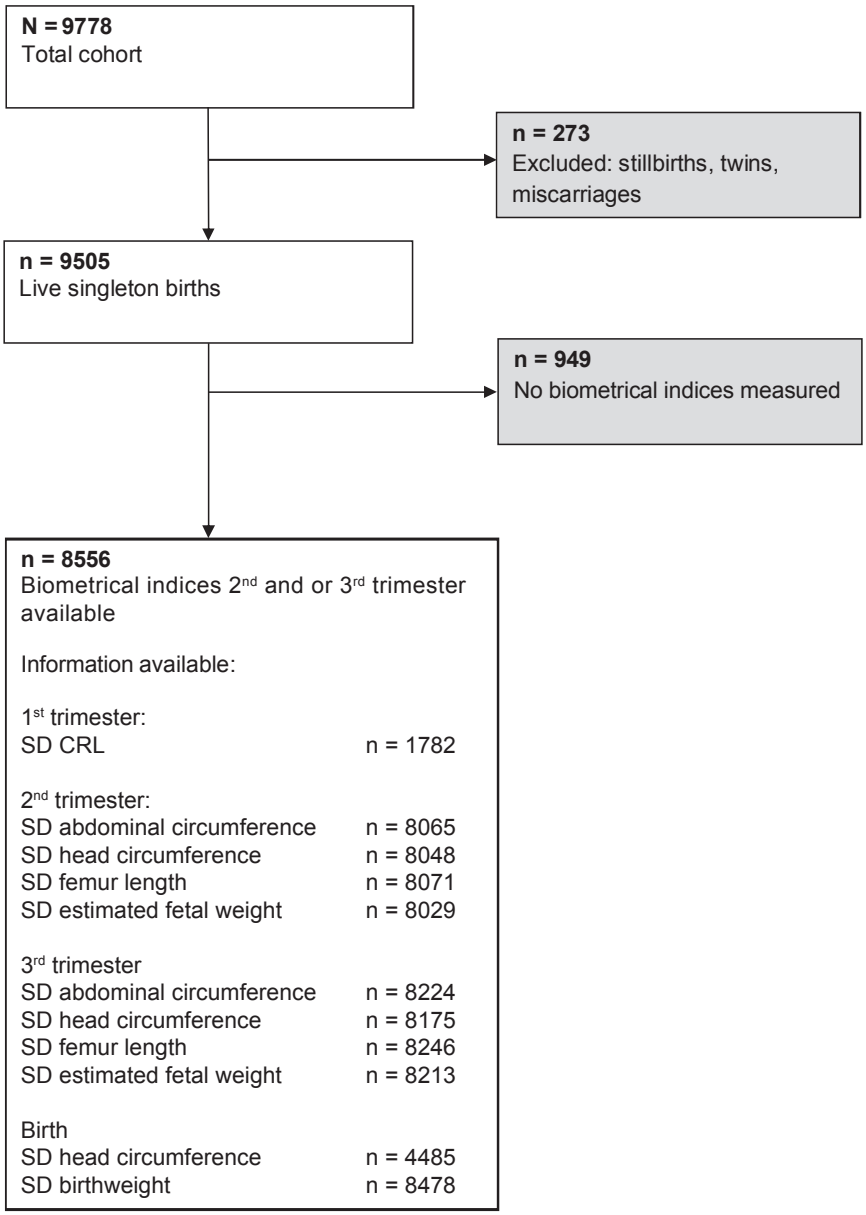
Since pregnancy dating was based on CRL, only CRL measurements of women with a known and reliable first day of the last menstrual period (LMP) with a regular cycle (lasting 28 days + / - 4 days) were included in the analyses (n = 1782). For the purpose of analyses on CRL measurement pregnancy dating in this subgroup of women was not based on CRL but on the LMP.

Second and third trimester

Fetal ultrasound examinations were performed in the second (median 20.5 weeks of gestation, 90% range 18.9 – 22.9) and third trimester (median 30.3 weeks of gestation, 90% range 28.7 – 32.4). Fetal biometry (HC, AC and FL) was measured trans abdominally during each ultrasound examination. Standardized ultrasound planes for HC, AC and FL are described elsewhere (9-11). Estimated fetal weight (EFW) was calculated using the formula of Hadlock with parameters

AC, HC and FL (in cm): EFW = 10**(1.326 - 0.00326*AC*FL + 0.0107*HC + 0.0438*AC + 0.158*FL (12).

Figure 4.1 Flowchart



Gestational age-adjusted standard deviation scores (SDS) were calculated for all fetal growth measurements, including CRL measurements. These gestational age-adjusted standard deviation scores were based on reference growth curves from the whole study population and represent the equivalent of Z-scores (13). Ultrasound examinations were performed using an Aloka® model SSD-1700 (Tokyo, Japan) or the ATL-Philips® Model HDI 5000 (Seattle, WA, USA).

Delivery and birth complications

Information on gestational age at birth, offspring sex and pregnancy complications (PE, SGA, sPTB) was obtained from medical records. sPTB was defined as a spontaneous onset of birth <37 weeks of gestation. SGA was defined as a gestational age and fetal sex adjusted birthweight below the 10th percentile (13). Pre-eclampsia was defined as the development of SBP ≥ 140 mmHg and / or DBP ≥ 90 mmHg after 20 weeks of gestation plus the presence of proteinuria (≥ 0.3 g in a 24-hour urine specimen or ≥ 2 + [1 g/L] on a voided specimen, or ≥ 1 + [0.3 g/L] on a catheterized specimen) in previously normotensive women (14).

Infant growth

Well-trained staff in the Community Health Centers obtained postnatal growth characteristics according to standard schedules and procedures at the median ages of 1.1 (90% range 0.8 - 1.6), 2.2 (90% range 2.0 - 2.9), 3.3 (90% range 3.0 - 3.9), 4.4 (90% range 4.0 - 4.9), 6.2 (90% range 5.4 - 7.6), 11.1 (90% range 10.2 - 12.3), 14.3 (90% range 13.7 - 15.7), 18.3 (90% range 17.5 - 20.8) and 24.8 (90% range 23.6 - 27.5) months. Growth characteristics included body weight, length and HC. SD scores were obtained with Dutch growth reference charts (Growth Analyzer 3.0, Dutch Growth Research Foundation, Rotterdam, the Netherlands).

Statistical analyses

Firstly, we performed student t-tests and Chi-square tests to test sex-specific differences in fetal growth characteristics. Linear regression analyses were then performed to relate fetal biometric indices to sex. To further explore growth patterns between female and male fetuses and infants repeated measurement regression models were performed using the mixed model procedure with fetal and infant growth as a repeated outcome measure. These models take the correlation between repeated measurements of the same subject into account. Regarding the repeated measurements analyses that we used to assess fetal growth patterns, we used SD-scores according to the Niklasson growth standards. This standard adjusts for fetal sex. In addition we stratified

for fetal sex in our analyses, which creates the potential risk of over-adjusting. The growth standard of Usher and McLean is to our knowledge the only standard available not adjusting for fetal sex (15). Repeated measurements analyses on weight using this standard instead of the Niklasson standard are shown in Supplemental Figure 1. Finally, to investigate differences in fetal growth in pregnancies with a suboptimal intrauterine environment we created the composite outcome scores 'complicated pregnancy' and 'uncomplicated pregnancy'. Pregnancies complicated by either PE and/or sPTB and/or SGA were classified as being complicated. Uncomplicated pregnancies were defined by the absence of all these complications. All above mentioned linear regression and repeated measurement analyses were also performed within strata of these composite scores. Since fetal sex does not know any true confounding factors (e.g. smoking, folic acid intake, maternal ethnicity etc) primary analyses weren't adjusted for any of these covariates. However, since including these covariates into the analyses may be informative we included them in additional analyses shown in **supplemental Table S.4.1**. By using SD scores of all outcomes we automatically adjusted for gestational age at the time of measurement.

Lastly effect modification was tested on a multiplicative scale with maternal smoking and ethnicity. If the interaction term was statistical significant regression or repeated measurements analyses were performed in strata of that specific determinant.

Statistical analyses were performed using either the Statistical Package of Social Sciences version 21.0 for Windows (SPSS Inc, Chicago, IL, USA) or the Statistical Analysis System version 9.3 (SAS, Institute Inc. Gary NC, USA).

Results

Study population

Baseline characteristics of all participants are presented in **Table 4.1**. There were no differences between women pregnant with a male or female fetus concerning maternal age, height, weight, BMI, ethnicity, educational level, folic acid use or parity. Women with a male fetus smoked more often ($p < 0.01$). Also gestational age at ultrasound and gestational age at birth differed between male and female fetuses.

Effect of sex on fetal growth

Already in the first trimester fetal growth differed between the two sexes. Male fetuses showed to have a larger CRL as compared to female fetuses with a difference of 0.12 SD (95% CI 0.03;0.22, p < 0.001). In the second trimester of pregnancy male fetuses had a lower EFW of 0.05 SD (95% CI 0.00;0.09, p = 0.03) calculated by the Hadlock formula. In the third trimester no differences concerning EFW were observed. At birth male neonates were on average 188 grams heavier than female neonates (95% CI 182;193, p < 0.001).

In **Table 4.2** the results of the linear regression analyses on biometrical indices are depicted. Male sex was associated with a larger AC and HC, but a smaller FL in both the second and the third trimester of pregnancy (all p < 0.001). Results of the repeated measurements in SD-scores are shown in **Figure 4.2 A – D**. Male fetuses have a larger AC, and a smaller FL compared with female fetuses. Both track during pregnancy. Additionally, male HC is larger than female HC. However, this difference changes during pregnancy as the difference between male and female HC decreases as pregnancy precedes. This indicates that the growth pattern of HC differs with fetal sex in which male fetuses have a slower growth rate of HC then female fetuses (p < 0.001).

Regarding analyses assessing the effects of a suboptimal intrauterine environment, i.e. complicated versus uncomplicated pregnancies similar trends were observed with larger AC and HC and smaller FL in male fetuses compared to female fetuses (all p < 0.001). The only exception being AC in the complicated group, which did not differ between male and female fetuses in both the second and third trimester of pregnancy (respectively p = 0.11 and p = 0.46).

Effect of fetal sex on infant growth

Results of the repeated measurements in SD-scores are shown in **Figure 4.3 A – C**. Males have a smaller HC from 3 months onwards. The difference in HC increases with advancing age. The growth pattern of HC was significantly different between the two sexes (p = 0.01). Males have a larger body length compared with females. This was statistically significant from 9 months onwards. Although it seems that the difference between males and females increases with advancing age, the pattern in body length between the two sexes was not statistically significant (p = 0.46). For the pattern of body weight a cross-over was observed. At the age of 3 months and from 18 months onwards the difference in body weight was statistically different between males and females. Due to the cross-over body weight patterns were statistically different for females compared with males (p = 0.002).

Table 4.1 Baseline characteristics stratified by fetal sex

	Total	Females	Males	p-value
	N = 8556	n = 4230	n = 4326	
Maternal age (yrs)	29.6 (5.3)	29.6 (5.3)	29.7 (5.3)	0.49
Anthropometrics				
Height (cm)	167.5 (7.4)	167.4 (7.5)	167.6 (7.3)	0.25
Weight (kg)	66.3 (12.9)	66.4 (12.9)	66.1 (12.8)	0.32
BMI (kg/m2)	23.9 (19.4 - 33.8)	24.0 (19.4 - 33.9)	23.8 (19.3 - 33.7)	0.12
Ethnicity (%)				0.44
Western	4664 (57.5%)	2316 (57.9%)	2348 (57.1%)	
Non-Western	3447 (42.5%)	1682 (42.1%)	1765 (42.9%)	
Educational level (%)				0.74
Low	907 (11.6%)	462 (12.0%)	445 (11.3%)	
Middle	3627 (46.4%)	1782 (46.2%)	1845 (46.7%)	
High	3275 (41.9%)	1611 (41.8%)	1664 (42.1%)	
Smoking habits (%)				0.004
No	5452 (72.8%)	2740 (73.9%)	2712 (71.6%)	
Yes - stopped	642 (8.6%)	330 (8.9%)	312 (8.2%)	
Yes - continued	1399 (18.7%)	636 (17.2%)	763 (20.1%)	
Folic acid use (%)				0.11
No	1863 (29.3%)	899 (28.5%)	964 (30.2%)	
Before 10 weeks	1978 (31.1%)	971 (30.7%)	1007 (31.5%)	
Preconception start	2512 (39.5%)	1289 (40.8%)	1223 (38.3%)	
Nulliparous (%)	4718 (55.8%)	2353 (56.4%)	2365 (55.2%)	0.89
Gestational age at sonography (wks)				
First trimester	12.5 (11.1 - 14.5)	12.5 (11.1 - 14.4)	12.5 (11.1 - 14.6)	0.04
Second trimester	20.5 (18.9 - 22.9)	20.4 (18.8 - 22.8)	20.6 (18.9 - 23.0)	<0.001
Third trimester	30.3 (28.7 - 32.4)	30.3 (28.6 - 32.4)	30.4 (28.9 - 32.4)	<0.001
Gestational age at birth (wks)	40.1 (36.9 - 42.0)	40.1 (36.9 - 42.0)	40.1 (36.7 - 42.1)	0.001

Data are represented as n (%) or as the mean (SD) or as the median with the 90% range. Differences in baseline characteristics were tested using Student's t, Mann-Whitney U and Chi-square test.

Table 4.2 Linear regression analyses on biometrical indices

	AC (SDS)		HC (SDS)		FL (SDS)	
	β (95% CI)	p-value	β (95% CI)	p-value	β (95% CI)	p-value
Total						
2nd trimester						
Females	reference		reference		reference	
Males	0.09 (0.05;0.14)	< 0,001	0.30 (0.26;0.34)	< 0.001	-0.21 (-0.26;-0.17)	< 0.001
3rd trimester						
Females	reference		reference		reference	
Males	0.09 (0.05;0.13)	< 0,001	0.38 (0.34;0.42)	< 0.001	-0.21 (-0.26;-0.17)	< 0.001
Uncomplicated						
2nd trimester						
Females	reference		reference		reference	
Males	0.10 (0.05;0.15)	< 0,001	0.29 (0.25;0.34)	< 0.001	-0.21 (-0.25;-0.16)	< 0.001
3rd trimester						
Females	reference		reference		reference	
Males	0.12 (0.07;0.16)	< 0,001	0.38 (0.34;0.43)	< 0.001	-0.20 (-0.25;-0.15)	< 0.001
Complicated						
2nd trimester						
Females	reference		reference		reference	
Males	0.09 (-0.03;0.21)	0,16	0.38 (0.26;0.50)	< 0.001	-0.26 (-0.38;-0.13)	< 0.001
3rd trimester						
Females	reference		reference		reference	
Males	0.04 (-0.08;0.17]	0.49	0.42 (0.30;0.54)	< 0.001	-0.27 (-0.40;-0.15)	< 0.001

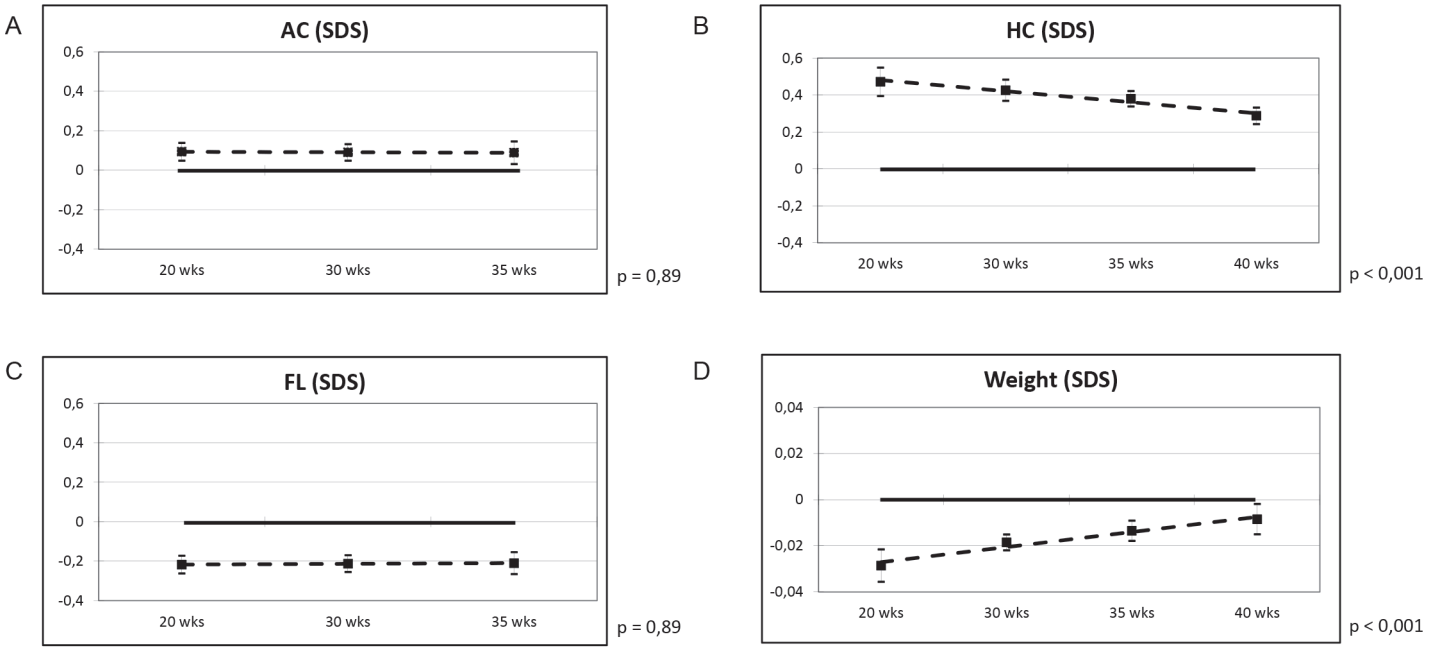
Values are regression coefficients (95% CI) and represent data in SD-score. Abbreviations: AC: abdominal circumference. HC: head circumference. FL: femur length.

Discussion

Main findings

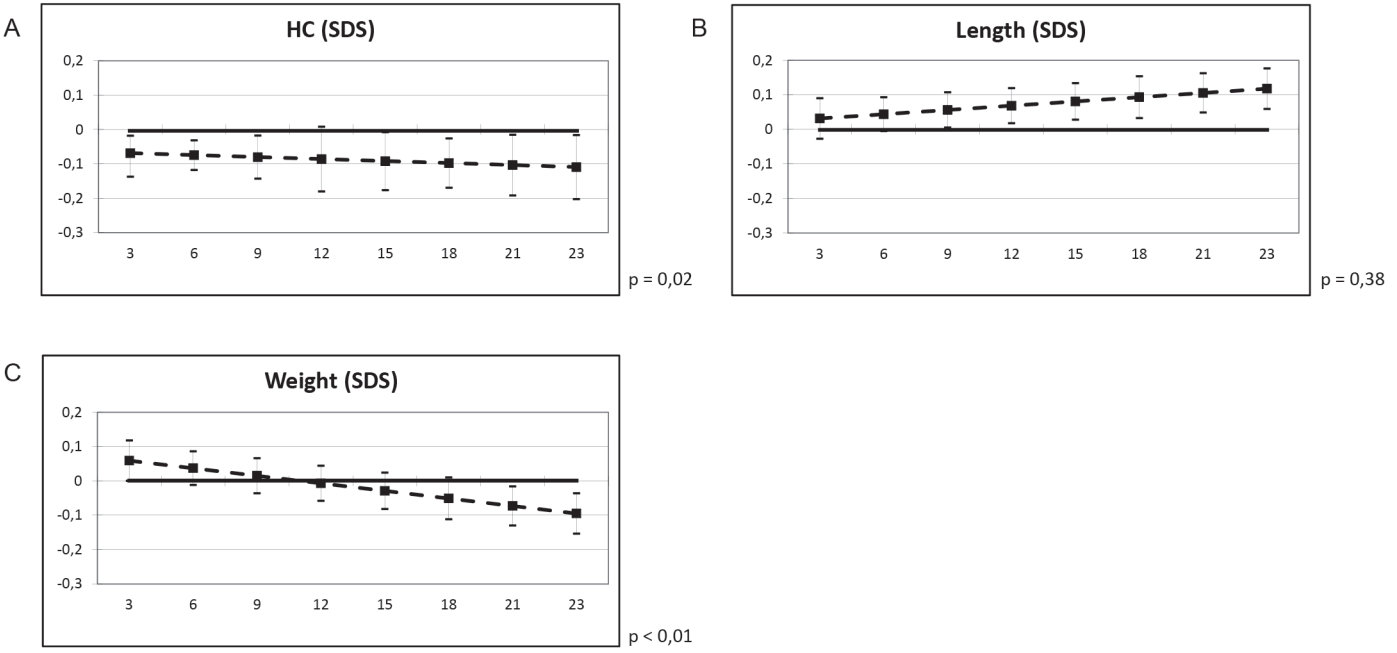
In this study we demonstrate fetal sex specific differences in fetal growth. These differences are already present from the first trimester of pregnancy onwards and track throughout pregnancy. Male and female fetuses do not only differ in weight, they also differ in biometric indices with a different body proportion as a consequence. Moreover, male fetuses follow a different growth pattern than female fetuses with a slower growth rate of the HC. The presence of PE, SGA or

Figure 4.2 Associations between sex and fetal growth – repeated measurements analyses adjusted for maternal smoking



Results from unbalanced repeated measurement regression analyses. Data are represented as gestational age and fetal sex adjusted SD scores with the 95% CI. Female fetal sex was used as a reference category. P-value represents the difference in growth pattern between male and female fetuses and is the p-value of the interactionterm sex * gestational age. Abbreviations: AC: abdominal circumference. HC: head circumference. FL: femur length. SDS: standard deviation score.

Figure 4.3 Associations between sex and infant growth – repeated measurements analyses



Results from unbalanced repeated measurements regression analyses. Data are represented as infant age and sex adjusted SD scores with the 95% CI. Female fetal sex was used as a reference category. P-value represents the difference in growth pattern between male and female infants and is the p-value of the interaction term sex * infant age. Abbreviations: HC: head circumference. SDS: standard deviation score.

sPTB does not affect this. During infancy the difference in HC growth patterns persists and a difference in weight patterns arises.

Interpretation

The Development and Origins of Health and Disease theory (DOHaD) states that in the case of adverse fetal exposure, the unborn fetus can modify its own development such that it will be prepared for survival in an environment in which resources are likely to be short. These early life adaptations help in survival by selecting an appropriate trajectory of growth in response to the environment. Although these adaptations may be beneficial for short term survival, they may have adverse consequences at birth or in later life (16, 17). The sex specific differences in body proportion and fetal growth shown in this study might therefore be one of the bases for sex differences shown in chronic diseases in later life. Especially since this study shows that sexual dimorphism in growth persists after birth until the age of two years. Little research has been done on differences in growth patterns between males and females. Some studies performed on a later age acknowledge differences in body proportions (18). Moreover peak velocity in growth differs between males and females since the timing of the beginning of puberty and therefore growth spurt is different (19, 20). This is one of the first studies showing that differences in growth patterns between males and females begin at a very early age.

Fetal sex specific differences in fetal growth and growth patterns may be explained by differences in placentation as previously suggested by our group (6). Maternal serum levels of s-Flt1, PIGF and PAI-2 were shown to be higher in the presence of a female fetus. However, Bouwland – Both et al. from our group showed positive associations between maternal sFlt-1, PIGF, plasminogen activator inhibitor-2 (PAI-2) in early pregnancy and CRL (21). According to these results one would expect that female CRL is increased compared with male CRL. However, this study showed that male embryos had a larger CRL as compared with females. Considering the effect of placental biomarkers on embryonic growth and the sex-specific differences in these biomarkers this conflicting result might be explained by effect modification. Hence, the effect of placental biomarkers on CRL is dependent on fetal sex. For this reason we performed additional interaction analyses showing that in a male embryo PAI-2 has a larger effect on CRL SD scores than in a female embryo (data not shown). Furthermore we added our data on placental biomarkers (first and second trimester s-Flt1 and PIGF) into the statistical models to test possible mediating effects of these biomarkers as a proxy for placentation (**Supplemental Table 4.1**). Although

results remained significant, several effect estimates changed with more than 10%. This implies an intermediate role for placental biomarkers. Moreover, extra-placental sources of these biomarkers exist. Previous studies show that s-Flt1 is also produced by maternal endothelial cells (22). These extra-placental sources could potentially contribute to differences in early embryonic growth since these biomarkers are associated with CRL.

Some studies state that fetal growth is the result of the availability of nutrients and therefore is mainly determined by placental function (23). However, until the 11th week of pregnancy cytotrophoblast plugs obliterate the tips of the utero-placental arteries preventing blood flow with the consequence that fetal growth is not dependent on hemotrophic nutrition during the first trimester. Hence, it is plausible that in early pregnancy not only placentation determines fetal growth but also other underlying factors such as intrinsic factors of the fetus (i.e. sex). We observed no differences between male and female fetal growth patterns in complicated and uncomplicated pregnancies. This may indicate that physiological placental regulatory mechanisms may be overruled by the pathophysiological sequelae in pregnancies complicated by PE, SGA and sPTB (6, 24).

Concerning biometrical indices little research has been performed focusing on fetal sex. One study reported on sex-specific antenatal growth charts (25). However, these growth charts were based on a population of 4234 women with just one antenatal measurement. Similar with our present study they showed larger HC and AC in male fetuses. In contrast with our results they showed that the difference between male and female HC increased with proceeding gestation. They did not show an effect of fetal sex on FL. Another study also assessed fetal sex specific differences in biometrical indices (26). They too found a larger HC in the case of a male fetus. This study had a smaller sample size of 427 measurements and analyses were performed cross-sectional.

In our study we observed a discrepancy in EFW and birth weight. Birth weight was higher in males, while EFW in the second and third trimester of pregnancy was higher in females. This inconsistency can either be explained by the applied formula estimating EFW or by the method used to determine gestational age of pregnancy. The Hadlock formula uses HC, AC, and FL to calculate the EFW. In our study male and female fetuses differed in body composition with different indices for male and female fetuses. The Hadlock formula could therefore be improved by adjusting for fetal sex as previously suggested by others (7, 27-

30). Melamed et al. constructed a fetal sex adjusted Hadlock formula which indeed showed a decrease in the systematic error (31). Secondly, to determine gestational age of pregnancy we used either first trimester CRL or BPD measurement. This study however shows that CRL of a male embryo is larger than that of a female embryo. A possible consequence is that male embryos were dated as being older what might explain why females have a higher EFW during second and third trimester. Lastly, we have to consider the possibility that the growth rate of female fetuses is relatively high in the second trimester while the growth rate of males is higher in the third trimester. This is confirmed by de Jong et al. who showed that the daily growth rate in the third trimester prior to birth was significantly higher for male fetuses (32). This would indeed result in a higher EFW in the second trimester but a lower birth weight for female fetuses as seen in this study.

Conclusion

In conclusion we can state that there are differences in fetal and infant growth between males and females. These findings help us in the understanding of the mechanism of growth which is not only important for birth outcome but also predisposes for possible adverse adult health. In clinical practice as well as future research concerning placentation and fetal development and growth, sex has to be taken into account.

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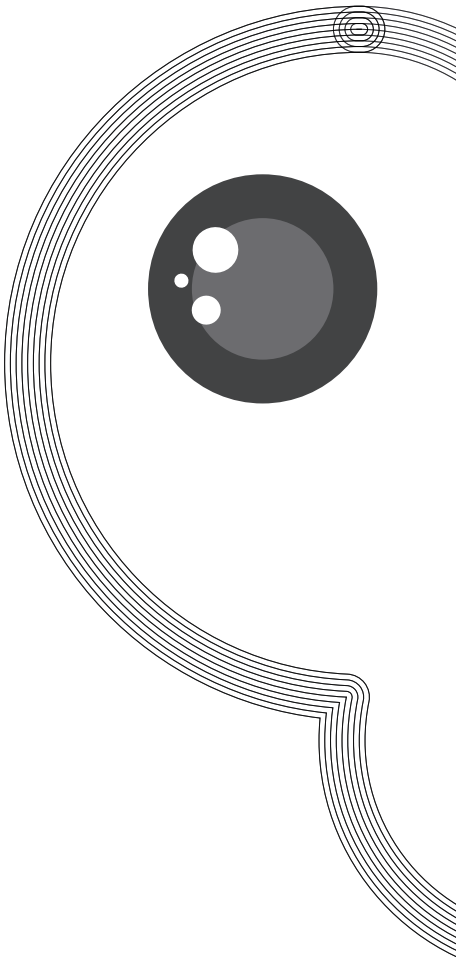
Table S.4.1 Adjusted linear regression analyses on fetal growth

	AC (SDS)			HC (SDS)			FL (SDS)		
	Model 1	**Model 2**	**Model 3**	**Model 1**	**Model 2**	**Model 3**	**Model 1**	**Model 2**	**Model 3**
Total									
2nd trimester									
Females	reference	reference	reference	reference	reference	reference	reference	reference	reference
Males	0.09 (0.04;0.13)***	0.10 (0.05;0.15)***	0.14 (0.09;0.19)***	0.30 (0.25;0.35)***	0.29 (0.24;0.34)***	0.36 (0.31;0.42)***	-0.22 (-0.27;-0.17)***	-0.21 (-0.26;-0.16)***	-0.14 (-0.19;-0.09)***
3rd trimester									
Females	reference	reference	reference	reference	reference	reference	reference	reference	reference
Males	0.09 (0.05;0.14)***	0.10 (0.05;0.15)***	0.11 (0.05;0.16)***	0.39 (0.34;0.43)***	0.41 (0.36;0.45)***	0.42 (0.36;0.47)***	-0.21 (-0.25;-0.16)***	-0.21 (-0.26;-0.16)***	-0.19 (-0.25;-0.14)***
Uncomplicated									
2nd trimester									
Females	reference	reference	reference	reference	reference	reference	reference	reference	reference
Males	0.08 (0.03;0.13)**	0.09 (0.04;0.15)***	0.14 (0.08;0.19)***	0.30 (0.25;0.35)***	0.29 (0.24;0.35)***	0.34 (0.28;0.40)***	-0.21 (-0.26;-0.16)***	-0.21 (-0.26;-0.15)***	-0.12 (-0.17;-0.06)***
3rd trimester									
Females	reference	reference	reference	reference	reference	reference	reference	reference	reference
Males	0.12 (0.07;0.16)***	0.11 (0.06;0.17)***	0.13 (0.07;0.19)***	0.39 (0.34;0.44)***	0.40 (0.35;0.45)***	0.42 (0.37;0.48)***	-0.20 (-0.25;-0.15)***	-0.21 (-0.26;-0.15)***	-0.18 (-0.23;-0.12)***

Table S.4.1 Continued

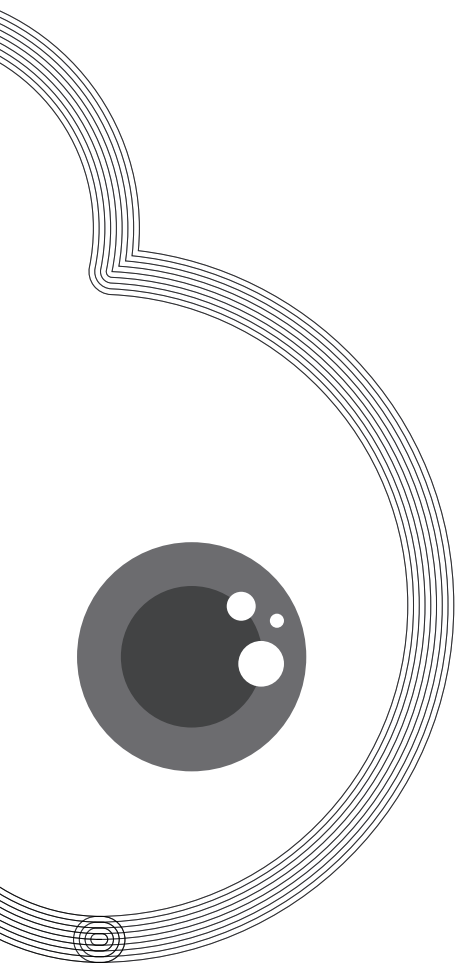
Complicated									
2nd trimester									
Females	reference	reference	reference	reference	reference	reference	reference	reference	reference
Males	0.12 (-0.01;0.25)	0.14 (-0.01;0.28)	0.18 (0.03;0.32)	0.36 (0.23;0.49)***	0.35 (0.20;0.49)***	0.49 (0.34;0.64)***	-0.26 (-0.40;-0.13)***	-0.22 (-0.37;-0.07)**	-0.20 (-0.35;-0.05)**
3rd trimester									
Females	reference	reference	reference	reference	reference	reference	reference	reference	reference
Males	0.07 (-0.07;0.21)	0.09 (-0.07;0.24)	0.09 (-0.07;0.29)	0.43 (0.30;0.55)***	0.46 (0.32;0.64)***	0.43 (0.28;0.59)***	-0.25 (-0.38;-0.13)***	-0.25 (-0.40;-0.11)***	-0.24 (-0.39;-0.09)**

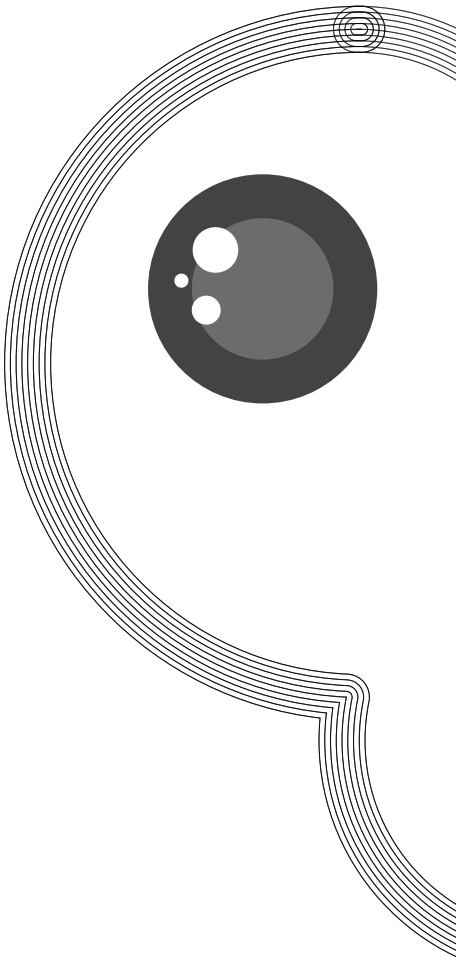
Values are regression coefficients (95% CI) and represent data in SD-score. Model 1: Adjusted for maternal smoking during pregnancy. Model 2: Adjusted for maternal smoking during pregnancy, folic acid intake, maternal ethnicity and parity. Model 3: Adjusted for first and second trimester PlGF and s-Flt1 plasma levels. * p < 0,05 ** p < 0,01 *** p < 0,001. Abbreviations: AC: abdominal circumference. HC: head circumference. FL: femur length.



Part III

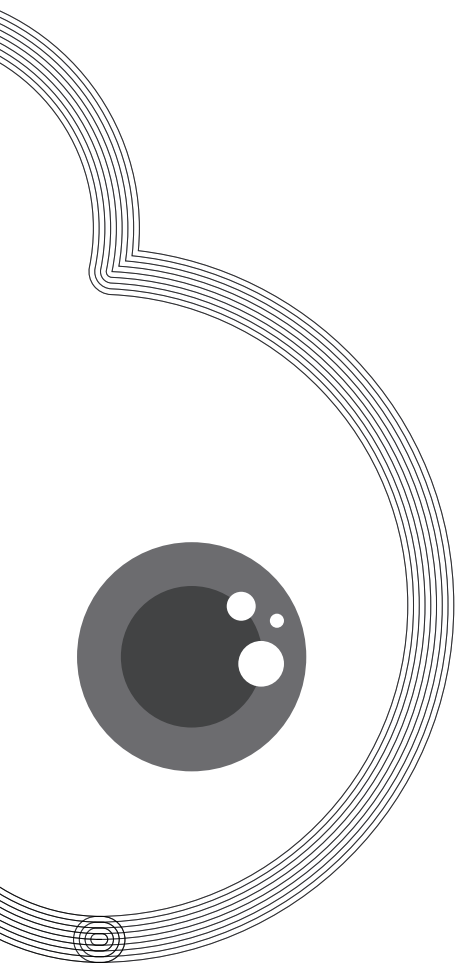
Maternal outcomes





Chapter 5

Fetal sex specific differences in human placentation



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Abstract

Introduction: Our objective was to assess fetal sex specific differences in first trimester placental biomarkers of both physiological and pathological pregnancies and their interaction with environmental influences. This study is embedded in the Generation R Study a, prospective cohort study.

Methods: Only live singleton births were included. Linear regression was performed to assess the effect of sex on first trimester placental biomarkers. Interaction analyses were performed to assess interaction of fetal sex with environmental influences. First trimester soluble fms-like tyrosine kinase (s-Flt1), placental growth factor (PlGF), plasminogen activator inhibitor (PAI-2) and homocysteine levels were assessed.

Results: Significant fetal sex specific differences in placental biomarkers were observed. S-Flt1, PAI-2 and PlGF log transformed concentrations were 0.08 ng/mL (95% CI 0.05;0.11), 0.07 ng/mL (95% CI 0.06;0.09) and 0.04 pg./mL (95% CI 0.01;0.06) higher in case of female as compared to male placentas. In pregnancies complicated by pre-eclampsia (PE), preterm birth (PTB) or a newborn being small for gestational age (SGA) no fetal sex specific differences were observed. Interaction analyses suggest that concentrations of s-Flt1, PlGF and PAI-2 decrease in male placentas in the case of hyperhomocysteinemia but remain equal in female placentas.

Conclusion: Fetal sex affects early placentation processes with discrepancies regarding pregnancies complicated by PE, PTB or a newborn being SGA. This suggests that other mechanisms causing these complications may dominate the fetal sex effect. The differences concerning homocysteine suggest that fetal sex dependent placental gene-environment interactions exist.

Introduction

Pregnancy induces several placental-mediated hemodynamic adaptations of maternal physiology to deal with the increased demands of the developing fetal-placental unit. Placental gene expression is known to be fetal sex specific (1, 2). Furthermore, earlier studies showed that the fetoplacental unit influences maternal physiology in a sex-specific manner with stronger expressions of placental cytokine mRNA among female placentas resulting in altered maternal asthma symptomatology between women carrying a female and women carrying a male fetus (3-5). Likewise, a different ratio of male to female fetus concerning miscarriages and pre-eclamptic pregnancies has led to the hypotheses that sex specific associations with respect to placentation exist (6). Given the knowledge that the placenta is affected by environmental influences that may modify epigenetic marks and gene expression and subsequently placental development and function (1, 7), we hypothesize that fetal sex specific alterations in early placentation exist, and that these alterations differ according to environmental influences. We examined sex specific differences among soluble fms-like tyrosine kinase 1 (s-Flt1), placental growth factor (PlGF) and plasminogen activator inhibitor-2 (PAI-2) representing important angiogenic and fibrinolytic factors implicated in placental development and function (8-10). Concerning environmental influences we assessed smoking, age, parity, folic acid use and educational level as a proxy of behavioural status. Furthermore we assessed homocysteine concentrations as a proxy for nutritional status. Homocysteine is an important substance regarding DNA methylation and crucial for placental development and functioning (11, 12).

Materials and methods

Study design

The study was embedded in The Generation R Study, a population-based prospective cohort study from early pregnancy onwards in Rotterdam, the Netherlands (13). The study has been approved by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam, the Netherlands. Written informed consent was obtained from all participants. Women were enrolled prenatally between 2001 and 2005 (n = 8880). For the present study, women with a live singleton birth were considered eligible (n = 8631). Only women of which at least one placental biomarker was assessed in the first trimester were included (n = 6040).

Birth outcomes

We firstly aimed to assess the fetal sex specific differences in the total study population. Secondly, we aimed to assess these differences in subgroups of our study population i.e. pregnancies complicated by the placental syndrome encompassing pre-eclampsia (PE, n = 125), spontaneous preterm birth (PTB, n = 221) and a newborn being small for gestational age (SGA, n = 609). PE was defined after the completion of the pregnancy according to the International Society for the Study of Hypertension in Pregnancy criteria (14). PE was defined as the novo hypertension (an absolute blood pressure of 140/90 mmHg or greater) after the 20th gestational week with concurrent proteinuria (0.3 g or greater in a 24-hour urine specimen or 2+ or greater [1g/L] on a voided specimen, or 1+ or greater [0.3 g/L] on a catheterized specimen). The occurrence of hypertension and hypertension related complications were cross validated using hospital registries (15). PTB was defined as a spontaneous onset of birth <37 weeks of gestation. SGA was defined as a birth weight under de 10th percentile based on our own cohort (16).

Placental biomarkers

Regarding markers of placentation, maternal non fasting venous blood samples were drawn in the first trimester (median 13.2 weeks of gestational age, 90% range 10.5 – 17.2 weeks). Details of processing procedures have been described previously (17, 18). Plasma s-Flt1 and PlGF concentrations were analyzed using an immunoelectrochemoluminence assay on the Architect System (Abbott Diagnostics B.V., Hoofddorp, the Netherlands) and plasma PAI-2 concentrations were determined by enzyme-linked immunosorbent assay. Serum homocysteine concentrations were analyzed using a microparticle-enhanced immunoassay on the AxSYM and Architect system. Data on maternal age, educational level, folic acid use smoking habits in early pregnancy, and parity were obtained through self-administered questionnaire at enrollment (response rate 93%)(13). Information on maternal outcomes, including PE, PTB and SGA was obtained from medical records, completed by community midwives and obstetricians.

Statistical analyses

To test differences in baseline characteristics between males and females Student's t, Mann-Whitney U and Chi-square tests were firstly performed. Second to assess fetal sex specific differences in s-Flt1, PlGF and PAI-2 concentrations Mann-Whitney U test was used as not all markers of placentation were normally distributed. Next, log transformation of s-Flt1, PlGF and PAI-2 concentrations were performed with subsequent linear regression analyses to relate the

placental biomarker concentrations to fetal sex, adjusted for the gestational age at sampling and placental weight. We tested fetal sex specific differences in markers of placentation in response to different environmental influences known to affect placentation. Effect modification was tested by multiplying fetal sex with the covariables maternal smoking, age, parity, educational level as a proxy of behavioural status and serum homocysteine concentrations as a proxy for nutritional status. If $p < 0.10$ was fulfilled linear regression analyses were performed in strata of that specific determinant for both sexes. All statistical analyses were performed using SPSS version 20.0 for Windows (IBM SPSS INC, Chicago, IL, USA).

Results

No differences between women carrying a male fetus and women carrying a female fetus concerning maternal age, ethnicity, education level, BMI at intake i.e. enrollment in the study, folic acid use, gravidity, parity, gestational age at birth and placental weight were observed (**Table 5.1**).

The non-adjusted plasma concentrations of s-Flt1, PlGF and PAI-2 are presented in **Table 5.2** in the first column of each biomarker. Associations between fetal sex and the plasma concentrations of the placental biomarkers in the first trimester of pregnancy are presented in the same table in the second column. In the total group consisting of 6040 participants, all placental biomarkers were higher in women carrying a female fetus compared with women carrying a male fetus. Subsequently, we stratified the total group into 4 groups: PE (n = 125), SGA (n = 609), PTB (n = 221) and women without the previous pregnancy complications (i.e. uncomplicated pregnancies, n = 5137). There were 52 women with more than one complication, these women contribute to both complication groups. In women with PE, SGA or PTB all fetal sex specific differences disappeared. In uncomplicated pregnancies all results were comparable with the total group i.e. all placental biomarkers were higher in women carrying a female fetus compared with women carrying a male fetus.

We did not find a fetal sex specific interaction concerning maternal smoking, folic acid use, and maternal age at intake, educational level and parity. However in the case of high homocysteine concentrations (> 97.7th percentile) interactions were found as demonstrated in **Figure 5.1**. In women carrying a male fetus plasma levels of PlGF and PAI-2 decreased in the case of hyperhomocysteinemia.

However, in women carrying a female fetus these effects were no longer present. Concerning plasma levels of s-Flt1 no effect modification was found.

Table 5.1 Baseline characteristics stratified by fetal gender

	Total	Males	Females	p-value
	N = 6040	n = 2986	n = 3054	
Maternal age (yrs)	29.8 (5.1)	29.8 (5.1)	29.7 (5.0)	0.33
< 20 (%)	204 (3.4%)	105 (3.4%)	99 (3.3%)	
20 – 30 (%)	2646 (43.8%)	1311 (42.9%)	1335 (44.7%)	
> 30 (%)	3190 (52.8%)	1638 (53.6%)	1552 (52.0%)	
Ethnicity				0.39
Western (%)	3582 (61.8%)	1801 (61.2%)	1781 (62.3%)	
Non-Western (%)	2218 (38.2%)	1141 (38.8%)	1077 (37.7%)	
Educational level				0.10
Low (%)	551 (9.8%)	262 (9.2%)	289 (10.4%)	
Mid (%)	2544 (45.3%)	1297 (45.7%)	1247 (45.0%)	
High (%)	2517 (44.9%)	1281 (45.1%)	1236 (44.6%)	
BMI at intake (kg/m^2)	23.5 (19.3-33.3)	23.5 (19.2 – 33.3)	23.6 (19.3 – 33.3)	0.29
< 20 (%)	594 (9.9%)	318 (10.5%)	276 (9.3%)	
20 – 25 (%)	3276 (54.6%)	1670 (55.0%)	1606 (54.1%)	
25 – 30 (%)	1462 (24.4%)	710 (23.4%)	752 (25.4%)	
> 30 (%)	671 (11.2%)	339 (11.2%)	332 (11.2%)	
Smoking habits				0.13
No	3869 (72.0%)	1941 (71.7%)	1928 (72.3%)	
Yes – stopped	503 (9.4%)	239 (8.8%)	264 (9.9%)	
Yes – continued	1002 (18.6%)	529 (19.5%)	473 (17.7%)	
Folic acid use				0.10
No	1155 (25.1%)	604 (26.0%)	551 (24.1%)	
Before 10 weeks	1468 (31.8%)	753 (32.4%)	715 (31.3%)	
Preconception start	1987 (43.1%)	966 (41.6%)	1021 (44.6%)	
Nulliparous (%)	3424 (57.2%)	1719 (56.7%)	1705 (57.6%))	0.88
Gestational age at birth (wks)	40.1 (36.9 – 42.0)	40.1 (37.0 – 42.1)	40.1 (36.9 – 42.0)	0.29
Birth weight (gr)	3419 (564)	3481 (570)	3355 (550)	<0.001
Placental weight (gr)	635 (148)	643 (150)	628 (145)	<0.01

Data are represented as n (%) or as the mean (SD) or as the median with the 90% range . Differences in baseline characteristics were tested using Student's t, Mann-Whitney U and Chi-Square tests.

Table 5.2 Associations between placental biomarkers in the first trimester of pregnancy and fetal sex

	Placental biomarkers					
	s-Flt1	**p-value**	**PlGF**	**p-value**	**PAI-2**	**p-value**
Total (N = 6040)						
Male (n = 3054)	4.9 (2.1 - 11.2)	<0.001	42.1 (17.1 - 157.4)	0.001	38.1 (19.3 - 70.8)	<0.001
Female (n = 2986)	5.2 (2.3 - 12.5)		43.9 (18.1 - 153.6)		41.4 (21.1 - 72.7)	
Pre-eclampsia (n = 125)						
Male (n = 58)	5.0 (1.7 - 10.7)	0.69	31.1 (10.6 - 205.2)	0.24	38.8 (16.7 - 68.9)	0.87
Female (n = 67)	5.0 (1.8 - 13.8)		37.5 (12.9 - 154.0)		40.4 (15.3 - 76.0)	
SGA (n = 609)						
Male (n = 310)	4.5 (1.9 - 12.0)	0.93	41.5 (14.9 - 162.8)	0.37	37.3 (18.5 - 65.9)	0.12
Female (n = 110)	4.5 (1.8 - 11.9)		42.3 (15.9 - 174.7)		40.5 (19.7 - 73.1)	
Preterm birth (n = 221)						
Male (n = 111)	4.7 (1.8 - 10.5)	0.4	37.5 (12.7 - 151.6)	0.85	37.8 (17.2 - 76.8)	0.35
Female (n = 110)	4.6 (2.2 - 11.5)		44.2 (17.0 - 202.5)		40.4 (23.6 - 75.7)	
Uncomplicated pregnancy (n = 5137)						
Male (n = 2601)	4.9 (2.2 - 12.1)	<0.001	42.4 (17.6 - 157.4)	0.004	38.3 (19.5 - 71.2)	<0.001
Female (n = 2536)	5.3 (2.4 - 12.5)		44.1 (18.5 - 150.9)		41.6 (21.6 - 72.4)	

Data are represented as the median with the 90% range. The p-value represents the result of the linear regression analyses, adjusted for gestational age at sampling.

Discussion

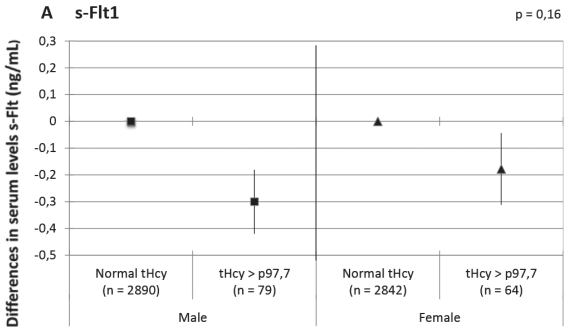
Main findings

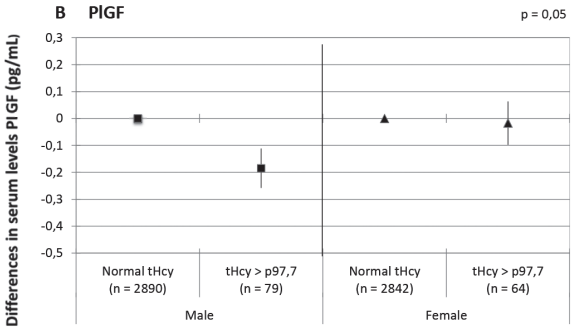
We demonstrate fetal sex related differences in biomarkers of early placentation among physiologic pregnancies as well as among pregnancies with complications belonging to the placental syndrome (19). We further show evidence of fetal sex specific placental gene-environmental interactions.

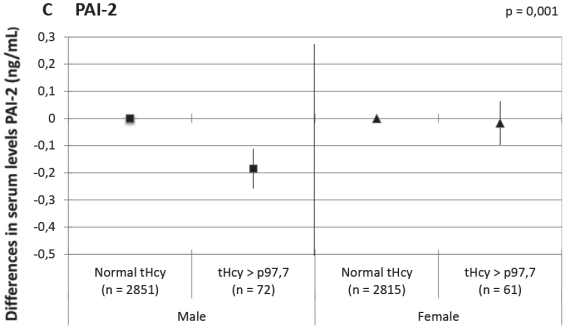
Interpretation

Normal human pregnancy involves important changes to maternal vascular function that allow for the large increase in blood flow to the fetoplacental unit (20). Abnormal vascular adaptations are associated with pregnancy complications including PE and SGA. The angiogenic and fibrinolytic growth

Figure 5.1 Associations between fetal sex and placental biomarkers stratified by hyperhomocysteinemia







Results from multiple linear regression analyses stratified per fetal sex / hyperhomocystein defined as > 97,7th percentile (> 12,3 µmol/L). The p-value represents the interactionterm in the regression model. Values are regression coefficients (95% Confidence Interval) and reflect the differences in plasma levels of s-Flt1, PAI-2 and PlGF, compared to reference. Since all placental biomarkers were not normally distributed, log transformation was applied. All values are adjusted for gestational age at sampling. Abbreviations: tHcy: homocystein.

factors PlGF, s-Flt1 and PAI-2 are well known for their associations with placental development and function and consequently placenta-mediated vascular adaptation to pregnancy (18). Previously it was demonstrated that maternal vascular function can be affected by the sex of the fetus and that this process varies in the presence of PE (1, 21). In normal physiologic pregnancies, maternal microvascular vasodilatation induced by placentally derived corticotrophin releasing hormone is enhanced in women with a male fetus compared to women with a female fetus (21). However, in pre-eclamptic pregnancies this process seems to be different with reduced microvascular vasodilatation in women pregnant with a male fetus compared to normotensive women pregnant with a male fetus. Importantly, in women pregnant with a female fetus, no differences are observed (1). As changes in maternal vascular function are associated with changes in placental metabolic function, these results are in line with our data showing not only that the placental release of circulating angiogenic and fibrinolytic factors is influenced by fetal sex, but also that these fetal sex associated differences in angiogenic and fibrinolytic factors alter in pregnancies complicated by the placental syndrome, including PE, SGA and PTB. Secondly, concerning the fact that we observed different associations in complicated versus uncomplicated pregnancies we know that placentation in the case of PE, SGA or PTB in the first trimester is already altered compared with an uncomplicated pregnancy (20). Impaired trophoblast invasion of myometrial arteries and poor spiral artery adaptation are some of the pathophysiological features preceding the placental syndrome (22). First trimester placental oxidative stress with an imbalance in cytokines and other inflammatory factors may be related to this (23). We hypothesize that due to this imbalance, the influence of fetal sex in pregnancies complicated by PE, SGA or PTB, is dominated and therefore not detectable anymore. Though the finding that the fetal sex specific differences in those pregnancies complicated with the placental syndrome are not statistical significant, could also be due to limited sample size compared with the uncomplicated pregnancies.

Lastly, we also have to take into account that other extra-placental sources exist of the investigated placental biomarkers. Previous studies show that s-Flt1 is also produced by endothelial cells and monocytes (24). These extra-placental sources of s-Flt1 could potentially contribute to the pathophysiology of PE. This is in line with other studies suggesting that plasma s-Flt1 levels are increased in postpartum women with a history of PE (25). Also PlGF is expressed in extra-placental tissues that are associated with a high grade of vascularization and cell growth such as tumorous tissue.

Concerning the prediction of pregnancy complication, a number of candidate biomarkers are currently under investigation, including s-Flt1 and PlGF. Numerous studies have consistently demonstrated elevated plasma levels of s-Flt1 in women with PE, even detectable up to 5 weeks before the onset of clinical symptoms and studies exploring the predictive value are currently being performed (26, 27). Likewise a decrease in plasma level of PlGF is seen and the potential of a predictive test is currently being assessed (28, 29). Given the results of this study, namely that fetal sex specific differences in these biomarkers exist, a new study exploring adding on the extra predictive value would be interesting. Though the design of our study does not enable us to perform such studies we performed some additional analyses and tried to explore this partly. We only found a fetal sex dependent decrease in plasma s-Flt1 levels in a SGA pregnancy, in which s-Flt1 decreases in the case of a female placenta and remains equal in the case of a male placenta (**Supplemental Table S.5.1** and **Supplemental Figure S.5.1**).

It is well known that the placenta can be affected by numerous environmental influences. These environmental influences may modify epigenetic marks and gene expression within the placenta and subsequent placental development and function (30). Studies focused on sex-specific effects of the Dutch hunger winter showed that changes in maternal diet during pregnancy can alter placental size with maternal under nutrition in early gestation resulting in a smaller placenta with the decrease in placental size being greater for males than for females (31). Gabory et al. further showed a sexually dimorphic basal placental gene expression in response to maternal diet with sex-specific alterations at CpG site throughout the genome (1).
Homocysteine is involved in cytotrophoblast apoptosis and thereby crucial for placental development (11). Higher concentrations of homocysteine have been associated with placental vasculopathy (18). Homocysteine is also an important key substance regarding DNA methylation. It is formed during the methionine cycle and is involved in the one carbon methyl group transfer metabolism where homocysteine acts as a methyl donor when it is converted to S-adenosyl-methionine. Previously, Fryer and colleagues reported a significant correlation between plasma total homocysteine and DNA methylation at numerous CpG sites throughout the genome, together with the dysregulation of clusters of imprinted genes (12). Female placental biomarkers were not affected by hyperhomocysteinemia while male placental biomarkers were. This might suggest that, when it concerns hyperhomocysteinemia, female placentas are more protected against the adverse effects of hyperhomocysteinemia. This is in line with above mentioned literature and the first to show that male and female

placentas have different mechanisms to cope with differential influences such as high homocysteine concentrations.

Strengths and limitations

This is the first study to address fetal sex specific differences in first trimester markers of placentation. The study was embedded in a large population-based prospective cohort study with an extensive data collection on both first trimester biomarkers as well as environmental influences. For our purpose a large sample size of 6040 participants was available. The response at baseline for participation in the Generation R cohort was 61%. The response rate reflects the number of children born to mothers living in the study area on their delivery date and participating in the study as a percentage of the total number of children born to mothers who fulfilled these eligibility criteria. Selective participation in this study did occur since pregnant women who participated were generally higher educated, more healthy, and more frequently of European origin than those who did not participate (13). This may have led to selection of a more affluent and relatively healthy population and might have affected the generalizability of our results.

Concerning our choice to use educational level as a proxy for behavioural status and homocysteine levels as proxy for nutritional status; earlier literature describes that educational level is an important determinant of employment and economic circumstances, and thus reflects material recourses. It also reflects non-economic social characteristics such as general and health-related knowledge which influences health behaviour (32). We therefore opted for educational level as a proxy for behavioural status. Concerning homocysteine levels it is well known that hyperhomocysteinemia can result from a suboptimal nutritional status. Shortage of B vitamins, increases homocysteine levels (33). Previous studies have found associations between serum levels of homocysteine and dietary intake (34). We therefore chose homocysteine levels as a proxy for nutritional status.

Conclusion

In conclusion we can state that there are sex specific differences in placental biomarkers that are measurable already in the first trimester of pregnancy. However, these differences are only detectable in physiological pregnancies. This suggests that in the case of the placental syndrome other mechanisms may

dominate the effect of fetal sex on placentation and placental function. As the success of early placentation mediates mechanisms of human hemodynamic adaptation to pregnancy as well as fetal growth, our results highlight again the importance of fetal sex as independent factor when studying placenta-mediated-diseases such as PE and SGA. In the future, these pregnancy complications may be investigated in a fetal sex specific way in order to provide specific interventions and recommendations. Moreover the placental syndrome is associated with adult cardiovascular disease of the child (35). The sex specific differences found in this study may be relevant to the pathogeneses of sex specific cardiovascular morbidity and mortality in later adult life.

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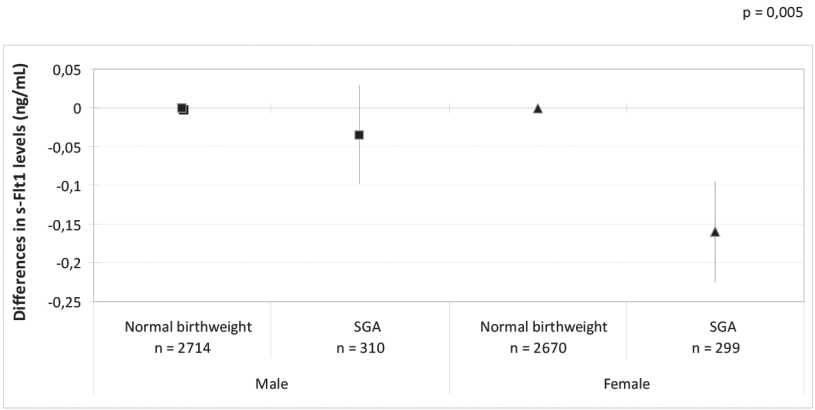
Figure S.5.1 Associations between fetal sex and plasma level s-Flt1 stratified by birthweight



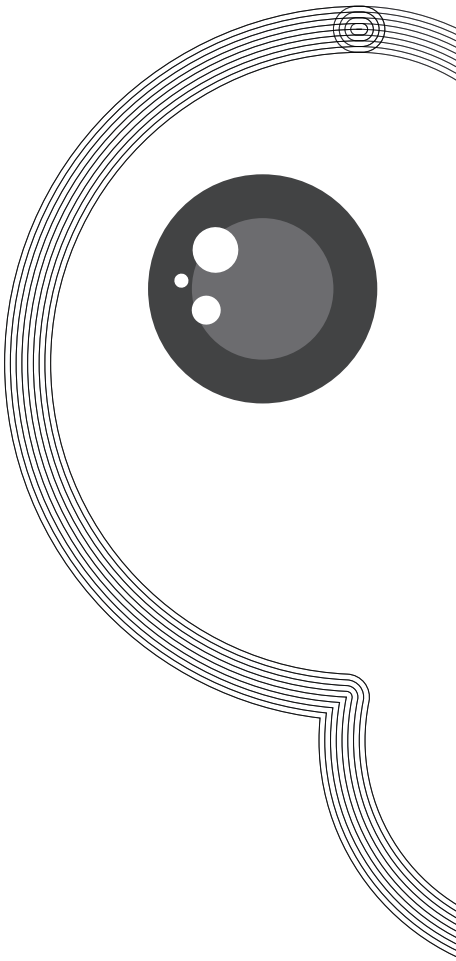
Results from multiple linear regression analyses stratified per fetal sex / SGA defined as birthweigh <10th percentile. The p-value represents the interactionterm in the regression model. Values are regression coefficients (95% Confidence Interval) and reflect the differences in plasma levels of s-Flt1, compared to reference. Since s-Flt1 was not normally distributed, log transformation was applied. All values are adjusted for gestational age at sampling. Abbreviations: SGA: small for gestational age.

Table S.5.1 Placental biomarkers stratified by pregnancy complication

	Males			
	Uncomplicated	**PE**	**SGA**	**PTB**
s-Flt1	4.9 (2.2 - 11.1)	5.0 (1.7 - 10.7)	4.5 (1.9 - 12.0)	4.7 (1.8 - 10.5)
PlGF	42.4 (17.6 - 157.4)	29.9 (10.6 - 155.2)**	41.5 (14.9 - 162.8)	37.5 (12.7 - 151.6)
PAI-2	38.3 (19.5 - 71.2)	38.8 (16.7 - 68.9)	37.3 (18.5 - 65.9)	37.8 (17.2 - 76.8)

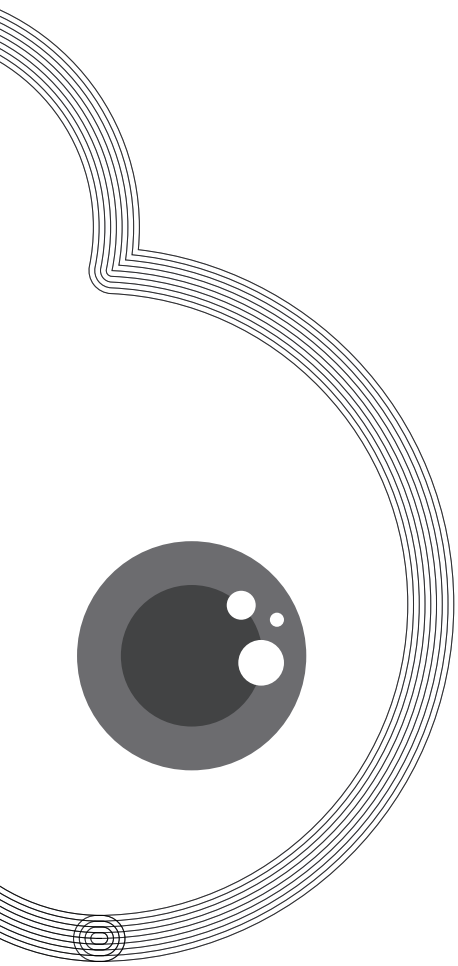
	Females			
	Uncomplicated	**PE**	**SGA**	**PTB**
s-Flt1	5.3 (2.4 - 12.5)	5.0 (1.8 - 13.8)	4.5 (1.8 - 11.9)***	4.6 (2.2 - 11.5)**
PlGF	44.1 (18.5 - 150.9)	37.5 (12.9 - 154.0)*	42.3 (15.9 - 174.7)	44.2 (17.0 - 202.5)
PAI-2	41.6 (21.6 - 72.4)	40.4 (15.3 - 76.0)	40.5 (19.7 - 73.1)	40.4 (23.6 - 75.7)

All presented values are medians with the 90% range. Using the Mann-Whitney U test, the plasma levels are compared with the uncomplicated group within the same gender. * $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$. Abbreviations: PE: pre-eclmapsia. SGA: small for gestational age. PTB: preterm birth.



Chapter 6

Fetal sex dependency of maternal vascular adaptation to pregnancy



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Abstract

Introduction: We aim to investigate fetal sex dependency of maternal vascular adaptation to pregnancy as assessed by uteroplacental vascular resistance and maternal blood pressure.

Methods: This prospective population-based cohort study was performed in Rotterdam, the Netherlands. In total, 8224 live singleton pregnancies were included. Maternal vascular adaptation was assessed in all trimesters of pregnancy. Pregnancies were stratified into being either complicated by the placental syndrome (i.e. pre-eclampsia, fetal growth restriction or preterm birth, n = 1229) or uncomplicated (n = 6995). Blood pressures, pulsatility index of the uterine artery (PI-UtA) and presence of notching in the uterine artery were assessed.

Results: In women carrying a male fetus PI-UtA was higher than in women with a female fetus in the total group (second trimester $p < 0.001$, third trimester $p = 0.005$). Effect estimates differed between subjects with or without the placental syndrome. In the total group, women with a male fetus showed more often notching in the Doppler resistance pattern (OR 1.42, 95% CI 1.17;1.72). Different blood pressure patterns were observed between pregnant women with a male fetus and pregnant women with a female fetus and between complicated pregnancies and uncomplicated pregnancies.

Conclusion: Fetal sex is significantly associated with maternal vascular adaptation to pregnancy with differential effects in uncomplicated pregnancies and in pregnancies complicated by the placental syndrome.

Introduction

Normal human pregnancy is characterized by a generalized state of vasodilatation and a depressed response to vasoconstrictors (1). The vascular endothelial growth factor family of angiogenic growth factors, which includes placental growth factor (PlGF) and the fms-like-tyrosine-kinase 1 receptor (sFlt-1), are important in the regulation of placental angiogenesis and maternal spiral artery remodeling. In addition sFlt-1, a splice variant of the Flt-1 receptor, is expressed in the placenta with known anti-angiogenic properties. This prevents vascular endothelial growth factor from catalyzing endothelial NO synthase and thereby reducing the concentration of PlGF.

When vascular remodeling is impaired, excess hypoxia is induced. This results in the suboptimal intra-uterine circumstances seen in three types of pregnancy complications: pre-eclampsia, fetal growth restriction and spontaneous preterm birth which together represent the "placental syndrome" (2-5). Both sFlt-1 and PlGF are correlated with the placental syndrome (6-8).

Previous research by our group identified clear fetal sex specific differences in maternal first trimester sFlt-1 and PlGF concentrations which seem to vary according to the presence or absence of the placental syndrome (9). On this basis, we hypothesized that placental function differs according to fetal sex, subsequently having differential effects on maternal vascular adaptation to pregnancy.

As uterine artery (UtA) Doppler abnormalities suggest increased impedance to flow in the uterine circulation and failure of physiologic transformation of the spiral arteries, we investigated the effects of fetal sex on UtA Doppler measurements in the second and third trimesters of pregnancy. Second, we determined fetal sex specific associations with maternal blood pressure. Lastly, we examined the specific effects of the presence or absence of the placental syndrome on these associations.

Material and methods

Study design

The study was embedded in The Generation R Study, a population-based prospective cohort study from early pregnancy onwards (10). The study was approved by the Medical Ethics Committee of the Erasmus Medical Center Rotterdam, the Netherlands. Written informed consent was obtained from all participants. For the present study we included women with a live born singleton with available information regarding the presence or absence of the placental syndrome (encompassing pre-eclampsia, fetal growth restriction and/or spontaneous preterm birth) (n = 8633). Women with chronic hypertension, hypercholesterolaemia, diabetes mellitus, systemic lupus erythematosus and/or thyroid disorders were excluded (n = 409). This resulted in a final population for analysis of n = 8224 (**Figure 6.1**).

Uterine artery Doppler measurements

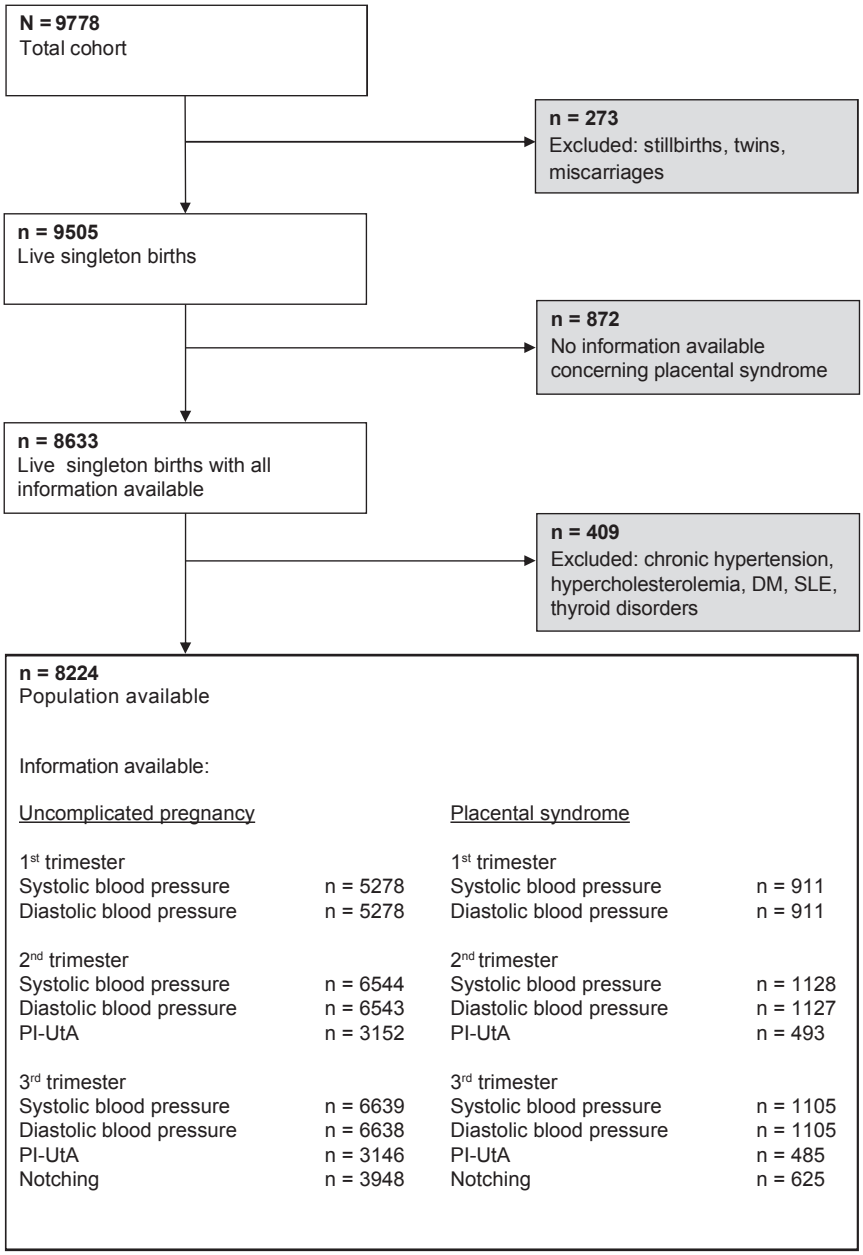
Doppler measurements of UtA were performed in the second (median 20.5 weeks, 90% range 18.9 – 22.9) and third (median 30.4 weeks, 90% range 28.8 – 32.9) trimester of pregnancy (11, 12). We measured the pulsatility index (PI) of left and right UtA near the crossover with the left or right external iliac artery. For each measurement, three consecutive uniform wave forms were recorded by pulsed Doppler ultrasound. The mean of three measurements and the mean of the left and right UtA was used for further analysis. Since PI-UtA varies with gestational age we constructed gestational age adjusted Z-scores. These Z-scores were used in all regression models. The presence of notching in flow velocity wave forms was also assessed in the third trimester. If present at the left or right side, presence was stated as 'yes'. If present at both sides, presence was stated as 'bilateral'. Ultrasound measurements were performed in a blinded fashion with regard to previous measurements and pregnancy outcomes. UtA Doppler measurements were performed at two of our three research centers. For this reason, PI-UtA measurements are available in a subgroup of women (n = 4574).

Blood pressure

Systolic and diastolic blood pressures (respectively SBP and DBP) were measured for each participant in all three trimester of pregnancy, with the validated Omron 907® automated digital oscillometric sphygmanometer (OMRON Healthcare Europe B.V. Hoofddorp, the Netherlands). All women were seated in upright position with back support and were asked to relax for 5 minutes. A cuff was placed around the nondominant upper arm. The arm was supported at the level

of the heart with the bladder midline over the brachial artery pulsation. In case of an upper arm exceeding 33 cm a larger cuff (32 - 42 cm) was used. The mean value of two blood pressure readings over a 60 second interval was documented.

Figure 6.1 Flowchart



The placental syndrome

Information on pre-eclampsia was obtained from medical records filled out by midwifes or obstetricians. For women who had suspected pre-eclampsia these records were cross-checked with the original hospital charts (13). Pre-eclampsia was defined as the development of SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg after 20 weeks of gestation plus the presence of proteinuria (≥ 0.3 g in a 24-hour urine specimen or ≥ 2 + [1 g/L] on a voided specimen, or ≥ 1 + [0.3 g/L] on a catheterized specimen) in previously normotensive women (14). Gestational age at birth, birth weight and fetal sex were obtained from midwife and hospital registries. Spontaneous preterm birth was defined as the spontaneous onset of birth before 37 weeks of gestation. Fetal growth restriction was defined as a gestational age and fetal sex adjusted birth weight below the 10th percentile (15).

Statistical analyses

First, to test differences in baseline characteristics between women carrying male and female fetuses Student's *t*, Mann-Whitney U and Chi-square tests were performed. Second, cross-sectional linear regression analyses were performed to relate fetal sex to PI-UtA in the second and third trimesters of pregnancy. Third, to analyse the associations of fetal sex with the presence of notching in the UtA, simple logistic regression models were used. Then, to explore blood pressure trajectories between male and female fetuses repeated measurement regression models were performed using the mixed model procedure with maternal blood pressure as repeated outcome measure. These models take the correlation between repeated measurements of the same subject into account. The best fitting models were constructed using fractional polynomials of gestational age (16). The models for SBP was as follows: $SBP = \beta_0 + \beta_1 * fetal\ sex + \beta_2 * gestational\ age + \beta_3 * gestational\ age^{-2} * fetal\ sex * gestational\ age.$ For DBP the next model was used: $DBP = \beta_0 + \beta_1 * fetal\ sex + \beta_2 * gestational\ age + \beta_3 * gestational\ age^{-5} * fetal\ sex * gestational\ age.$ Finally, mediation and interaction analyses for the covariate smoking were performed. In a mediation analyses the percentage change of the effect estimate was calculated using a bootstrap method with 1000 resamplings (17, 18). Interaction analyses were performed by multiplying fetal sex with the variable smoking. A p-value below 0.10 was considered statistically significant. Finally, all analyses were repeated after stratification into the presence or absence of the placental syndrome. Statistical analyses were performed using Statistical Package of Social Sciences version 21.0 for Windows (SPSS Inc. Chicago, IL, USA) , the Statistical Analysis System version 9.3 (SAS, Institute Inc. Gary NC, USA) and R version 3.0.0 (libraries meta and metaphor; The R foundation for Statistical Computing).

Results

No differences between women carrying a male or female fetus concerning maternal age, ethnicity, education, BMI, folic acid use and parity were observed (**Table 6.1**). Women carrying a male fetus smoked more often. Male fetuses had a higher birth weight compared to female fetuses.

Table 6.1 Baseline characteristics stratified by fetal sex

	Total	Males	Females	p-value
	N = 8224	n = 4157	n = 4067	
Maternal age (yrs)	29.6 (5.3)	29.6 (5.3)	29.5 (5.3)	0.31
< 20 (%)	361 (4.4%)	172 (4.1%)	189 (4.6%)	
20 - 30 (%)	3662 (44.5%)	1837 (44.2%)	1824 (44.9%)	
> 30 (%)	4200 (51.1%)	2147 (51.7%)	2053 (50.5%)	
Ethnicity (%)				0.33
Western	4443 (57.3%)	2232 (56.8%)	2210 (57.8%)	
Non-Western	3313 (42.7%)	1701 (43.2%)	1611 (42.2%)	
Educational level (%)				0.62
Low	441 (6.3%)	223 (6.3%)	218 (6.3%)	
Mid	3067 (43.6%)	1532 (43.0%)	1534 (44.1%)	
High	3533 (50.2%)	1808 (50.7%)	1725 (49.6%)	
BMI at intake (kg/m2)	23.8 (19.4 - 33.3)	23.8 (19.3 - 33.3)	23.9 (19.4 - 33.4)	0.18
< 20 (%)	728 (8.9%)	385 (9.3%)	343 (8.5%)	
20 - 25 (%)	4274 (52.4%)	2176 (52.7%)	2097 (52.0%)	
25 - 30 (%)	2162 (26.5%)	1062 (25.7%)	1100 (27.3%)	
> 30 (%)	994 (12.2%)	503 (12.2%)	491 (12.2%)	
Smoking habits (%)				0.008
No	5181 (72.6%)	2584 (71.6%)	2596 (73.6%)	
Yes - stopped	611 (8.6%)	295 (8.2%)	315 (8.9%)	
Yes - continued	1344 (18.8%)	730 (20.2%)	614 (17.4%)	
Folic acid use (%)				0.07
No	1786 (29.5%)	926 (30.4%)	859 (28.6%)	
Before 10 weeks	1875 (31.0%)	959 (31.5%)	916 (30.5%)	
Preconception start	2384 (39.4%)	1157 (38.0%)	1227 (40.9%)	
Nulliparous (%)	4529 (55.8%)	2265 (55.1%)	2263 (56.5%)	0.84
Gestational age at birth (wks)	40.1 (36.9 - 42.0)	40.1 (36.9 - 42.1)	40.2 (36.9 - 42.00)	0.001
Birth weight (gr)	3413 (558)	3476 (572)	3348 (536)	<0.001

Data are represented as the mean (SD) or as the median with the 90% range. Differences in baseline characteristics were tested using Student's t, Mann-Whitney U and Chi-square tests.

Uterine artery Doppler measurements

Second trimester UtA-PI was higher in women carrying a male fetus (**Table 6.2**). After stratification into the presence or absence of the placental syndrome, similar results were observed in both groups with a trend towards a higher UtA in women pregnant with a male fetus compared to women pregnant with a female fetus in the second trimester and third trimester of pregnancy. As women with a male fetus tended to smoke more often, an additional analysis was performed with adjustment for smoking. All effect estimates increased but the conclusions remained the same. The presence of a male fetus was associated with a higher frequency of notching of the uterine artery (**Table 6.3**).

Blood pressure

Differential blood pressure patterns were observed between women carrying a male fetus and women carrying a female fetus as well as between uncomplicated pregnancies and pregnancies complicated by the placental syndrome (**Figure 6.2**). In uncomplicated pregnancies a significant different SBP ($p = 0.03$) but not DBP ($p = 0.65$) pattern was observed for women with a male fetus compared to women with a female fetus, with a cross-over in the SBP pattern at the end of the second trimester. In pregnancies that were complicated by the placental syndrome a significantly different DBP ($p = 0.003$) pattern was seen between women carrying a male and women carrying a female fetus. At the beginning of pregnancy, women with a male fetus had a lower DBP compared to women with a female fetus. At the end of the second trimester in women with a complicated pregnancy a cross-over in DBP was observed with higher DBPs for women with a male fetus compared to women with a female fetus at the end of term.

The mediation and interaction analyses on the covariate maternal smoking were both not significant, indicating that smoking behavior is neither a confounding factor nor a mediator of the effect of fetal sex on maternal vascular adaptation.

Table 6.2 Regression analyses of the pulsatility index and notching of the uterine artery

		Second trimester PI-UtA			
		Crude		Adjusted	
	Mean (SD)	β (95% CI)	p-value	β (95% CI)	p-value
Total group					
Male (n = 1834)	0.07 (1.01)	0.136 (0.071;0.201)	<0.001	0.156 (0.086;0.226)	<0.001
Female (n = 1791)	-0.07 (0.98)	ref		ref	
Uncomplicated					
Male (n = 1576)	0.00 (0.96)	0.106 (0.040;0.172)	0.002	0.116 (0.044;0.187)	0.001
Female (n = 1561)	-0.11 (0.93)	ref		ref	
Placental syndrome					
Male (n = 258)	0.48 (1.24)	0.294 (0.076;0.513)	0.009	0.379 (0.145;0.613)	0.002
Female (n = 230)	0.18 (1.22)	ref		ref	
		Third trimester PI-UtA			
		Crude		Adjusted	
	Mean (SD)	β (95% CI)	p-value	β (95% CI)	p-value
Total group					
Male (n = 1797)	0.06 (1.02)	0.110 (0.045;0.175)	0.001	0.147 (0.078;0.216)	<0.001
Female (n = 1830)	-0.05 (0.97)	ref		ref	
Uncomplicated					
Male (n = 1565)	-0.03 (0.92)	0.097 (0.035;0.160)	0.002	0.120 (0.052;0.187)	<0.001
Female (n = 1577)	-0.12 (0.87)	ref		ref	
Placental syndrome					
Male (n = 232)	0.62 (1.40)	0.233 (-0.017;0.483)	0.07	0.338 (0.073;0.602)	0.01
Female (n = 253)	0.38 (1.40)	ref		ref	

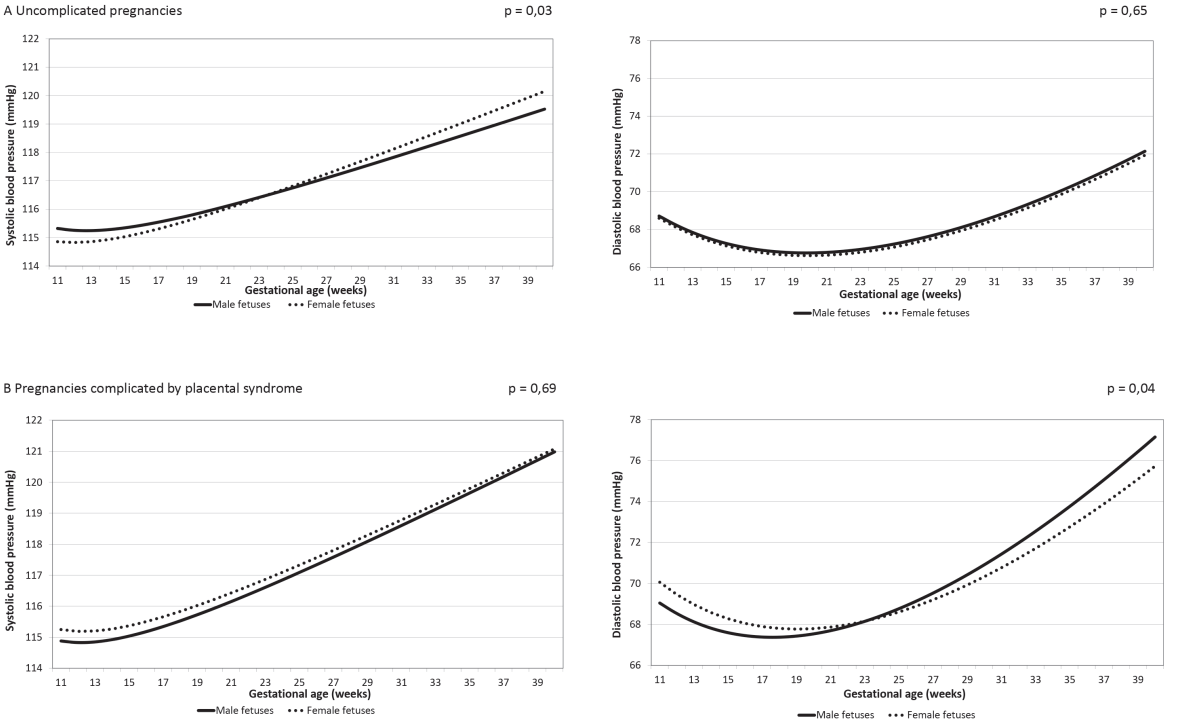
The uterine artery pulsatility index is represented as a gestational age adjusted Z-score. Data are represented as the mean with the standard deviation or as the difference compared to the reference (ref) category with the 95% CI. Crude: unadjusted. Adjusted: adjusted for maternal smoking. The p-value represents the result of the linear regression analyses. Abbreviations: PI-UtA: pulsatility index of the uterine artery

Table 6.3 Logistic regression analyses of notching of the uterine artery

Present yes / no				
	crude OR (95% CI)	**adjusted OR (95% CI)**	**change OR (%)**	**p-value**
Total group (N = 4573)				
Male (n = 2303)	1.42 (1.17;1.72)	1.50 (1.17;1.93)	5.6%	0.001
Female (n = 2270)	ref			
Uncomplicated (n = 3948)				
Male (n = 1985)	1.36 (1.08;1.71)	1.58 (1.17;2.13)	16.2%	0.003
Female (n = 1963)	ref			
Placental syndrome (n = 625)				
Male (n = 318)	1.61 (1.10;2.35)	1.51 (0.94;2.43)	6.2%	0.09
Female (n = 307)	ref			
Bilateral yes / no				
	crude OR (95% CI)	**adjusted OR (95% CI)**	**change OR (%)**	**p-value**
Total group (N = 4573)				
Male (n = 2303)	1.76 (1.25;2.49)	2.03 (1.32;3.11)	15.3%	0.001
Female (n = 2270)	ref			
Uncomplicated (n = 3948)				
Male (n = 1985)	1.83 (1.17;2.88)	2.43 (1.34;4.39)	32.8%	0.003
Female (n = 1963)	ref			
Placental syndrome (n = 625)				
Male (n = 318)	1.66 (0.95;2.90)	1.74 (0.91;3.34)	4.8%	0.09
Female (n = 307)	ref			

Data are represented as the odds ratio (OR) with the 95% CI. All analyses were adjusted for gestational age at the time of measurement.

Figure 6.2 Repeated measurements on blood pressure



Change in systolic and diastolic blood pressure in mmHg per fetal seks category based on repeated measurements analyses. Systolic blood pressure = $\beta_0 + \beta_1 * \text{fetal sex} + \beta_2 * \text{gestational age} + \beta_3 * \text{fetal sex} * \text{gestational age} + \beta_4 * \text{gestational age}^{-2}$. Diastolic blood pressure = $\beta_0 + \beta_1 * \text{fetal sex} + \beta_2 * \text{gestational age} + \beta_3 * \text{fetal sex} * \text{gestational age} + \beta_4 * \text{gestational age}^{0,5}$. Abbreviations: GA = gestational age in weeks.

Discussion

Main findings

This study shows that there are fetal sex specific differences in maternal vascular adaptation to pregnancy. This is shown by significant differences in Doppler measurements of the UtA and differences in SBP and DBP patterns with differential effects according to the presence or absence of the placental syndrome.

Strengths and limitations

The main strength of this study is its prospective data collection from early pregnancy onwards. Sample size was large, involving data of 8224 participants. Doppler measurements of the UtA were performed in a subgroup of enrolled women (n = 5819). Non-response analyses showed that women without UtA Doppler measurements tended to have a lower level of education, to be of non-European descent and to have a higher BMI (19). Women without UtA Doppler measurements were also more likely to have had adverse pregnancy outcomes. If the selection mechanisms had been related to both determinant and outcome, this may have led to biased effect estimates. However, given the prospective nature of the study, this seems unlikely.

Interpretation

One of our key findings is that PI-UtA was higher in women with a male fetus than in those with a female fetus. This was accompanied by a higher prevalence of UtA notching in males, a finding that is in agreement with results previously reported by Stark et al. who showed that the presence of a male fetus is associated with a more vasoconstricted state in the maternal microvascular circulation, with a greater vasodilatory response than in female pregnancies (20). Our results on increased uteroplacental vascular resistance in male fetuses are also in line with a theory previously proposed by Vatten and Skjaerven, whereby male embryos are more susceptible to the process of implantation than female embryos (21). According to their concept, this is reflected in a higher miscarriage rate among male embryos (22). As consequence, relative to female fetuses, male fetuses that survive the period of placentation have a higher risk for perinatal mortality and morbidity if their mother develops PE. This theory is confirmed by a recent publication in which male biased mortality in the first weeks of pregnancy is described (23). This suggests that embryonic implantation may be more often suboptimal in the case of a male embryo. The serum markers human chorionic gonadotrophin hormone (hCG) reflects embryonic implantation. Secreted by the syncytiotrophoblast, hCG continues the production of progesterone by maintaining the corpus luteum(24).

The corpus luteum closely regulates endometrial function. Hence, hCG might therefore indirectly affect endometrial receptivity. Furthermore hCG promotes angiogenesis in the uterine vasculature and blocks any immunological action by the mother on foreign invading placental cells (25). Previous studies show that women carrying a female fetus have higher serum levels of hCG compared with women carrying a male fetus (26-31). In our own birth cohort the same phenomenon is observed (data not shown). We hypothesize that due to lower levels of hCG male embryos experience lower endometrial receptivity, resulting in increased suboptimal placentation and higher mortality rates in early pregnancy.

The higher PI-UtA and the increased presence of notching reflect an increased utero-placental resistance among male pregnancies. This may originate from suboptimal implantation and placentation. Many studies have reported a higher degree of placental inflammation in the presence of a male fetus. Histological examination has shown that male placentas obtained from pregnancies that led to spontaneous premature births have more severe lesions of chronic inflammation than placentas from matched females (32). Mothers of male neonates born preterm have higher circulating levels of pro-inflammatory cytokines but lower levels of the anti-inflammatory cytokine interleukin-10 and granulocyte colony-stimulating factor (G-CSF) (33). Interleukin-10 is of particular interest, since lower levels of interleukin-10 are associated with pregnancy loss (34). This is in line with the higher rate of miscarriages in male embryos, and so with the possibility of impaired placentation. This is confirmed by our own reported results on fetal sex specific differences in maternal first trimester placental biomarkers which showed lower PlGF concentrations in women carrying a male fetus (9). To explore whether the placental biomarkers PlGF and s-Flt1 are mediating factors of the association between fetal sex and the uterine artery pulsatility index, we performed an additional mediation analyses using first trimester plasma levels of s-Flt1 and PlGF. These results indeed show an intermediate role for placental biomarkers (**Table S.6.1**). First trimester PlGF and s-Flt1 explained 33.0 and 44.3% respectively, of the association between fetal sex and second trimester uterine PE. Lastly, we hypothesized that, if the theory of Vatten and Skjaerven is correct, then pregnancies with a male embryo susceptible to developing pre-eclampsia due to impaired placentation would already have miscarried in the first trimester. Male fetuses who survive the period of placentation therefore represent a relatively healthy group of fetuses. As this is not the case for the female fetuses one could expect a female-biased prevalence of PE. For this reason we compared the frequency of PE between male and female fetuses in our Generation R cohort, which showed a male/female ratio of 0.31 among early onset PE. This is in line with

previously reported results by Vatten and Skjaerven, who studied over 1.8 million births from 1967 to 1998 and found a strong female dominance among women with PE and preterm birth with a male/female sex ratio of 0.87 (21). Interestingly, in women with PE with a delivery at term, the proportion of males was higher (1.06) than for females. These results are underlain by our results on differential blood pressure patterns with crossing over at the end of the second trimester in the blood pressure patterns between women carrying a male and women carrying a female fetus, as well as between pregnancies complicated by the placental syndrome and uncomplicated pregnancies. They show that maternal adaptation mechanisms to pregnancy differ between male and female fetuses.

The previously mentioned theory of Vatten and Skjaerven also states that female fetuses more easily adapt to their environment while male fetuses are more focused on continued growth. One mechanism to ensure continued male growth may be the redirection of blood flow from the maternal peripheral circulation to the utero-placental circulation (22). This may explain why in the first half of pregnancy blood pressures of women carrying a male fetus are lower compared with women carrying a female fetus.

Conclusion

This study demonstrated that fetal sex specific maternal vascular adaptation to pregnancy exists, with differential effects according to sex and the presence or absence of pre-eclampsia, fetal growth restriction or spontaneous preterm birth. These findings help us clarify vascular adaptation to pregnancy. Clinical practice will not be affected, but we strongly feel that new clinical insights start with increased understanding of human fetal and maternal physiology. As the underlying mechanisms for these fetal sex specific differences need to be identified, future research should examine the physiologic and pathophysiologic mechanisms.

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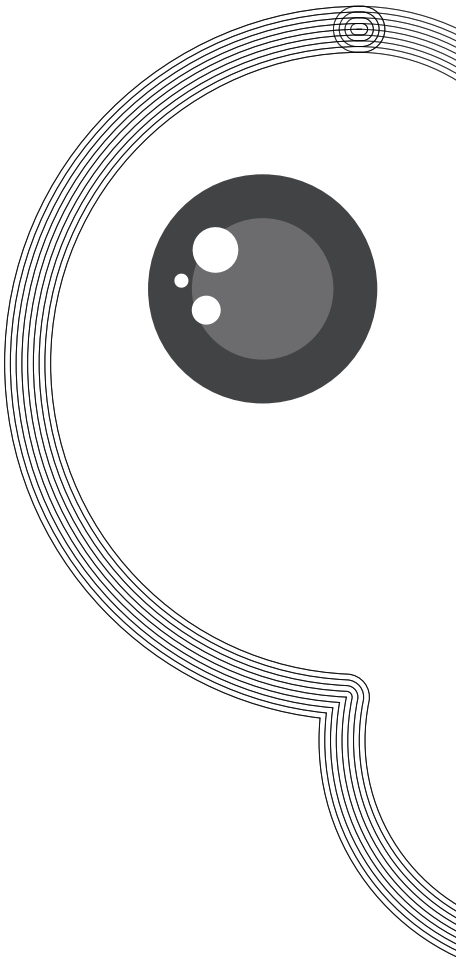
Table S.6.1 Mediation analyses of placental biomarkers on uterine artery pulsatility index

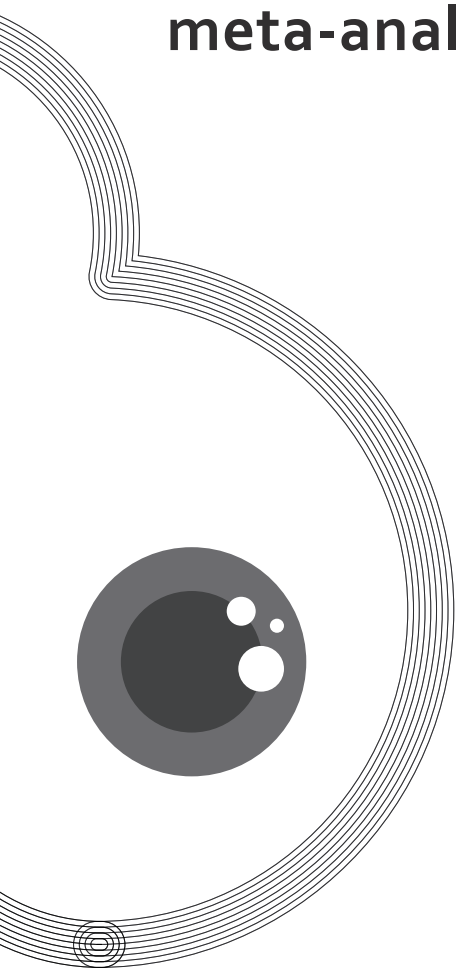
	Second trimester PI-UtA			
	% change (95% CI) PlGF	p-value	% change (95% CI) s-Flt1	p-value
Total group				
Male (n = 1388)	-33.0 (-88.9;-1.5)	0.04	-44.3 (-103.7;-12.7)	0.006
Female (n = 1317)				
Uncomplicated				
Male (n = 1198)	-30.9 (-110.0;5.53)	0.09	-43.6 (-129.8;-5.8)	0.024
Female (n = 1139)				
Placental syndrome				
Male (n = 190)	-33.1 (-146.7;13.3)	0.13	-39.2 (-156.5;7.8)	0.11
Female (n = 178)				
	Third trimester PI-UtA			
	% change (95% CI) PlGF 1st trimester	p-value	% change (95% CI) s-Flt1 1st trimester	p-value
Total group				
Male (n = 1326)	-7.7 (-51.8;31.9)	0.71	-23.1 (-71.2;13.8)	0.18
Female (n = 1339)				
Uncomplicated				
Male (n = 1169)	-12.1 (-75.9;35.5)	0.51	-26.5 (-103.9;15.4)	0.22
Female (n = 1151)				
Placental syndrome				
Male (n = 157)	28.5 (-144.6;353.5)	0.45	1.9 (-116.7;206.7)	0.81
Female (n = 188)				

Data represents the attenuations of effect estimates relative to the unadjusted model given in Table 6.2. Abbreviations: PI-UtA: uterine artery pulsatility index.



Chapter 7

Fetal sex and maternal pregnancy outcomes: a systematic review and meta-analysis



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Abstract

Introduction: Multiple maternal pregnancy complications are placenta mediated. Since the placenta also has a sex, fetal sex-specific differences could exist. We aimed to determine the association of fetal sex with maternal pregnancy complications.

Methods: Six electronic databases (Ovid MEDLINE, EMBASE, Cochrane Central, Web-of-Science, Pubmed and Google scholar) were systematically searched to identify eligible studies published before March 14, 2017 together with reference lists of the included studies and contact with experts in the field. Studies that assessed associations of fetal sex and the presence of maternal pregnancy complications within singleton pregnancies were included. Data were extracted by two independent reviewers using a predesigned data collection form. Pooled meta-analyses were performed. Our main outcomes were gestational hypertension, pre-eclampsia, eclampsia, gestational diabetes, placental abruption, postpartum hemorrhage and miscarriage.

Results: From 5528 unique references, 63 studies were selected, including over 12 million women. Male fetal sex was associated with term pre-eclampsia (pooled OR 1.07 [95%CI 1.05;1.10]) and gestational diabetes (pooled OR 1.04 95% CI [1.02;1.07]). All other pregnancy complications tended to be associated with male fetal sex, except for pre-term pre-eclampsia, which was associated with female fetal sex and miscarriages which were not associated with fetal sex. Overall quality of the included studies was good.

Conclusion: This meta-analysis suggests that the occurrence of pregnancy complications differ according to fetal sex which may be related with a higher risk for the mother in the presence of a male fetus. Future research on placental-mediated pregnancy complications should take this sexual dimorphism into account.

Introduction

In pregnancy, the placenta constitutes the active interface between the maternal and fetal blood circulation. It regulates important physiological changes during pregnancy and accounts for fetal development and nutrient supply. Impaired placentation leading to abnormal placental perfusion and hence placental dysfunction is believed to be etiologically related to several pregnancy complications such as pre-eclampsia (1, 2). The central role of the placenta in maternal health suggests an intensive interplay between the mother and the placenta. Since also the placenta has a sex, during pregnancy clear fetal sex specific differences are noticeable in maternal vascular adaptation to pregnancy and even the occurrence of different pregnancy complications such as pre-eclampsia and gestational diabetes (3-5). Despite increasing evidence that placentation and maternal adaptation to pregnancy are influenced by fetal sex, in studies that assess possible pathophysiological mechanisms during pregnancy, fetal sex is not taken into account.

We conducted a comprehensive systematic review and meta-analysis of studies evaluating maternal pregnancy complications in which we considered a wide range of pregnancy complications. Moreover we explore the worldwide impact of fetal sex on these maternal pregnancy complications by calculating population attributable fraction (PAF).

Materials and methods

Data sources and search strategy

This review was conducted using a predefined protocol and reported in accordance with PRISMA and MOOSE guidelines (**Table S.7.1 and S.7.2**) (6, 7). With the help of an experienced biomedical information specialist five electronic databases (Ovid MEDLINE, EMBASE.com, Cochrane CENTRAL, Web-of-Science Core Collection and Google scholar) were searched from inception until March 14, 2017, without publication date restriction. The computer-based searches combined terms related to (1) the exposure (gender, sex of the fetus, embryo and baby); and (2) maternal pregnancy complications as outcome (e.g. gestational hypertension, pre-eclampsia, eclampsia, gestational diabetes, placental abruption, postpartum hemorrhage and miscarriage) (**Table S.7.3**). Two independent reviewers screened the titles and abstracts of all studies initially identified, according to predefined selection criteria. Any disagreement was resolved through consensus or consultation with a third independent reviewer. Full texts were retrieved from studies that satisfied all selection criteria. Reference lists of the 20% selected studies most recently published and reviews identified on the topic were screened to identify additional publications.

Study selection and eligibility criteria

Observational studies were eligible if they assessed fetal sex as primary exposure in singleton pregnancies and if they examined this in relation to end points for maternal pregnancy complications. Eligible study populations included women recruited from health care settings or general populations. Studies on newborns with an abnormal karyogram, congenital conditions involving sex steroids and/or sex characteristics were excluded.

Data extraction

Two authors independently extracted data and consensus was reached in case of any inconsistency with involvement of a third author. A predesigned electronic data extraction form was used to collect relevant information. The data collection form included questions on qualitative aspects of the study (e.g. date of publication, design, geographical origin and setting, selection criteria, patient samplings), participant characteristics (e.g. number included in the analysis, age distribution, ethnicity, comorbidities), information on the reported outcome (type of outcome and assessment method) and on how associations were examined (statistical analysis, adjustment variables). In instances of

multiple publications on the same study population, the most up-to-date and comprehensive information was extracted.

Assessing study quality

Two reviewers independently rated the quality of studies using the Newcastle–Ottowa Quality Assessment Scale (**Table S.7.4**). This quality score system is applicable for case-control and cohort studies and allocates points for information on participants, comparability and outcome with a maximum of eight points.

Statistical analysis

We pooled the reported differences between pregnancies with a male and female fetus on maternal pregnancy complications. To enable a consistent approach to the meta-analysis and enhance interpretation of the findings, effect estimates were converted into odds ratios where appropriate. The inverse variance weighted method was used to combine summary measures using random-effects models to minimize effects of between-study heterogeneity (8). We also conducted sensitivity analyses using fixed-effects models. Heterogeneity was assessed using the Cochrane $_{X}^{2}$ statistic and the I^2 statistic and was distinguished as low ($I^2 \leq 25\%$), moderate ($I^2 > 25\%$ and $< 75\%$), or high ($I^2 \geq 75\%$) (9).

Sensitivity analyses were performed by excluding from the analysis studies with very strict inclusion or exclusion criteria resulting in a specific participant population (e.g. only inclusion of nulligravid women, women who were admitted with hyperemesis gravidarum, or women who had gestational diabetes, placental abruption, or a neonate born small for gestational age [SGA] etc). Stratified analyses were pre-specified as characteristics of assessment of heterogeneity and performed on geographical location (Western vs. non-Western), number of participants (< 10,000 vs. ≥ 10,000), study design (case-control vs retrospective cohorts vs prospective cohort) and on quality score (<7 vs. ≥ 7). Population attributable fractions (PAF) were calculated as PAF = (p (RR – 1)) / (p (RR – 1) + 1), with p reflecting the proportion of exposed and RR the relative risk (10). A narrative synthesis and construction of descriptive summary tables were performed for these studies that could not be quantitatively pooled, since they did not report quantitative effect estimates.

All tests were 2-tailed; $p \leq 0.05$ was considered statistically significant. Stata release 13 (StataCorp) was used for all analyses.

Results

Study identification and selection

After deduplication, the database searches identified 5528 citations. After screening titles and abstracts, 339 references were selected for detailed evaluation of their full texts. Of those, 64 articles met our inclusion criteria and were included in the review, of which 55 could also be included in meta-analyses (**Table 7.1**, **Figure 7.1**).

Characteristics of included studies

The 64 included studies reported results for 12,065,168 unique women (**Table S.7.5**). Fourty-three were retrospective cohort studies, nine prospective cohort studies and the remaining 12 studies were case-control studies. The majority of studies were performed in Western countries (21 in Northern America, 19 in Europe, two in Australia and one study in both Europe and Australia). Of the remaining studies, ten were performed in Asia, nine in the Middle East, and two in Africa. More than one outcome was measured in 21 studies, and for these the measure of association for each outcome was included in the analysis. In total 95 associations were included.

Association of fetal sex with maternal pregnancy outcomes

Fetal sex and gestational hypertension

Of the included studies, 18 investigated gestational hypertension with a total of 5,752,496 participants (**Table 7.1, Table S.7.6**) (11-28). Of these studies five found an association with male fetal sex (13, 14, 18, 21, 26), one with female fetal sex (24) and 12 found no association (11, 12, 15-17, 19, 20, 22, 23, 25, 27, 28). Four studies stratified their results (15, 20, 21, 26). One study stratified for severity of gestational hypertension (mild, moderate and severe), however none of the subgroups were associated with fetal sex (15). Another study stratified for parity in which no association was found for both primiparous as well as multiparous women (20). Persson et al stratified for comorbidity (gestational diabetes, diabetes mellitus type 1 or 2) (21). Only in the healthy group an association was found between male fetal sex and gestational hypertension. No such association was found for women with diabetes. The last study stratified for gestational age at birth and found that gestational hypertension was associated with male fetal sex only in term and post-term pregnancies (26).

Figure 7.1 Literature search for identification of studies on the association between fetal sex and maternal pregnancy complications

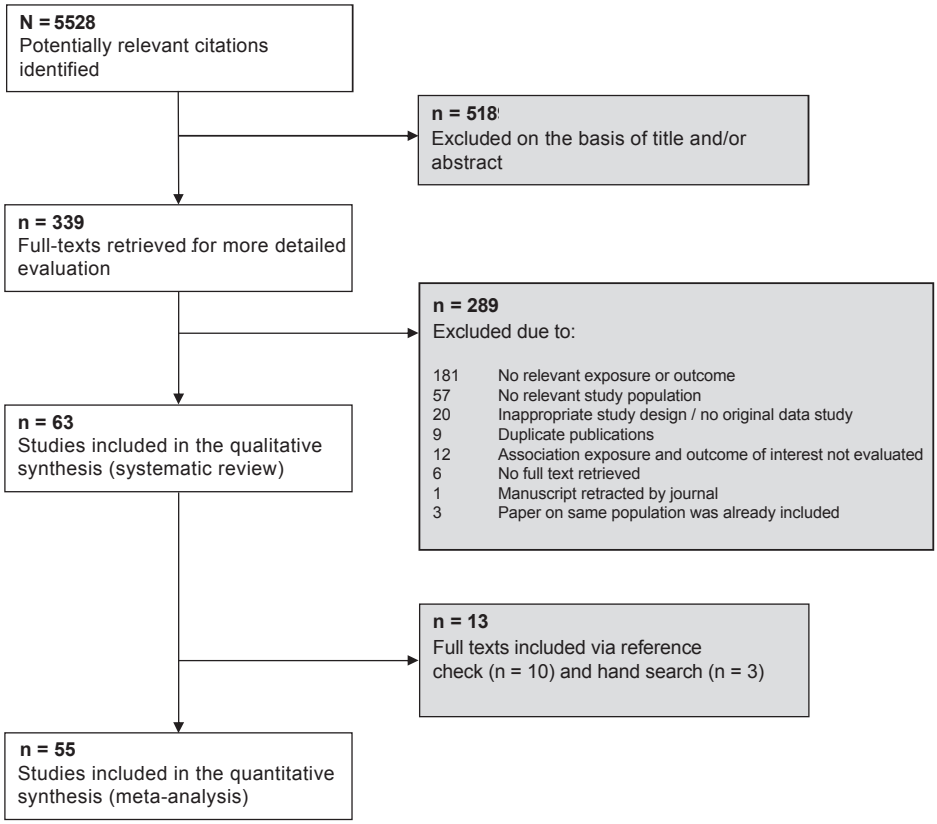


Table 7.1 Characteristics of the 63 studies included in the systematic review

	Gestational hypertension	Pre-eclampsia	Eclampsia	Gestational diabetes	Placental abruption	Postpartum hemorrhage	Miscarriage
No of unique studies	18	31	8	25	14	3	3
Participants							
Total	5,752,496	4,174,035	4,931,754	2,031,629	3,130,530	103,123	1217
Median (IQR), No.	9,504 (2205 – 118,130)	13,432 (727 - 84,875)	21,481 (3,086 - 713,055)	17,794 (800 - 65,808)	46,820 (4,645 - 193,463)	37,327 (623 - 65,173)**	313 (221 - 683)**
Location							
Europe	6	9*	3	6	5	1	1
North America	3	7	3	10	2	0	1
Australia	2	2*	0	2	1	0	0
Asia	4	9	1	4	1	1	1
Middle East	3	3	0	2	4	1	0
Africa	0	2	1	1	1	0	0
Quality score							
Median (IQR), No.	6 (6-7)	6 (6 - 7)	6 (6 - 6)	6 (6 - 7)	7 (6 - 8)	6 (5-8)**	6 (6 - 6)**

* One study was performed in both Europe and Australia ** Range instead of IQR due to limited number of studies

In our pooled meta-analyses (n = 16 studies) that compared the occurrence of gestational hypertension in women carrying a male fetus compared with women carrying a female fetus, the OR was 1.01 (0.98;1.04) (I^2 = 76.0%, p < 0.001) (**Figure 7.1A**). The PAF for total gestational hypertension was 1.31% (95% CI [-0.22;2.84], p = 0.09).

Fetal sex and pre-eclampsia

Of the included studies, 31 investigated pre-eclampsia with a total of 4,174,035 participants (**Table 7.1, Table S.7.6**) (11, 13, 17-21, 23, 24, 26, 27, 29-48). Six studies found an association with male fetal sex (21, 26, 31, 37, 45, 47), six with female fetal sex (19, 24, 27, 32, 33, 48) and the remaining 19 studies did not find a statistical significant association (11, 13, 17, 18, 20, 23, 29, 34-36, 38-44, 46, 49). Interestingly, the association between fetal sex and pre-eclampsia was dependent of gestational age. Eight studies stratified their results for gestational age (11, 26, 35, 37, 38, 42, 43, 47). Two studies stratified their results not only in term vs pre-term but additionally investigated several gestational age periods showing a strong association between female pregnancies and very early pre-eclampsia (26, 47). This association attenuated with gestational age. At term and post-term the association is reversed and male fetal sex is associated with pre-eclampsia. Two studies stratified into severity of pre-eclampsia with the more severe pre-eclampsia being associated with pregnancies with a female fetus (24, 27).

In our pooled meta-analyses (n = 29) that compared the occurrence of overall pre-eclampsia (i.e. pre-term, term and post-term) in women carrying a male fetus compared with women carrying a female fetus, the OR was 0.97 (0.94 – 1.00) (I^2 = 78.1%, p < 0.001) (**Figure 7.1B**). For pre-term, term and post-term pre-eclampsia the pooled ORs were 0.90 (0.75;1.09) (I^2 = 94.5%, p < 0.001), 1.07 (1.05;1.10) (I^2 = 14.0%, p = 0.32) and 1.76 (0.56;5.48) (I^2 = 84.4%, p = 0.011) including 6, 5 and 2 studies respectively (**Figure 7.1C, 7.1D** and **7.1E** respectively). The PAF for total pre-eclampsia was 1.23% (95% CI [-0.64;3.11], p = 0.20).

Fetal sex and eclampsia

Of the included studies, eight investigated eclampsia with a total of 4,931,754 participants (**Table 7.1, Table S.7.6**) (13, 14, 17, 21, 29, 30, 48, 50). Two studies found an association with male fetal sex (21, 50), one study with female fetal sex (48), and the remaining studies did not find a significant association (13, 14, 17, 29, 49).

In our pooled meta-analyses (n = 7 studies), that compared the occurrence of eclampsia in women carrying a male fetus compared with women carrying a female fetus, the OR was 1.00 (0.95;1.04) (I^2 = 47.0%, p = 0.08) (**Figure 7.1E**). The PAF for eclampsia was 0.71% (95% CI [-3.60;5.02], p = 0.75).

Fetal sex and gestational diabetes

Of the included studies, 25 investigated gestational diabetes, with a total of 2,030,653 participants (**Table 7.1, Table S.7.6**) (15-17, 22, 23, 26, 29, 35, 36, 38, 40, 46, 51-63). Of the included studies, seven studies found an association between fetal sex and gestational diabetes all showing a higher rate of gestational diabetes within women carrying a male fetus (23, 26, 29, 35, 38, 60, 64).

In our pooled meta-analyses (n = 24 studies), that compared the occurrence of gestational diabetes in women carrying a male fetus compared with women carrying a female fetus, the OR was 1.04 (1.02;1.07) (I^2 = 43.5%, p = 0.013) (**Figure 7.1G**). The PAF of male fetal sex for gestational diabetes was 1.75% (95% CI [1.05;2.46], p < 0.001). Assuming a worldwide prevalence of 4%, this resembles almost 150,000 cases of gestational diabetes worldwide due to the presence of a male fetus.

Fetal sex and placental abruption

Of the included studies, 14 investigated placental abruption, with a total of 3,130,530 participants (**Table 7.1, Table S.7.6**) (15, 17, 23, 32, 40, 49, 50, 65-72). Three studies found a significant association between placental abruption and women carrying a male fetus (66, 69, 70). Two studies found an association with female fetal sex (15, 67). Two studies stratified their results according to maternal age (65, 71). In the majority of age groups placental abruption was associated with the presence of a male fetus. One study stratified their analyses for parity (nulliparous vs multiparous) (66). In both groups placental abruption was associated with the presence of a male fetus.

In our pooled meta-analyses (n = 13 studies), that compared the occurrence of placental abruption in women carrying a male fetus vs women carrying a female fetus, the OR was 1.07 (0.93;1.23) (I^2 = 92.9%, p < 0.001) (**Figure 7.1H**). The PAF for placental abruption was 1.18% (95% CI [1.05;2.46], p < 0.001). Assuming a worldwide prevalence of 4%, this resembles almost 150,000 cases of gestational diabetes worldwide due to the presence of a male fetus.

Fetal sex and postpartum hemorrhage

Of the included studies, three investigated post-partum hemorrhage, with a total of 103,123 participants (**Table 7.1, Table S.7.6**) (16, 38, 71). One study found an association with the presence of a female fetus (38). This study however excluded preterm births. The other two studies did not find an association (16, 71).

Fetal sex and miscarriage

Of the included studies, three investigated miscarriage, with a total of 1,217 participants (**Table 7.1, Table S.7.6**) (73-75). Fetal or embryonic sex was assessed by karyotyping and/or inspection of the external genitalia. Two studies found an association between miscarriages and female embryonic sex (74, 75). One study stratified for morphological normal and abnormal embryos showing an association with male sex within the morphological normal embryos (73). One study stratified their analyses for gestational age (75). In the total group and in the group 4 - 10 weeks an association was found for female sex.

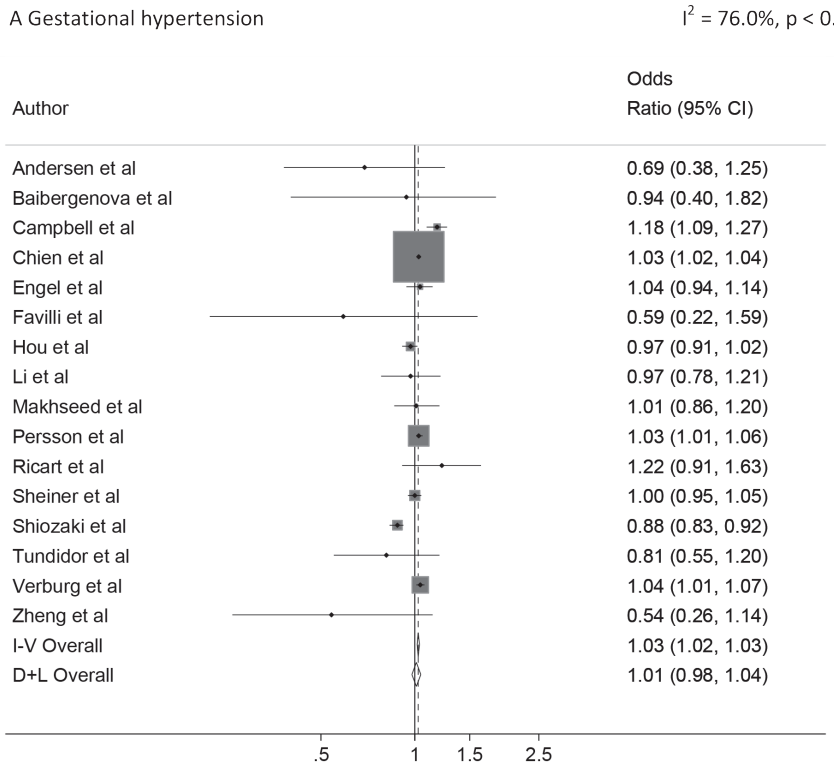
Study quality, heterogeneity and sensitivity analyses

Study quality according to the Newcastle-Ottowa scale was good. On third of all included studies had a quality score of ≥ 7 out of 8 and 15% percent of studies had the maximum score of 8 (**Table S.7.5**).

In separate sensitivity analyses all studies with specific inclusion or exclusion criteria were excluded from the meta-analyses. All results remained the same except for pre-term pre-eclampsia, which became slightly stronger (OR 0.85 [0.81;0.89] vs. OR 0.90 [0.75;1.09]). Furthermore all analyses were stratified according to geographical location, number of participants, study design and quality score (**Table S.7.7**). Stratified analysis for gestational hypertension by the level of quality score showed that only in the low quality studies (i.e. quality score < 7) an association with male fetal sex was found ($p < 0.001$). In the case of total occurrence of pre-eclampsia an association with male fetal sex was found for studies performed in Western countries, whereas in non-Western countries an association with female fetal sex was found ($p < 0.001$). For eclampsia stratification by number of participants showed no association with fetal sex in the larger studies (i.e. ≥ 10,000 participants) and an association with female fetal sex in the one smaller study ($p = 0.02$). When stratifying by study design an association between female fetal sex and eclampsia was found in the one included case-control study, with male fetal sex in the one included prospective cohort study and no association in the five included retrospective cohort ($p = 0.01$).

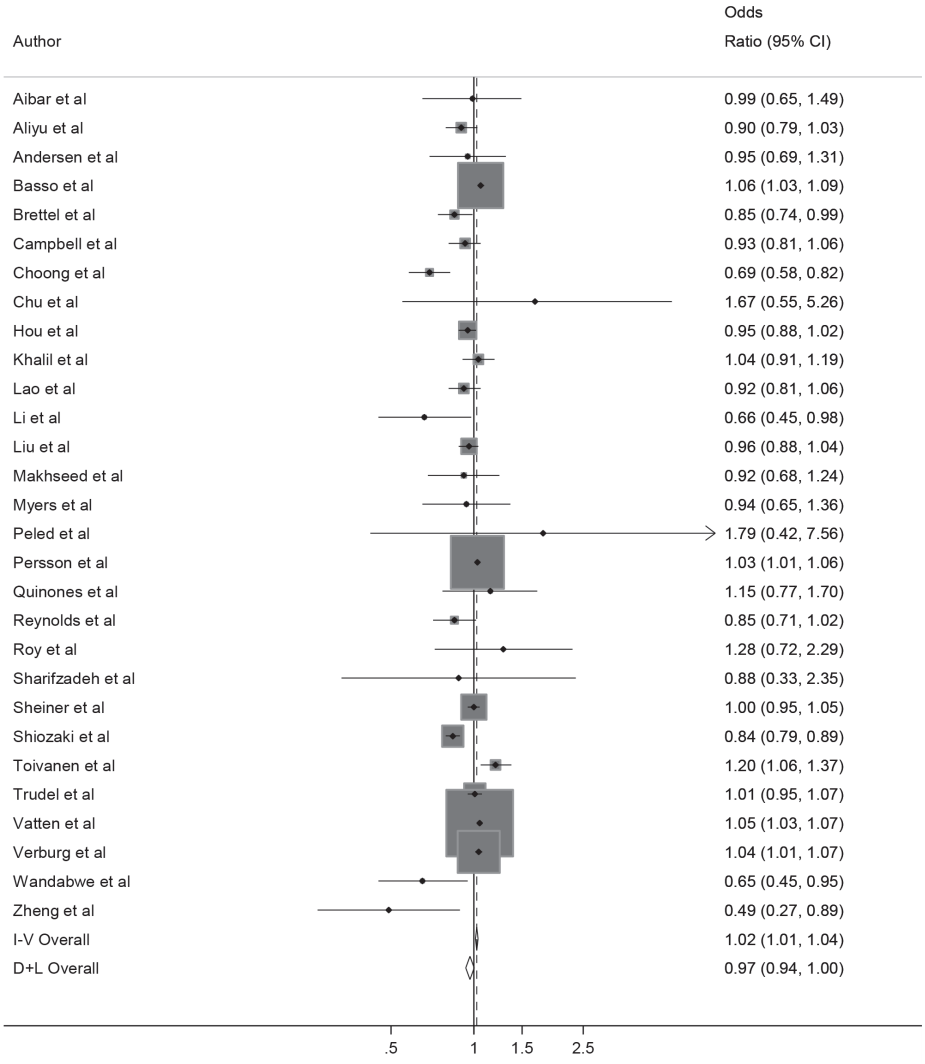
Five of eight analyses showed high between study heterogeneity, with an I^2 estimate exceeding 75% ($p < 0.05$ for the Cochrane X2 statistic) (**Figure 7.2**). This level of heterogeneity might be explained by differences between studies attributable to heterogeneous study populations, methods and outcome definition. Within the stratified analyses in some subgroups the I^2 exceeding 75% decreased. High heterogeneity was specifically found in studies with > 10,000 including participants, retrospective cohort studies and studies with a quality score < 7.

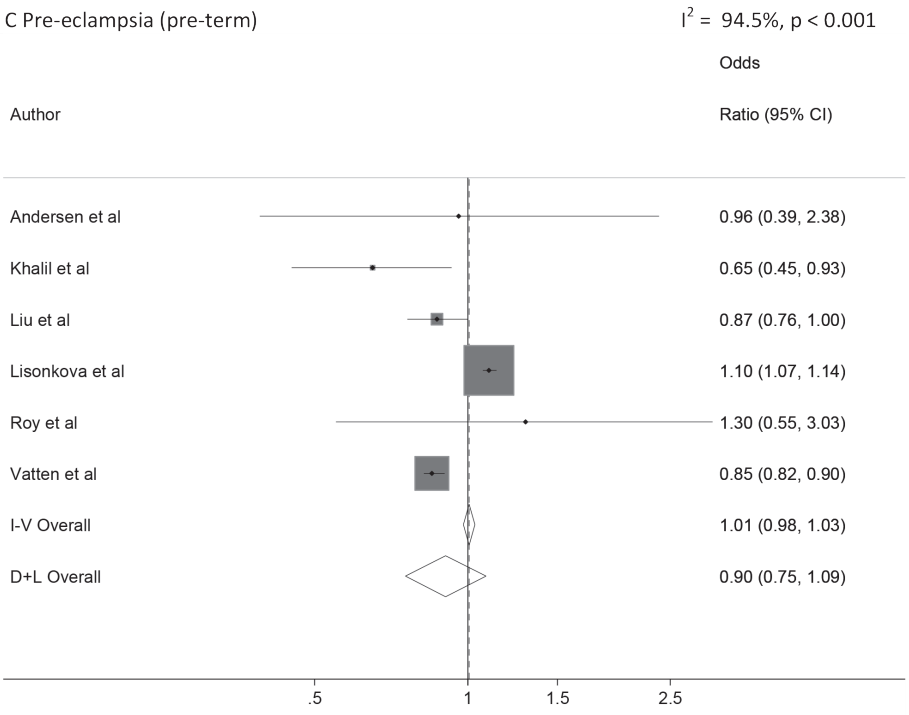
Figure 7.2 Forrest plots of meta-analyses on the association between female versus male fetal sex and maternal pregnancy outcome

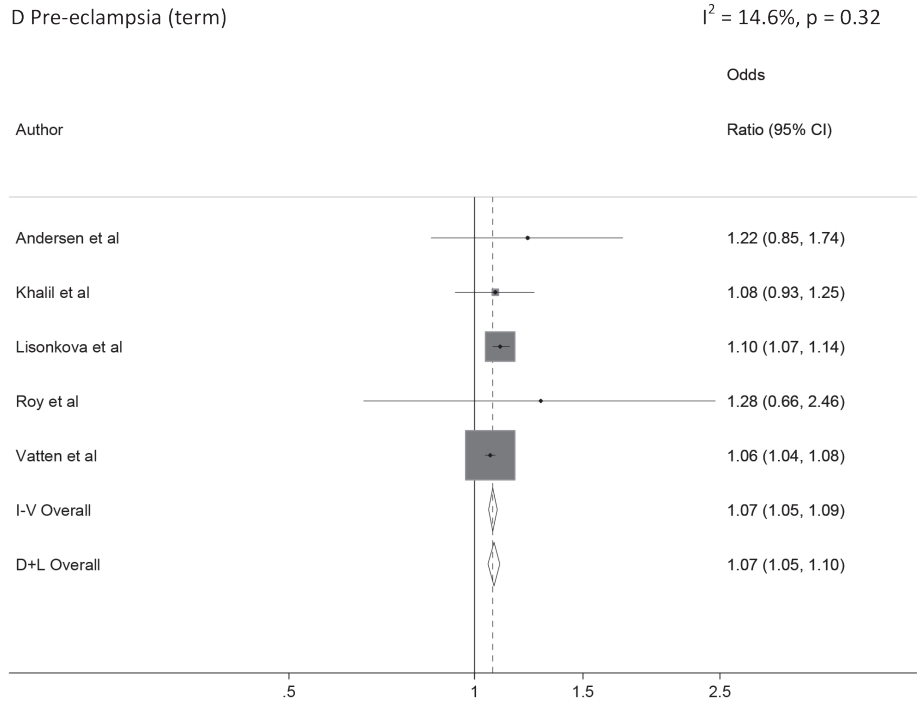


B Pre-eclampsia (overall)

I^2 = 78.1%, p < 0.001

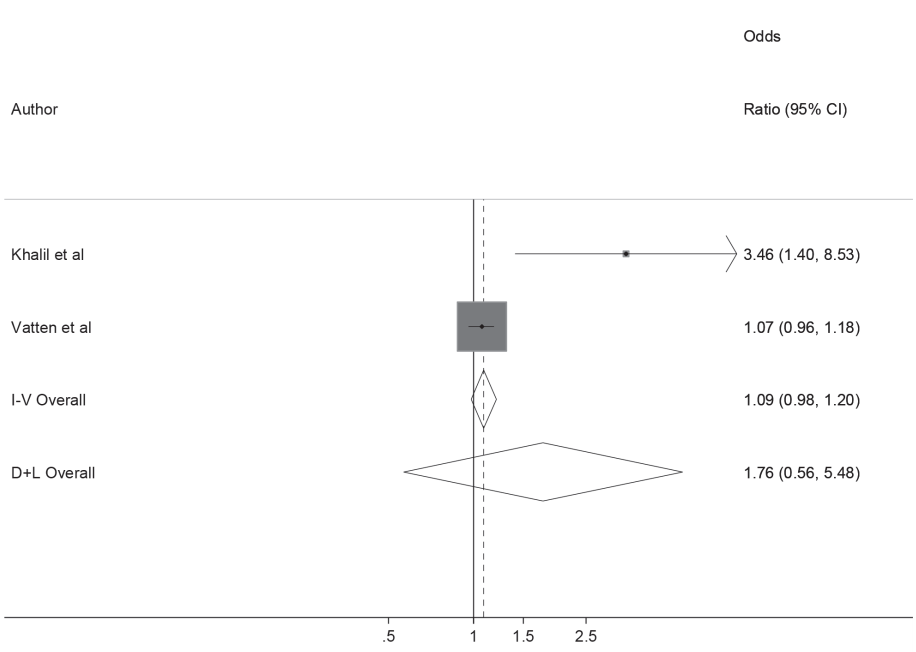



C Pre-eclampsia (pre-term)
I^2 = 94.5%, p < 0.001
Author
Odds Ratio (95% CI)
Andersen et al
0.96 (0.39, 2.38)
Khalil et al
0.65 (0.45, 0.93)
Liu et al
0.87 (0.76, 1.00)
Lisonkova et al
1.10 (1.07, 1.14)
Roy et al
1.30 (0.55, 3.03)
Vatten et al
0.85 (0.82, 0.90)
I-V Overall
1.01 (0.98, 1.03)
D+L Overall
0.90 (0.75, 1.09)
.5
1
1.5
2.5


D Pre-eclampsia (term)
I^2 = 14.6%, p = 0.32
Author
Odds Ratio (95% CI)
Andersen et al
1.22 (0.85, 1.74)
Khalil et al
1.08 (0.93, 1.25)
Lisonkova et al
1.10 (1.07, 1.14)
Roy et al
1.28 (0.66, 2.46)
Vatten et al
1.06 (1.04, 1.08)
I-V Overall
1.07 (1.05, 1.09)
D+L Overall
1.07 (1.05, 1.10)
.5
1
1.5
2.5

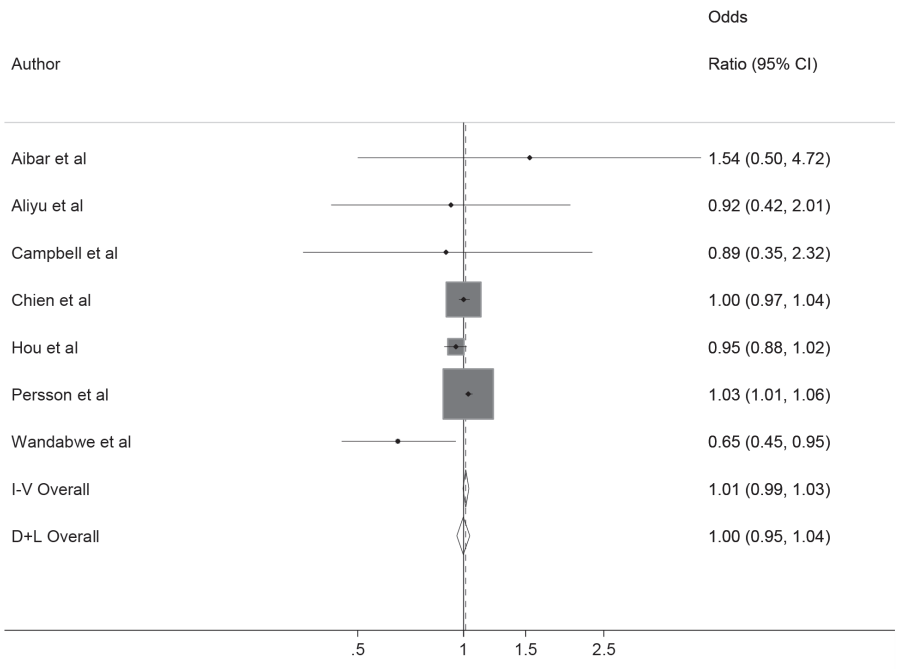
E Pre-eclampsia (post-term)

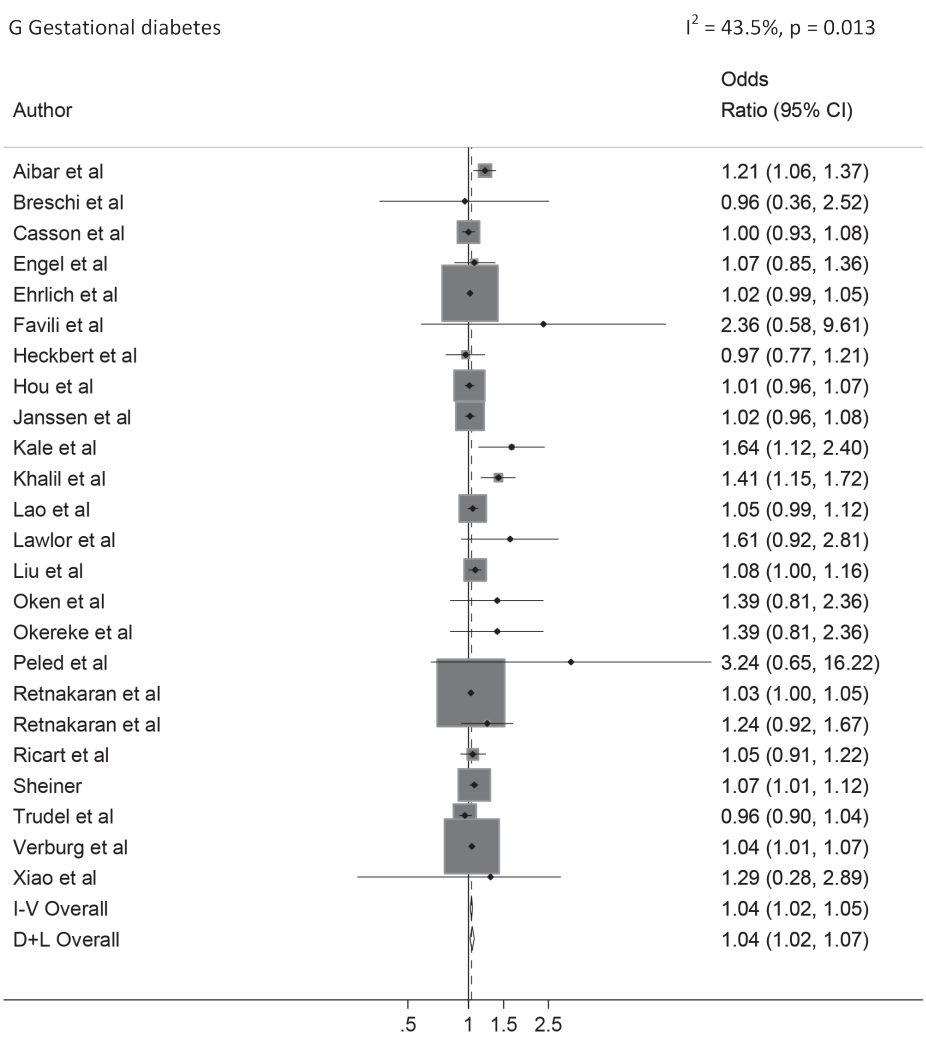
I^2 = 84.4%, p = 0.011



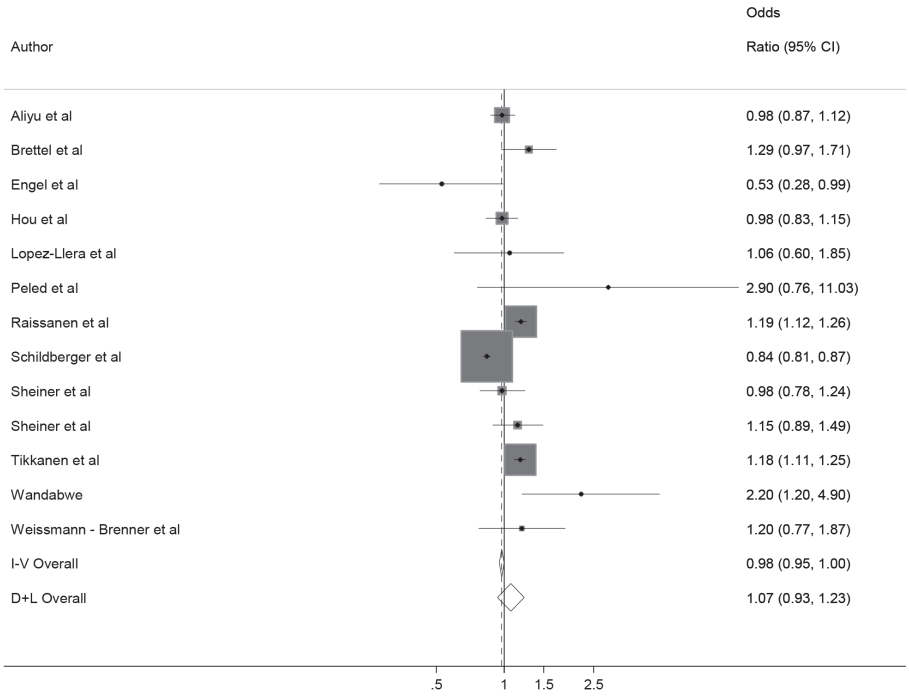
F Eclampsia

I^2 = 47.0%, p = 0.08




G Gestational diabetes
I^2 = 43.5%, p = 0.013
Author
Odds Ratio (95% CI)
Aibar et al
1.21 (1.06, 1.37)
Breschi et al
0.96 (0.36, 2.52)
Casson et al
1.00 (0.93, 1.08)
Engel et al
1.07 (0.85, 1.36)
Ehrlich et al
1.02 (0.99, 1.05)
Favili et al
2.36 (0.58, 9.61)
Heckbert et al
0.97 (0.77, 1.21)
Hou et al
1.01 (0.96, 1.07)
Janssen et al
1.02 (0.96, 1.08)
Kale et al
1.64 (1.12, 2.40)
Khalil et al
1.41 (1.15, 1.72)
Lao et al
1.05 (0.99, 1.12)
Lawlor et al
1.61 (0.92, 2.81)
Liu et al
1.08 (1.00, 1.16)
Oken et al
1.39 (0.81, 2.36)
Okereke et al
1.39 (0.81, 2.36)
Peled et al
3.24 (0.65, 16.22)
Retnakaran et al
1.03 (1.00, 1.05)
Retnakaran et al
1.24 (0.92, 1.67)
Ricart et al
1.05 (0.91, 1.22)
Sheiner
1.07 (1.01, 1.12)
Trudel et al
0.96 (0.90, 1.04)
Verburg et al
1.04 (1.01, 1.07)
Xiao et al
1.29 (0.28, 2.89)
I-V Overall
1.04 (1.02, 1.05)
D+L Overall
1.04 (1.02, 1.07)
.5
1
1.5
2.5

H Placental abruption $I^2 = 92.9\%, p < 0.001$



Discussion

Main findings

This is the first systematic review and meta-analyses investigating fetal sex and the association to multiple major pregnancy outcomes showing that sexual dimorphisms in maternal pregnancy complications exist. The majority of pregnancy complications tended to be associated with pregnancies with a male fetus, with the exception of preterm pre-eclampsia in which an association with female fetuses was found.

Interpretation

Within pre-eclampsia diverse results were found when stratifying for gestational age. A similar phenomenon has been observed for geographical location. Previous studies observed that in non-Western countries pre-term pre-eclampsia is much more prevalent compared with Western countries (76). This is reflected in our stratified meta-analyses on the total prevalence of pre-eclampsia stratified by geographical location. In non-Western countries pre-eclampsia was associated with female fetal sex while in Western countries pre-eclampsia was associated with male fetal sex. This could well be explained by the higher prevalence of pre-term pre-eclampsia in non-Western countries and term pre-eclampsia in Western countries. The same phenomenon has been observed before in a recent individual patient meta-analyses performed by the Global Pregnancy Collaboration (4). Our results confirm their observation. A similar time diverse pattern was seen in previous research on fetal sex specific differences in blood pressure patterns during pregnancy (3). Within complicated pregnancies (including pre-eclampsia) a different diastolic blood pressure pattern was observed for women with a male fetus compared with women with a female fetus, with crossing patterns in the second trimester. Women carrying a female fetus started with a higher diastolic blood pressure compared with women carrying a male fetus. However, from 24 weeks of gestation onwards these women had a lower diastolic blood pressure (3). This phenomenon is in line with our results. Although the exact underlying mechanisms of these changing pattern are still subject of investigation it might imply that male embryos are more susceptible to suboptimal implantation or abnormal placental development. Those pregnancies with a male embryo susceptible for pre-eclampsia due to impaired placentation might already have miscarried in the first trimester (77). The surviving population of male fetuses will thereby represent a relatively healthy group of fetuses leading to a female-biased prevalence of the overall and preterm risk of pre-eclampsia (77). Since especially early-onset pre-eclampsia is thought to originate from abnormal placentation a cross-over is observed for the pre-term and term pre-eclampsia (2).

The implication that male embryos are more susceptible to placental development is in line with the results described in this systematic review since other placental related pregnancy complications were also found to be mainly associated with the presence of a male fetus. We hypothesize that carrying a male fetus demands a higher degree of metabolic and vascular maternal adaptation to pregnancy compared with carrying a female fetus since sons are heavier and larger at birth and require more energy during gestation and lactation (78). For example, women carrying a male fetus have poorer pancreatic beta cell function in pregnancy (60). This is in line with our finding that women carrying a male fetus are at higher risk for developing gestational diabetes. Previous research also showed that within women who experienced gestational diabetes, those women who carried a male fetus are at higher risk of developing diabetes type 2 in later life than women who carried a female fetus (61). Adverse effects of carrying a male fetus are also shown in vascular adaptation to pregnancy. Women carrying a male fetus have higher uterine artery pulsatility during the second trimester and more often present themselves with notching in the third trimester of pregnancy (3). This reflects an increased utero-placental resistance among male pregnancies which may originate from suboptimal implantation and placentation (3). The process of implantation is difficult to examine in human subjects. However, studies on biomarkers as human chorionic gonadotrophin hormone (hCG), reflecting embryonic implantation show that women carrying a female fetus have higher serum levels of hCG than women carrying a male fetus (79-85). It could be hypothesized that due to lower levels of hCG, male embryos experience lower endometrial receptivity, resulting in increased suboptimal placentation and higher mortality in early pregnancy.

Not only during pregnancy the consequences of carrying a male fetus for maternal health are evident, also long term adverse health outcomes have been found. Helle et al were the first to suggest that a shorter maternal lifespan is associated with the number of sons born (86). More recently, research has shown that the maternal lifespan declines with the number of sons they gave birth to whereas the number of daughters born was not associated with women's post-reproductive survival (87, 88). Helle et al validated their results by demonstrating that this effect was independent of the number of sons and daughters surviving to adulthood and by showing that the number of sons and daughters was not associated with post-reproductive survival in men (87). These findings support the hypothesis that baring sons is cardiometabolically more costly than baring daughters.

Conclusions on fetal sex and miscarriage rates are difficult to draw from the included studies. One of our exclusion criteria was an abnormal karyogram, which is highly prevalent in miscarriages (89). This could have introduced a selection bias if an abnormal karyogram occurs more often in male pregnancies and give rise to a female dominance in miscarriages with a normal karyogram while in the total group of miscarriages there is a male dominance. Furthermore investigation of the pregnancy product after a miscarriage is not part of daily practice and only performed specific cases like recurrent miscarriages. To investigate if a sexual dimorphism in miscarriages exists, all future research should focus on the total rate of miscarriages, stratified for chromosomal abnormalities.

To our knowledge, this is the first comprehensive review that assessed the quantitative association between fetal sex and multiple major pregnancy outcomes. The studies included in our analyses included over 12 million women combined and assessed seven adverse pregnancy outcomes. Some systematic reviews exist focusing on one pregnancy complication (5, 90). Eleven of our 25 included studies on gestational diabetes and 22 of our 31 included studies on pre-eclampsia were not included in these systematic reviews previously published. For pre-eclampsia this resulted in one million more participants included in the current review. In the previous systematic review and meta-analyses on pre-eclampsia the authors however did not stratify into pre-term, term and post-term pre-eclampsia.

Strengths and limitations

Strengths and limitations of the current also study merit careful consideration. First, as are all systematic reviews, our review is prone to reporting bias, owing to the possibility that studies with more extreme results are more likely to be published. In this systematic review, multiple included articles did not primarily investigate the effect of fetal sex on pregnancy outcome. However, due to the fact that the information was given anyway in the manuscript, odds ratios could be calculated. Nevertheless studies could have been missed in our search if fetal sex was not mentioned in the abstract of the study. Additionally, meta-analyses are always limited by the quality of the individual published studies. The majority of studies included in the current analyses were of high quality, with a low risk of bias. However, the majority of studies did not give a clear definition of the pregnancy outcome which was assessed. Also definitions changed internationally across time. This might have introduced some heterogeneity into the analyses.

Most studies that were included did not adjust for any confounders. From an epidemiological point of view, when using fetal sex as an exposure we do not have to deal with any confounding factors since there are no factors described influencing fetal sex.

Conclusion

Our findings support the emerging concept of a sexual dimorphism in the maternal-fetal-placental interplay. Most importantly all results are consistent with each other and validate the hypothesis that carrying a male fetus is accompanied with a higher cardiovascular and metabolic load for the mother resulting in an increased risk of maternal pregnancy complications. Although the effect sizes in this meta-analysis are modest, it holds important implications for our understanding of maternal-fetal physiology. Furthermore, the absolute numbers of pregnancy complications worldwide occurring due to the presence of a male fetus are high. Fetal sex should therefore be taken into account when assessing risks of pregnancy complications and adverse cardiovascular health in later life. Since maternal vascular adaptation differs between women carrying a male or female fetus it is plausible that also the treatment efficacy of placental mediated pregnancy complications is dependent on fetal sex. Further research to confirm this pharmacological sexual dimorphism is necessary.

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Supplemental information

Table S.7.1 PRISMA checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	4
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	NA
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	5,6
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	5
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	5 and Appendix 1
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	5, 6
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	6

Table S.7.1 Continued

Section/topic	#	Checklist item	Reported on page #
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	7
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	7
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	7
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	7
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	8 and Figure 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	8 and Table 1 and Appendix 5
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	12, 13 and Appendix 6
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	8-12, Appendices 5 and 6
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	8-12, Figure 2
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	12,13
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	13, Appendix 7
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	14-18
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	17-18
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	18
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	NA

Table S.7.2 MOOSE checklist

Criteria		Brief description of how the criteria were handled in the meta-analysis
Reporting of background should include		
√	Problem definition	Multiple maternal pregnancy complications are placenta mediated. Since the placenta also has a sex, fetal sex-specific differences in the occurrence of these complications could exist. A meta-analysis on multiple pregnancy complications and fetal-sex differences has not been performed previously.
√	Hypothesis statement	Male fetal sex is associated with more maternal pregnancy complications than female fetal sex.
√	Description of study outcomes	We included studies on the following maternal pregnancy complications: gestational hypertension, pre-eclampsia, eclampsia, gestational diabetes, placental abruption, post-partum haemorrhage and miscarriage.
√	Type of exposure or intervention used	Fetal sex.
√	Type of study designs used	All observational study designs including cohort,case-controls and cross-sectional studies.
√	Study population	Only studies carried out in singleton pregnancies were included. Studies on newborns with an abnormal karyogram, congenital conditions involving sex steroids and/or sex characteristics were excluded.
Reporting of search strategy should include		
√	Qualifications of searchers	The credentials of the investigators are indicated in the authors list and in the methods section.
√	Search strategy, including time period included in the synthesis and keywords	Search strategy and time periods are detailed in page 5 of the manuscript,in Figure 1 and the full search strategy is available in appendix 1.
√	Databases and registries searched	Ovid MEDLINE, EMBASE, Cochrane Central, Web-of-Science, Google Scholar are mentioned in the methods.
√	Search software used, name and version, including special features	We did not employ a search software. Endnote X7 was used to merge retrieved citations and eliminate duplications and to screen references for relevance.
√	Use of hand searching	We hand-searched bibliographies of retrieved systematic reviews and meta-analysis for additional references.
√	List of citations located and those excluded, including justifications	Details of the literature search process are outlined in the flow chart. Citations for the included studies are included in the text and Appendices 5 and 6. The citation list for excluded studies is available upon request.
√	Method of addressing articles published in languages other than English	We placed no restrictions on language; local translation services were available. Conference abstracts were excluded in the search process.
√	Method of handling abstracts and unpublished studies	Systematic reviews were used to identify further references. Authors of included studies were contacted to retrieve missing full texts and to identify any missing studies.
√	Description of any contact with authors	Authors of included studies were contacted to retrieve missing full texts and to identify any missing studies.
Reporting of methods should include		
√	Description of relevance or appropriateness of studies assembled for assessing the hypothesis to be tested	Detailed inclusion and exclusion criteria are described in the methods section.

Table S.7.2 Continued

√	Rationale for the selection and coding of data	A predesigned data collection form was prepared to extract the relevant information from the included full texts, including study design, characteristics of the study participants and information on the reported outcome.
√	Assessment of confounding	We performed qualitative analyses to evaluate differences between studies.
√	Assessment of study quality, including blinding of quality assessors; stratification or regression on possible predictors of study results	We used the Newcastle- Ottawa Scale (NOS) to evaluate the quality of case-control and cohort studies included in this review.
√	Assessment of heterogeneity	I2 was calculated and reported in Figure 2 and discussed in the text.
√	Description of statistical methods in sufficient detail to be replicated	We conducted qualitative analysis of the data with sensitivity analyses and stratification on multiple variables.
√	Provision of appropriate tables and graphics	We included 2 main figures, 1 main table, and 7 appendices.
Reporting of results should include		
√	Graph summarizing individual study estimates and overall estimate	Figure 2.
√	Table giving descriptive information for each study included	Appendices 5 and 6.
√	Results of sensitivity testing	The results on sensitivity analyses are described on page 14 and 15.
√	Indication of statistical uncertainty of findings	95% confidence intervals were presented if available.
Reporting of discussion should include		
√	Quantitative assessment of bias	Not applicable.
√	Justification for exclusion	We excluded studies that had no or unclear definition of exposure and outcome, or data extraction was not feasible. Studies on newborns with an abnormal karyogram, congenital conditions involving sex steroids and/or sex characteristics were excluded.
√	Assessment of quality of included studies	We used the Newcastle-Ottawa Scale (NOS) to evaluate the quality of case-control and cohort studies included in this review.
Reporting of conclusions should include		
√	Consideration of alternative explanations for observed results	Non-confounded alternative explanations for the observed results are unlikely since fetal sex is random.
√	Generalization of the conclusions	The generalizability of our findings has been enhanced by the involvement of data from multiple regions in the world, including North-America, Europe, the Middle-East, Australia, Asia and Africa. However there is a clear lack of evidence from South-America.
√	Guidelines for future research	Further work is necessary to elucidate the pathophysiological background of the observed sexual dimorphism in maternal-fetal-interplay.
√	Disclosure of funding source	M.J. Tielemans, J. Schoufour, T. Voortman and O.H. Franco work in ErasmusAGE, a center for aging research across the life course funded by Nestlé Nutrition and Metagenics Inc. Nestlé Nutrition and Metagenics Inc. had no role in design and conduct of the study, collection, management, analysis and interpretation of the data; or preparation, review or approval of the manuscript.

S.7.3 Search strategy

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(((gender/de OR 'sex difference'/exp) AND fetus/exp) OR (((gender* OR femal* OR male* OR sex OR boy* OR girl*) NEAR/6 (fetus* OR fetal OR foetus* OR foetal OR embryo* OR baby OR babies))):ab,ti) AND ('pregnancy outcome'/exp OR 'pregnancy disorder'/exp OR 'pregnancy complication'/exp OR 'Doppler flowmetry'/exp OR 'Doppler flowmeter'/exp OR 'birth weight'/exp OR 'intrauterine growth retardation'/exp OR 'prenatal growth'/exp OR mortality/de OR 'fetus mortality'/de OR 'embryo mortality'/de OR 'perinatal mortality'/exp OR 'prenatal mortality'/exp OR (((pregnan* OR gravid* OR gestation* OR maternal* OR mother* OR intrauterin* OR neonat* OR newborn* OR prenatal*) NEAR/6 (mortalit* OR surviv* OR fatal* OR outcome* OR growth* OR disorder* OR disease* OR complication* OR toxemi* OR hypertens* OR 'blood pressure' OR small* OR large* OR duration OR prolong*)) OR eclamp* OR preeclamp* OR Doppler OR notching OR ((birth) NEAR/3 (weight OR length*)) OR birthweight OR lbw OR elbw OR vlbw OR iugr OR fgr OR sga OR macrosomi* OR stillbirth OR stillborn OR (live NEXT/1 birth*) OR prematur* OR postmatur* OR imematur* OR preterm OR postterm OR dysmatur* OR serotinit* OR miscarriage* OR mortalit* OR (spontaneous NEAR/3 abortion*)):ab,ti) NOT ([animals]/lim NOT [humans]/lim) NOT ([Conference Abstract]/lim OR [Letter]/lim OR [Note]/lim OR [Conference Paper]/lim OR [Editorial]/lim OR 'case report'/exp OR 'case report':ti) AND [english]/lim

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((sex/ AND fetus/) OR (((gender* OR femal* OR male* OR sex OR boy* OR girl*) ADJ6 (fetus* OR fetal OR foetus* OR foetal OR embryo* OR baby OR babies))).ab,ti.) AND ("Fetal Mortality"/ OR "Maternal Mortality"/ OR "Perinatal Mortality"/ OR exp pregnancy outcome/ OR pregnancy complications/ OR exp Ultrasonography, Doppler/ OR birth weight/ OR exp Infant, Low Birth Weight/ OR Fetal Growth Retardation/ OR (((pregnan* OR gravid* OR gestation* OR maternal* OR mother* OR intrauterin* OR neonat* OR newborn* OR prenatal*) ADJ6 (mortalit* OR surviv* OR fatal* OR outcome* OR growth* OR disorder* OR disease* OR complication* OR toxemi* OR hypertens* OR "blood pressure" OR small* OR large* OR duration OR prolong*)) OR eclamp* OR preeclamp* OR Doppler OR notching OR ((birth) ADJ3 (weight OR length*)) OR birthweight OR lbw OR elbw OR vlbw OR iugr OR fgr OR sga OR macrosomi* OR stillbirth OR stillborn OR (live ADJ birth*) OR prematur* OR postmatur* OR imematur* OR preterm OR postterm OR dysmatur* OR serotinit* OR miscarriage* OR mortalit* OR (spontaneous ADJ3 abortion*)).ab,ti.) NOT ((letter OR news OR comment OR editorial OR congresses OR abstracts OR case reports).pt. OR case report.ti.) AND english.la.

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((((gender* OR femal* OR male* OR sex OR boy* OR girl*) NEAR/6 (fetus* OR fetal OR foetus* OR foetal OR embryo* OR baby OR babies))):ab,ti) AND ((((pregnan* OR gravid* OR gestation* OR maternal* OR mother* OR intrauterin* OR neonat* OR newborn* OR prenatal*) NEAR/6 (mortalit* OR surviv* OR fatal* OR outcome* OR growth* OR disorder* OR disease* OR complication* OR toxemi* OR hypertens* OR 'blood pressure' OR small* OR large* OR duration OR prolong*)) OR eclamp* OR preeclamp* OR Doppler OR notching OR ((birth) NEAR/3 (weight OR length*)) OR birthweight OR lbw OR elbw OR vlbw OR iugr OR fgr OR sga OR macrosomi* OR stillbirth OR stillborn OR (live NEXT/1 birth*) OR prematur* OR postmatur* OR imematur* OR preterm OR postterm OR dysmatur* OR serotinit* OR miscarriage* OR mortalit* OR (spontaneous NEAR/3 abortion*)):ab,ti)

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TS=(((((gender* OR femal* OR male* OR sex OR boy* OR girl*) NEAR/5 (fetus* OR fetal OR foetus* OR foetal OR embryo* OR baby OR babies)))) AND ((((pregnan* OR gravid* OR gestation* OR maternal* OR mother* OR intrauterin* OR neonat* OR newborn* OR prenatal*) NEAR/5 (mortalit* OR surviv* OR fatal* OR outcome* OR growth* OR disorder* OR disease* OR complication* OR toxemi* OR hypertens* OR "blood pressure" OR small* OR large* OR duration OR prolong*)) OR eclamp* OR preeclamp* OR Doppler OR notching OR ((birth) NEAR/2 (weight OR length*)) OR birthweight OR lbw OR elbw OR vlbw OR iugr OR fgr OR sga OR macrosomi* OR stillbirth OR stillborn OR (live NEAR/1 birth*) OR prematur* OR postmatur* OR imematur* OR preterm OR postterm OR dysmatur* OR serotinit* OR miscarriage* OR mortalit* OR (spontaneous NEAR/2 abortion*))) NOT ((animal* OR mouse* OR mice OR rat OR rats OR murine OR bovine OR ovine OR porcine OR cow OR pig OR sheep OR rabbit*) NOT (human* OR patient* OR woman* OR women* OR baby OR babies))) AND DT=(Article) AND LA=(english)

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"female|male fetus|fetal|embryo"|"fetal|embryonal gender|sex"
"pregnancy|fetus|fetal|maternal|neonatal|newborn mortality
|survival|outcome|growth|disorders|diseases|complications"|stillbirth

Tabel S.7.4 Newcastle-Ottawa Quality Assessment Scale

A. Case control studies

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Exposure categories. A maximum of two stars can be given for the category Comparability.

Selection

1) Is the case definition adequate
   a) Yes, with independent validation *
   b) Yes, eg record linkage or based on self reports
   c) No description
2) Representativeness of the cases
   a) Consecutive or obviously representative series of cases *
   b) Potential for selection biases or not stated
3) Selection of Controls
   a) Community records *
   b) Hospital controls
   c) No description
4) Definition of Controls
   a) No history of disease (endpoint) *
   b) No description of source

Comparability

1) Comparability of cases and controls on the basis of the design or analyses
   a) Study controls for ... (Select the most important factor) *
   b) Study controls for any additional factor * (This criteria could be modified to indicate specific control for a second important factor)

Exposure

1) Ascertainment of exposure
   a) Secure record (eg surgical records) *
   b) Structured interview where blind to case/control status *
   c) Interview not blinded to case/control status
   d) Written self report or medical record only
   e) No description
2) Same method of ascertainment for cases and controls
   a) Yes *
   b) No

3) Non-Response rate
   a) Same rate for both groups *
   b) Non respondents described
   c) Rate different and no designation

B. Cohort studies

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Exposure categories. A maximum of two starts can be given for Comparability.

Selection

1) Representativeness of the exposed cohort
   a) Truly representative of the average ... (describe) in the community *
   b) Somewhat representative of the average ... in the community *
   c) Selected group of users eg nurses, volunteers
   d) No description of the derivation of the cohort
2) Selection of the non exposed cohort
   a) Drawn from the same community as the exposed cohort *
   b) Drawn from a different source
   c) No description of the derivation of the non exposed cohort
3) Ascertainment of exposure
   a) Secure record (eg surgical records) *
   b) Structured interview *
   c) Written self report
   d) No description
4) Demonstration that outcome of interest was not present at start of study
   a) Yes *
   b) No

Comparability

1) Comparability of cohorts on the basis of the design or analysis
   a) Study controls for ... (select the most important factor) *
   b) Study controls for any additional factor * (This criteria could be modified to indicate specific control for a second important factor)

Outcome

1) Assessment of outcome
   a) Independent blind assessment *
   b) Record linkage *
   c) Self report
   d) No description
2) Was follow-up long enough for outcome to occur
   a) Yes (select an adequate follow up period for outcome of interest) *
   b) No
3) Adequacy of follow up of cohorts
   a) Complete follow up – all subjects accounted for *
   b) Subjects lost to follow up unlikely to introduce bias – small number lost -> ... % (select and adequate %) follow up, or description provided of those lost) *
   c) Follow up rate < ... % (select an adequate %) and no description of those lost
   d) No statement

Table S.7.5 Study characteristics

First author, year	Country	Study design	Population characteristics	Pregnancy outcome	n	Definition of pregnancy outcome	Quality score
Aibar et al, 2012 [29]	Spain	RCS	Singleton pregnancies with delivery > 24 weeks	PE	29,530	NR	6
				E	29,530	NR	6
				GD	29,530	NR	6
Aliyu et al, 2012 [30]	USA	RCS	Singleton births with placental abruption 20 - 42 weeks	PE	10,014	DBP ≥ 90 mmHg and proteinuria	7
				E	10,014	Seizures associated with pre-eclamptic features	7
Aliyu et al, 2012 [49]	USA	RCS	Singleton births with pre-eclampsia or eclampsia 20 - 42 weeks	PA	56,313	All or part of the placenta has been pulled away from the uterine wall, disrupting the flow of blood and oxygen to the fetus.	7
Andersen et al, 2016 [11]	Denmark	PCS	Singleton pregnancies without early fetal loss	GH	2,110	De novo hypertension defined as >140/90 mmHg with at least 4h between after gestational week 20+0.	8
				PE	2,110	Gestational hypertension with de novo onset of proteinuria (> 0.3 g/24 h)	8
Ashwal et al, 2016 [28]	Israel	RCS	All women with unfavorable cervix admitted for induction of labor between 34+0 and 41+6 weeks of gestation.	GH / PE	1,062	NR	8
Baibergenova et al, 2006 [12]	Canada	RCS	Pregnant women with asthma and an emergency department visit	GH	530	ICD coding (013-014)	6
Basso et al, 2001 [31]	Denmark	RCS	Danish citizins with delivery > 28 weeks	PE	84,875	NR	6

Table S.7.5 Continued

Breschi et al, 1993 [51]	Italy	PCS	Singleton pregnancies with hospital visit in 3rd trimester of pregnancy without diabetes type 1 or 2 or any other systemic disease	GD	529	Fasting venous blood glucose of 5,8 mmol/mL or more and a 2-h value of 9.2 mmol/mL or more.	6
Brettell et al, 2008 [32]	UK	RCS	Singleton hospital delivery > 24 weeks without major congenital abnormalities	PE	75,725	NR	6
				PA	75,725	NR	6
Byrne et al, 1987 [73]	USA	RCS	Singleton pregnancies ending in a spontaneous abortion before 28 weeks	M	683	Spontaneous expulsion of a recognized intrauterine pregnancy in which the fetus is dead when expelled	6
Campbell et al, 1983 [13]	UK	RCS	Primigravid with delivery of live- or stillbirth > 28 weeks	GH	13,432	Nelson 1955 criteria	6
				PE	13,432	Nelson 1955 criteria	6
				E	13,432	Nelson 1955 criteria	6
Cheng et al, 2014 [74]	Taiwan	RCS	Miscarriage with dilation and curettage and cytogenetic test of product	M	221	Embryonic or anembryonic loss of pregnancy	6
Chien et al, 2011 [14]	USA	RCS	Live singleton births in the whole country with delivery > 20 weeks	GH	3,853,678	NR	6
				E	3,853,678	NR	6
Choong et al, 1995 [33]	Hong Kong	RCS	Live singleton births in hospital without eclampsia and/or chronic hypertension.	PE	19,383	RR > 130/90 mmHg after 20 wks of pregnancy twice while resting in the hospital at least 6 h apart, with or without the presence of protein in a clear-catch midstreum urine specimen (greater than 300 mg 24 h)	6
Chu et al, 2014 [34]	USA	CCS	Women delivering in hospital	PE	48	RR ≥ 140/90 mmHg after 20 weeks with proteinuria (≥300 mg in 24 h urine, 2+ dipstick or 1+ catheterized sample, or protein:creatinine ≥0,3.	6

Table S.7.5 Continued

Cosson et al, 2016 [52]	France	RCS	Singleton births without pre-existing diabetes.	GD	20,149	Fasting plasma glucose value ≥5,3 mmol/L and/or a 2-h plasma glucose value ≥7,8 mmol/L	8
Del Fabro, 2011 [75]	Italy	RCS	Women with ≥ 1 previous miscarriage and a current singleton pregnancy ending in a miscarriage with hospital admittance	M	313	Spontaneous abortion < 22 weeks	6
Ehrlich et al, 2012 [53]	USA	RCS	Live singleton births with a maternal age 15 - 45 yrs at time of delivery	GD	250,120	First screening test, if value ≥7.8 mmol/L then diagnosic test (3h oral glucose tolerance test). If two or more values meeting or exceeding the following cut point: fasting 5.3, 1h 10.0, 2h 8.6, 3h 7.8 mmol/L then diagnosis GD.	7
Engel et al, 2008 [15]	Australia	RCS	Singleton pregnancies in women 13 - 47 yrs	GH	16,445	Mild: BP > 140/90. Moderate: BP > 160/100. Severe: BP > 170/110	6
				GD	16,445	A positive glucose tolerance test and either diet or insulin controlled	6
				PA	15,790	NR	8
Favilli et al, 2013 [16]	Italy	Matched RCS	All singleton deliveries in hospital	GH	623	NR	6
				GD	623	NR	6
				PPH	623	NR	5
Heckbert et al, 1988 [54]	USA	CCS	Live singleton births in the state of Washington	GD	1,278	NR	5
Hou et al, 2014 [17]	China	PCS	Singleton births > 27 weeks	GH	109,722	NR	6
				PE / E	109,722	NR	6
				GD	109,722	Fasting level and levels 1 and 2 h after OGTT above: 5.1, 10.0, and 8.5 mmol/L respectivley	7
				PA	109,722	NR	6

Table S.7.5 Continued

Jakobovits et al, 1988 [65]	Hungary	RCS	Singleton pregnancies	PA	26,858	NR	5
Janssen et al, 1996 [55]	USA	CCS	Live singleton births without Down syndrome	GD	17,794	NR	5
Juberg et al, 1976 [18]	USA	RCS	Live singleton deliveries	GH	3,246	Rise in the SBP of at least 30 mmHg or in the DBP of 15 mmHg or when the SBP was ≥ 140 or DBP ≥ 90 mmHg	7
				PE	3,246	Gestational hypertension with proteinuria (0.3 g / L in 24 hours)	7
Kale et al, 2005 [56]	India	CCS	Live singleton births	GD	439	WHO 1998 criteria	5
Khalil et al, 2013 [35]	Libya	RCS	Live singleton deliveries > 28 weeks without neonatal death and congenital anomalies	PE	28,140	NR	6
				GD	28,140	NR	6
Lao et al, 2011 [36]	Hong Kong	RCS	Singleton deliveries > 23 weeks	PE	66,443	NR	6
				GD	66,443	NR	6
Lawlor et al, 2009 [57]	UK	PCS	Live singleton births with ≥ 1 year infant survival	GD	10,179	Diagnosis in the medical records of gestational diabetes in any women with no history of existing diabetes	7
Li et al, 2016 [19]	China	PCS	Singleton birth without pre-existing (chronic) hypertension	GH	6,223	De novo hypertension (blood pressure ≥ 140 mmHg systolic, and/ or 90 mmHg diastolic) after 20 week of gestation.	8
				PE	6,223	Gestational hypertension accompanied by proteinuria (≥0,3 g/24 h)	8
Lisonkova et al, 2013 [37]	Canada	RCS	Singleton deliveries > 20 weeks	PE	456,668	ICD coding (642,2, 642,5, 642,6, 642,7)	7

Table S.7.5 Continued

Liu et al, 2016 [38]	China	RCS	Singleton pregnancies with delivery > 28 weeks without neonatal death, congenital anomalies or after assisted reproductive technology	PE	65,173	NR	8
				GD	65,173	NR	8
				PPH	65,173	NR	8
López-Llera et al, 1990 [50]	Mexico	RCS	All singleton pregnancies with eclampsia and convulsions with delivery > 24 weeks	E	777	Hypertension, proteinuria and convulsions	5
				PA	777	NR	6
Makhseed et al, 1998 [20]	Kuwait	RCS	Singleton and twin deliveries (stratified in results)	GH	9,504	ACOG definition	6
				PE	9,504	ACOG definition	6
Myers et al, 2015 [39]	UK, Ireland, Australia and New Zealand	CCS	Singleton pregnancies	PE	600	SBP >140 mmHg or DBP >90 mmHg or both on at least 2 occasions 4 hours apart after 20 wks of gestation with either proteinuria (24-h >300 mg/mmol creatinine or urine dipstick protein > ++ or any multisystem complication of PE.	6
Oken et al, 2016 [58]	USA	PCS	Singleton pregnancies	GD	976	Two or more abnormal values on the OGTT. Abnormal values were: fasting > 95 mg/dL, 1 h > 180 mg/dL, 2h > 155 mg/dL, 3h > 140 mg/dL	8
Okereke et al, 2002 [59]	USA	CCS	Singleton pregnancies without pre-pregnancy diabetes mellitus, chronic hypertension, congenital malformation in newborn or pre-eclampsia. Exclusion of smokers.	GD	78	Criteria of Carpenter and Coustand	6

Table S.7.5 Continued

Peled et al, 2013 [40]	Israel	CCS	Singleton pregnancies admitted for hyperemesis gravidarum and delivery > 24 weeks	PE	545	NR	6
				GD	545	NR	6
				PA	545	NR	6
Persson et al, 2014 [21]	Sweden	RCS	All singleton pregnancies	GH / PE	914,167	NR	6
Quiñones et al, 2005 [41]	USA	RCS	All singleton pregnancies with neonatal birthweight <p10 without structural anomalies and aneuploidy	PE	727	NR	5
Räisänen et al, 2013 [66]	Finland	RCS	All live or stillborn singleton births wit delivery > 22 weeks or birthweight > 500 gr during the first neonatal week	PA	1,162,126	ICD-9 coding (641,2 and ICD-19 045)	7
Retnakaran et al, 2015 [61]	Canada	RCS	Nulligravid women with live singleton birth	GD	642,987	Women without pregestational DM who had diabetes coded on the delivery record.	6
Retnakaran et al, 2015 [60]	Canada	PCS	Singleton pregnancies with indication for OGTT between 24 - 34 weeks.	GD	1,074	Fasting blood glucose ≥ 5,8 mmol/L, 1-h glucose ≥ 10,6 mmol/L, 2-h blood glucose ≥ 9,2 mmol/L, or 3-h blood glucose ≥ 8,1 mmol/L.	7
Reynolds et al, 2012 [42]	USA	PCS	Nulliparous women with live singleton birth without chronich hypertension, gestational hypertension, pre-eclampsia, proteinuria, diabetes and delivery between 25 and 42 weeks. No hispanic and Asian women.	PE	9,317	NR	6
Ricart et al, 2008 [22]	Spain	PCS	Singelton pregnancies without former diagnosis of diabetes mellitus and maternal age between 14-45 years	GH	9,270	NR	7

Table S.7.5 Continued

				GD	9,270	National Diabetes Data Group criteria	7
Roy et al, 2015 [43]	India	CCS	Singleton pregnancies without medical or obstetrical complicated delivering at term. Preterm deliveries were included when women had pre-eclampsia.	PE	189	ACOG definition	6
Schildberger et al, 2016 [67]	Austria	RCS	Inpatient singleton births	PA	444,685	NR	8
Sharifzadeh et al, 2012 [44]	Iran	CCS	Live singelton births between with maternal age 18-35 yrs without PCOS, hyper-andorgenism, oligo-ovulation, systemic disorders like hypertension, reno-vascular disorders, diabetes mellitus, medication use, smoking and drug use	PE	64	Blood pressure of ≥ 140/90 mmHg on two occasions 6h apart plus 24 h urine protein ≥ 300 mg or a random urine protein of 2+ on 2 occasions 6 h apart.	5
Sheiner et al, 2002 [68]	Israel	RCS	Singleton preterm deliveries (22 - 36 weeks)	PA	5,934	Clinical findings such as vaginal bleeding, abdominal pain, uterine contractions, uterine tenderness and fetal distress or death. The diagnosis was confirmed after delivery by the observation of a blood clot behind the placenta	8
Sheiner et al, 2003 [72]	Israel	RCS	Singleton term deliveries (≥ 37 weeks)	PA	72,995	Clinical findings such as vaginal bleeding, abdominal pain, uterine contractions, uterine tenderness and fetal distress or death. The diagnosis was confirmed after delivery by the observation of a blood clot behind the placenta	8
Sheiner et al, 2004 [23]	Israel	RCS	All singleton pregnancies with delivery > 22 weeks and no previous cesarean section	GH / PE	108,995	NR	6

Table S.7.5 Continued

				GD	108,995	NR	6
Shiozaki et al, 2011 [24]	Japan	RCS	Singleton and twin deliveries (stratified in results)	GH	126,538	SBP ≥140 mmHg or DBP≥90 mmHg on two occasions	
				PE	126,538	Hypertension (SBP ≥140 mmHg or DBP≥90 mmHg on two occasions) and proteinuria (≥300 mg/24h or ≥1+ on dipstick on 2 occasions). Severe PE: SBP ≥160 and DBP≥100 and proteinuria ≥2g/24 h or ≥3+ on a dipstick.	7
Spellacy et al, 1985 [62]	USA	RCS	Singleton pregnancies	GD	19,313	NR	7
Tikkanen et al, 2012 [69]	Finland	RCS	All singleton and multiple pregnancies (stratified in results)	PA	1,121,188	ICD-9 (years 1987-1995) or ICD-10 (years 1996-2005)	7
Toivanen et al, 1970 [45]	Finland	RCS	All singleton pregnancies	PE	9,318	Dieckmann criteria	6
Trudell et al, 2015 [46]	USA	RCS	Singleton pregnancies without fetal anomalies and aneuploidy	PE	57,170	NR	6
				GD	57,170	NR	6
Tundidor et al, 2012 [25]	Spain	RCS	Singleton pregnancies with gestational diabetes	GH	2,299	BP >140/90 mmHgde novo after 20 wks	7
Vatten et al, 2004 [47]	Norway	RCS	Singleton pregnancies	PE	1,691,053	An increase in blood pressure after the 20th week of gestation. Either the DBP had > 15 mmHg higher than the level measured before the 20th week, or the SBP had to be > 30 mmHg higher than the level measured before the 20th week in combination with proteinuria.	7

Table S.7.5 Continued

Verburg et al, 2016 [26]	Australia	RCS	Live singleton births	GH / PE	574,358	Blood pressure ≥ 140/90 on two occasions at least 4 h apart or ≥ 170/110 on one occasion ± proteinuria.	8
				GD	574,358	Hospital or laboratory criteria where the test was performed. After 1987: fasting glucose ≥ 5,5 mmol/L and/or 2 hr value ≥ 8 mmol/L.	8
Wandabwe et al, 2005 [70]	Uganda	CCS	Live singleton births	PA	545	Women with abruptio who developed an episode of shock with SBP <90 mmHg and pulse of >100 mmHgwith a small volume. And if she had any one of the following: IV therapy of 2 unit of blood or IV therapy of 2 or more liters fluid. Delivery after 24 weeks.	6
Wandabwe et al, 2010 [48]	Uganda	CCS	Live singleton births	PE / E	434	After 20 wks development of hypertension of 160/110 mmHg or more on 2 occasions, proteinuria of 2+ or more on dipstick and epigastric pain or right upper quadrant pain or tenderness, visual disturbances, severe headache, oliguria, pulmonary oedema, episode of jaundice. Severe pre-eclampsia: eclampsia with a convulsion or generalised fit.	6
Weissmann-Brenner et al, 2015 [71]	Israel	RCS	Term and postterm live singleton births	PA	37,327	NR	6
				PPH	37,327	NR	6

Table S.7.5 Continued

Xiao et al, 2014 [63]	Canada	RCS	Singleton pregnancies with delivery > 24 weeks without pre-existing diabetes, endocrine disorders or other severe maternal illnesses	GD	299	American Diabetes Association 2003	6
Zheng et al, 2016 [27]	China	CCS	Live term singleton births of phenotypically normal neonates without aneuploidy, major fetal abnormalities and without any pregnancy complications	GH	294	Onset of BP ≥ 140 mmHg systolic or ≥ 90 mmHg diastolic without evidence of proteinuria.	6
				PE	294	ACOG definition	6

Quality score assessed by the Newcaste-Ottowa Scale. CCS = case control study. E = eclampsia.
GD = gestational diabetes. GH = gestational hypertension. M = miscarrige. PA = placental abruption.
PCS = prospective cohort study. PE = pre-eclampsia. PPH = postpartum hemorrhage.
RCS = retrospective cohort study.

Table S.7.6 Associations between fetal sex and maternal pregnancy outcomes

First author	Statistical analyses	Subgroups	Tendency towards which sex (M / F / =)	Crude effect estimate (95% CI)	p-value	Covariate adjustment	Adjusted effect estimate (95% CI)	p-value
Gestational hypertension								
Andersen et al. 2016	Logistic regression		F	0.69 (0.38 - 1.25)	0.22			
Baibergenova et al. 2006	Logistic regression		F	1.06 (0.55 - 2.50)	0.87			
Campbell et al. 1983	Logistic regression		M	1.18 (1.09 - 1.27)	< 0.0001			
Chien et al. 2011	Logistic regression		M	0.97 (0.96 - 0.98)	< 0.0001			
Engel et al. 2008	Chi-square	Total	M	1.04 (0.94 - 1.14)	0.46			
		Mild	M	1.04 (0.94 - 1.16)	0.44			
		Moderate	F	0.99 (0.80 - 1.24)	0.95			
		Severe	F	0.94 (0.62 - 1.42)	0.76			
Favilli et al. 2013	Logistic regression		F	1.69 (0.63 - 4.57)	0.43	Maternal age > 40 yrs, weight gain, BMI, gestational diabetes	0.98 (0.43 - 2.25)	0.97
Hou et al. 2014	Logistic regression		F	0.97 (0.91 - 1.02)	0.25			
Juberg et al. 1976	Chi-square		M		0.03			
Li et al. 2016	Logistic regression		F	0.97 (0.78 - 1.21)	0.79			
Makhseed et al. 1998	Logistic regression	Total	M	1.01 (0.86 - 1.20)	0.87			
		Primiparous	F	0.87 (0.65 - 1.17)	0.36			
		Multiparous	M	1.09 (0.89 - 1.33)	0.42			
Persson et al. 2014	Logistic regression	Healthy population	M	1.03 (1.01 - 1.06)	0.003			
		Gestational diabetes	M	1.08 (0.93 - 1.26)	0.31			
		Diabetes mellitus type I	F	0.93 (0.79 - 1.09)	0.35			
		Diabetes mellitus type II	F	0.83 (0.44 - 1.57)	0.56			

Table S.7.6 Continued

Ricart et al. 2008	Logistic regression		M	1.22 (0.91 - 1.63)	0.19
Sheiner et al. 2004	Logistic regression		=	1.00 (0.95 - 1.05)	0.96
Shiozaki et al. 2011	Logistic regression		F	0.88 (0.83 - 0.92)	< 0.0001
Tundidor et al. 2012	Relative risk		F	0.81 (0.55 - 1.20)	NR
Verburg et al. 2016	Relative risk	Total	M	1.05 (1.03 - 1.07)	NR
		25 - 29 wks	F	0.69 (0.58 - 0.81)	NR
		30 - 33 wks	F	0.87 (0.79 - 0.97)	NR
		34 - 36 wks	F	0.93 (0.87 - 0.98)	NR
		37 - 39 wks	M	1.06 (1.04 - 1.09)	NR
		40 - 42 wks	M	1.07 (1.04 - 1.11)	NR
Zheng et al. 2016	Logistic regression		F	0.54 (0.26 - 1.14)	0.11
Pre-eclampsia					
Aibar et al. 2012	Logistic regression		F	0.99 (0.65 - 1.49)	0.94
Aliyu et al. 2012	Logistic regression		F	0.90 (0.79 - 1.03)	0.12
Andersen et al. 2016	Logistic regression	Total	F	0.95 (0.69 - 1.31)	0.76
		Preterm	F	1.04 (0.42 - 2.56)	0.94
		Term	M	1.22 (0.85 - 1.74)	0.29
Basso et al. 2001	Logistic regression		M	0.94 (0.92 - 0.97)	< 0.05
Brettel et al. 2008	Logistic regression		F	1.17 (1.01 - 1.35)	0.03
Campbell et al. 1983	Logistic regression		F	1.08 (0.94 - 1.24)	0.3
Choong et al. 1995	Logistic regression		F	1.45 (1.22 - 1.71)	< 0.0001
Chu et al. 2014	Logistic regression		M	0.60 (0.19 - 1.83)	0.39
Hou et al. 2014	Logistic regression		F	0.95 (0.88 - 1.02)	0.13
Juberg et al. 1976	Chi-square		M		0.06
Khalil et al. 2013	Logistic regression	Total	M	1.04 (0.91 - 1.19)	0.57

Table S.7.6 Continued

		Preterm	F	1.53 (1.07 - 2.20)	0.02			
		Term	M	1.08 (0.93 - 1.25)	0.31			
		Postterm	M	3.46 (1.40 - 8.53)	0.007			
Lao et al. 2011	Logistic regression		F	0.92 (0.81 - 1.06)	0.26			
Li et al. 2016	Logistic regression		F	0.66 (0.45 - 0.98)	0.04			
Lisonkova et al. 2013	Cox regression	< 34 weeks	M	1.10 (1.07 - 1.14)	NR	NR	1.10 (1.06 - 1.14)	NR
		> 34 weeks	M	1.10 (1.07 - 1.14)	NR	NR	1.10 (1.06 - 1.14)	NR
Liu et al. 2016	Logistic regression	Total		0.96 (0.88 - 1.04)	0.31			
		Preterm		1.15 (1.00 - 1.32)	0.046			
Makhseed et al. 1998	Logistic regression	Total	F	0.92 (0.68 - 1.24)	0.57			
		Nulliparous	F	0.74 (0.49 - 1.10)	0.13			
		Multiparous	M	1.20 (0.76 - 1.90)	0.43			
Myers et al. 2015	Logistic regression		=	0.94 (0.65 - 1.36)	0.74			
Peled et al. 2013	Logistic regression		M	1.79 (0.42 - 7.56)	0.43			
Persson et al. 2014	Logistic regression	Healthy population	M	1.03 (1.01 - 1.06)	0.003			
		Gestational diabetes	M	1.08 (0.93 - 1.26)	0.31			
		Diabetes mellitus type I	F	0.93 (0.79 - 1.09)	0.35			
		Diabetes mellitus type II	F	0.83 (0.44 - 1.57)	0.56			
Quiñones et al. 2005	Logistic regression		M	1.15 (0.77 - 1.70)	0.5			
Reynolds et al. 2012	Logistic regression	Total	F	0.85 (0.71 - 1.02)	0.08			
		Preterm	F	1.25 (0.79 - 1.97)	0.34			
		Term	F	0.86 (0.71 - 1.04)	0.13			
Roy et al. 2015	Logistic regression	Total	M	1.28 (0.72 - 2.29)	0.4			
		Preterm	M	0.77 (0.33 - 1.81)	0.55			

Table S.7.6 Continued

		Term	M	1.28 (0.66 - 2.46)	0.46
Sharifzadeh et al. 2012			F	0.88 (0.33 - 2.35)	0.8
Sheiner et al. 2004	Logistic regression		=	1.00 (0.95 - 1.05)	0.96
Shiozaki et al. 2011	Chi-square	Pre-eclampsia	F	0.84 (0.79 - 0.89)	< 0.001
		Pre-eclampsia with fetal death	M	1.21 (0.70 - 1.48)	0.95
		Severe pre-eclampsia	F	1.21 (1.10 - 1.33)	0.001
		Severe pre-eclampsia with fetal death	F	1.14 (0.67 - 1.93)	0.63
Toivanen et al. 1970	Logistic regression		M	1.20 (1.06 - 1.37)	0.005
Trudel et al. 2015	Logistic regression		M	1.01 (0.95 - 1.07)	0.82
Vatten et al. 2004	Logistic regression	Total	M	1.05 (1.03 - 1.07)	< 0.0001
		Preterm (< 37 wks)	F	1.17 (1.11 - 1.22)	< 0.0001
		Term (37 - 42 wks)	M	1.06 (1.04 - 1.08	< 0.0001
		Postterm (>42 wks)	M	1.07 (0.96 - 1.18)	0.23
		25 - 29 weeks	F	1.55 (1.31 - 1.83)	< 0.0001
		30 - 33 wks	F	1.33 (1.21 - 1.46)	< 0.0001
		34 - 36 wls	F	1.07 (1.01 - 1.14)	0.03
		37 - 39 wks	F	0.98 (0.85 - 1.01)	0.18
		40 - 42 wks	M	1.10 (1.07 - 1.13)	< 0.0001
Verburg et al. 2016	Relative risk	Total	M	1.05 (1.03 - 1.07)	NR
		25 - 29 wks	F	0.69 (0.58 - 0.81)	NR
		30 - 33 wks	F	0.87 (0.79 - 0.97)	NR
		34 - 36 wks	F	0.93 (0.87 - 0.98)	NR

Table S.7.6 Continued

		37 - 39 wks	M	1.06 (1.04 - 1.09)	NR			
		40 - 42 wks	M	1.07 (1.04 - 1.11)	NR			
Wandabwe et al. 2010	Logistic regression		F	0.65 (0.45 - 0.95)	0.03			
Zheng et al. 2016	Logistic regression	Total	F	0.49 (0.27 - 0.89)	0.02			
		Mild	F	0.65 (0.30 - 1.43)	0.29			
		Severe	F	2.60 (1.18 - 5.73)	0.02			
Eclampsia								
Aibar et al. 2012	Logistic regression		M	1.54 (0.50 - 4.72)	0.45			
Aliyu et al. 2012	Logistic regression		F	0.92 (0.42 - 2.01)	0.83			
Campbell et al. 1983	Logistic regression		F	0.89 (0.35 - 2.32)	0.82			
Chien et al. 2011	Logistic regression		=	1.00 (0.97 - 1.04)	0.89			
Hou et al. 2014	Chi-square		M		0.13			
Llopez-Lera et al. 1990	Chi-square		M		< 0.05			
Persson et al. 2014	Logistic regression	Healthy population	M	1.03 (1.01 - 1.06)	0.003			
		Gestational diabetes	M	1.08 (0.93 - 1.26)	0.31			
		Diabetes mellitus type I	F	0.93 (0.79 - 1.09)	0.35			
		Diabetes mellitus type II	F	0.83 (0.44 - 1.57)	0.56			
Wandabwe et al. 2010	Logistic regression		F	0.65 (0.45 - 0.95)	0.03			
Gestational diabetes								
Aibar et al. 2012	Logistic regression		M	1.21 (1.06 - 1.37)	0.0034			
Breschi et al. 1993	Logistic regression		F	0.96 (0.36 - 2.52)	0.93			
Cosson et al. 2016	Logistic regression		=	1.00 (0.93 - 1.08)	0.96			
Ehrlich et al. 2012	Logistic regression		M	1.02 (0.99 - 1.05)	NR	Maternal ethnicity	1.02 (0.99 - 1.05)	NR
						Maternal ethnicity, education and age	1.02 (0.99 - 1.05)	NR

Table S.7.6 Continued

Engel et al. 2008	Logistic regression	M	1.07 (0.85 - 1.36)	0.54			
Favili et al. 2013	Logistic regression	M	2.36 (0.58 - 9.61)	0.37	Maternal age >40 yrs, BMI, weight gain, gestational hypertension	0.95 (0.37 - 2.46)	0.92
Heckbert et al. 1988	Logistic regression	F	0.97 (0.77 - 1.21)	0.79			
Hou et al. 2014	Logistic regression	M	1.01 (0.96 - 1.07)	0.61			
Janssen et al. 1996	Logistic regression	M	1.02 (0.96 - 1.08)	0.5			
Kale et al. 2005	Logistic regression	M	1.64 (1.12 - 2.40)	0.01			
Khalil et al. 2013	Logistic regression	M	1.41 (1.15 - 1.72)	< 0.001			
Lao et al. 2013	Logistic regression	M	1.05 (0.99 - 1.12	0.12			
Lawlor et al. 2009	Logistic regression	M	1.61 (0.92 - 2.81)	0.09			
Liu et al. 2016	Logistic regression	M	1.08 (1.00 - 1.16)	0.048			
Oken et al. 2016	Logistic regression	M	1.39 (0.81 - 2.36)	0.23			
Okereke et al. 2002	Logistic regression	M	1.39 (0.81 - 2.36)	0.23			
Peled et al. 2013	Logistic regression	M	3.24 (0.65 - 16.22)	0.15			
Retnakaran et al. 2015	Logistic regression	M	1.03 (1.00 - 1.05)	0.047			
Retnakaran et al. 2015	Logistic regression	M	1.24 (0.92 - 1.67)	0.16			
Ricart et al. 2008	Logistic regression	M	1.05 (0.91 - 1.22)	0.17			
Sheiner et al. 2004	Logistic regression	M	1.07 (1.01 - 1.12)	0.01			
Spellacy et al. 1985	Chi-square	M		NS			
Trudel et al. 2015	Logistic regression	F	0.96 (0.90 - 1.04)	0.32			
Verbug et al. 2016	RR	M	1.04 (1.01 + 1.07)	NR			
Xiao et al. 2014	Logistic regression	M	1.29 (0.58 - 2.89)	0.53			
Placental abruption							
Aliyu et al. 2012	Logistic regression	F	0.98 (0.87 - 1.12)	0.8			
Brettel et al. 2008	Logistic regression	M	1.29 (0.97 - 1.71)	0.08			
Engel et al. 2008	Logistic regression	F	0.53 (0.28 - 0.99)	0.049			

Table S.7.6 Continued

Hou et al. 2014	Logistic regression		F	0.98 (0.83 - 1.15)	0.76			
Jakobovits et al. 1988	Chi-square	Total	M		NS			
		17 - 20 yrs	M		< 0.001			
		21 - 25 yrs	M		< 0.01			
		26 - 30 yrs	F		NS			
		31 - 35 yrs	M		< 0.05			
		36 - 40 yrs	M		< 0.05			
		41 - 42 yrs	=		NS			
Lopez-Llera et al. 1990	Logistic regression		M	0.94 (0.54 - 1.66)	0.84			
Peled et al. 2013	Logistic regression		M	2.90 (0.76 - 11.03)	0.12			
Raissanen et al. 2013	Logistic regression	Total	M	1.19 (1.12 - 1.26)	< 0.0001			
		Nulliparous	M	1.23 (1.12 - 1.36)	< 0.0001	NR	1.36 (1.23 - 1.51)	
		Multiparous	M	1.16 (1.08 - 1.26)	0.001	NR	1.38 (1.27 - 1.50)	
Schildberger et al. 2016	Logistic regression		F	0.84 (0.81 - 0.87)	< 0.0001			
Sheiner et al. 2002	Logistic regression		F	0.98 (0.78 - 1.24)	0.88			
Sheiner et al. 2004	Logistic regression		M	1.15 (0.89 - 1.49)	0.28			
Tikkanen et al. 2012	Logistic regression		M	1.18 (1.11 - 1.25)	< 0.0001			
Wandabwe et al. 2005	Logistic regression		M	2.20 (1.20 - 4.90)	< 0.01	Distance to hospital, age, type of house, hypertension, previous caesarean section, previous stillbirth	1.90 (1.00 - 3.80)	NR
Weissmann - Brenner et al. 2015	Logistic regression	Total	M	1.20 (0.77 - 1.87)	0.42			
		Age < 40 yrs	M	1.14 (0.73 - 1.79)	0.56			
		Age > 40 yrs	M	5.08 (0.24 - 106.0)	0.29			

Table S.7.6 Continued

Postpartum hemorrhage					
Favili et al. 2013	Logistic regression	Total	M	1.12 (0.34 - 3.72)	0.85
		Age ≥ 40 yrs	M	2.10 (0.40 - 11.01)	0.38
		Age < 40 yrs	F	0.35 (0.04 - 3.37)	0.36
Weissmann - Brenner et al. 2015	Logistic regression	Total	M	1.20 (0.88 - 1.65)	0.25
		Age ≥ 40 yrs	M	1.16 (0.84 - 1.61)	0.35
		Age < 40 yrs	M	4.07 (0.45 - 36.5)	0.21
Liu et al. 2016	Logistic regression		F	0.91 (0.83 - 0.99)	0.0046
Miscarriage					
Byrne et al. 1987	Risk ratio	Total	M		< 0.05
		Morphological normal	M		< 0.05
		Morphological abnormal	F		> 0.05
Cheng et al. 2014	Risk ratio		F		< 0.001
Del Fabro et al. 2011	Risk ratio	Total	F		< 0.05
		4 - 10 wks	F		< 0.001
		11 - 15 wks	F		0.07
		16 - 20 wks	F		0.06

Table S.7.7 Pooled odds ratios of the occurrence of maternal pregnancy complications by study characteristics

	No.				p value for
Subgroup	**Studies**	**Participants**	**OR (95% CI)**	**I^2 (p-value)**	**Heterogeneity**
Gestational hypertension					
Geographical location					
Western	11	5,511,340	1.02 (0.98;1.06)	81.6% (<0.001)	0.30
Non-Western	5	125,016	0.99 (0.95;1.02)	0% (0.51)	
No of participants					
< 10.000	8	30,853	1.01 (0.98;1.05)	13.2% (0.33)	0.47
≥ 10.000	8	5,605,503	0.96 (0.85;1.10)	86.9% (<0.001)	
Study design					
Case-control	1	294	0.54 (0.26;1.14)	NA	
Retrospective cohort	11	5,508,737	1.02 (0.98;1.05)	80.9% (<0.001)	0.19
Prospective cohort	4	127,325	0.98 (0.89;1.08)	16.7% (0.31)	
Quality score					
< 7	11	5,489,916	1.03 (1.01;1.05)	55.8% (0.012)	< 0.001
≥ 7	5	146,440	0.92 (0.81;1.05)	35.5% (0.19)	
Pre-eclampsia (total)					
Geographical location					
Western	15	3,472,444	1.03 (1.00;1.05)	53.3% (0.008)	< 0.001
Non-Western	14	541,647	0.90 (0.83;0.97)	72.5% (<0.001)	
No of participants					
< 10.000	13	39,373	0.92 (0.78;1.08)	58.5% (0.004)	0.84
≥ 10.000	16	3,974,718	0.97 (0.94;1.01)	84.7% (<0.001)	
Study design					
Case-control	7	2,174	0.86 (0.64;1.16)	38.8% (0.13)	
Retrospective cohort	18	3,884,545	0.98 (0.95;1.02)	83.0% (<0.001)	0.12
Prospective cohort	4	127,372	0.90 (0.81;1.00)	29.5% (0.24)	
Quality score					
< 7	22	1,538,622	0.97 (0.93;1.02)	55.6% (0.046)	0.71
≥ 7	7	2,475,469	0.95 (0.88;1.02)	NA	

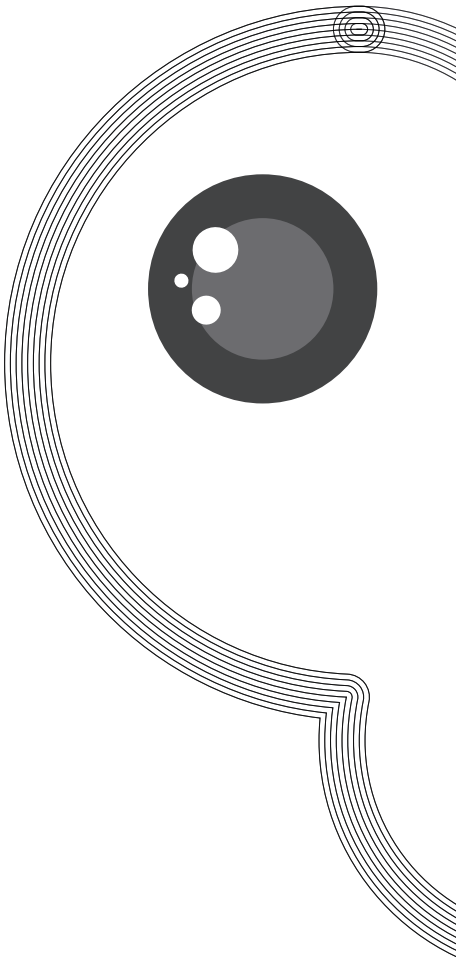
Table S.7.7 Continued

Eclampsia					
Geographical location					
Western	5	4,820,821	1.02 (1.00;1.04)	0% (0.64)	0.05
Non-Western	2	110,156	0.82 (0.57;1.18)	73.8% (0.05)	
No of participants					
< 10.000	1	434	0.65 (0.45;0.94)	NA	0.02
≥ 10.000	6	4,930,534	1.01 (0.99;1.04)	14.8% (0.32)	
Study design					
Case-control	1	434	0.65 (0.45;0.95)	NA	
Retrospective cohort	5	4,820,821	0.95 (0.88;1.02)	0% (0.64)	0.01
Prospective cohort	1	109,722	1.02 (1.00;1.04)	NA	
Quality score					
< 7	6	4,920,963	1.00 (0.95;1.04)	55.6% (0.046)	0.84
≥ 7	1	10,014	0.92 (0.42;2.01)	NA	
Gestational diabetes					
Geographical location					
Western	16	1,632,560	1.03 (1.01;1.05)	23.2% (0.19)	0.17
Non-Western	8	379,756	1.09 (1.02;1.15)	39.9% (0.14)	
No of participants					
< 10.000	10	15,111	1.16 (1.02;1.33)	16.3% (0.29)	0.14
≥ 10.000	14	1,997,205	1.04 (1.02;1.06)	52.8% (0.01)	
Study design					
Case-control	5	1,062	1.15 (0.94;1.40)	56.8% (0.06)	
Retrospective cohort	12	2,009,749	1.04 (1.02;1.06)	33.5% (0.12)	0.66
Prospective cohort	7	1,505	1.16 (1.01;1.33)	59.2% (0.02)	
Quality score					
< 7	18	1,091,263	1.05 (1.02;1.09)	49.5% (0.009)	0.75
≥ 7	6	921,053	1.04 (1.01;1.07)	28.6% (0.22)	
Placental abruption					
Geographical location					
Western	7	2,876,604	1.03 (0.86;1.23)	39.9% (0.14)	0.45
Non-Western	6	227,068	1.10 (0.93;1.31)	96.2% (<0.001)	
No of participants					
< 10.000	4	7,801	1.31 (0.85;2.02)	55.2% (0.08)	0.40
≥ 10.000	9	3,095,871	1.04 (0.90;1.22)	95.0% (<0.001)	
Study design					

Table S.7.7 Continued

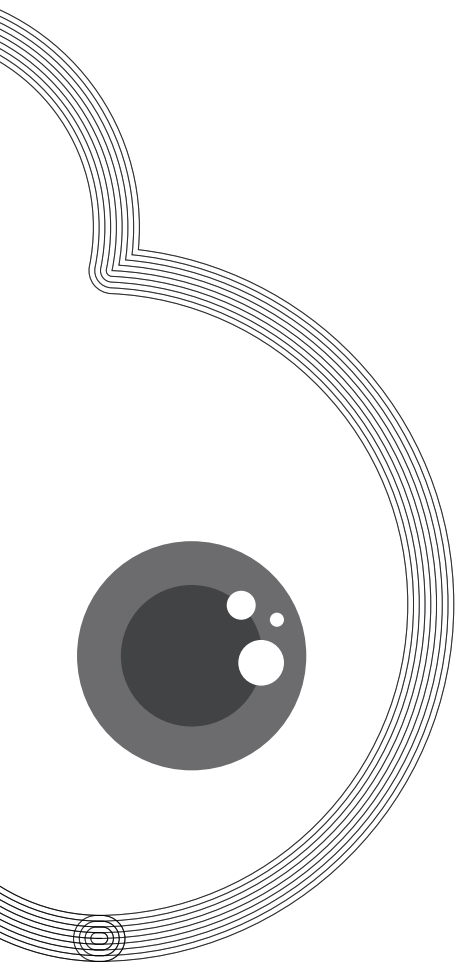
Case-control	2	1,090	2.34 (1.25;4.35)	0% (0.72)	
Retrospective cohort	10	2,992,860	1.05 (0.90;1.22)	94.4% (<0.001)	0.08
Prospective cohort	1	109,722	0.98 (0.83;1.15)	NA	
Quality score					
< 7	6	224,641	1.21 (0.96;1.51)	44.8% (0.11)	0.20
≥ 7	7	2,879,031	1.01 (0.85;1.19)	96.1% (<0.001)	

NA = not available



Chapter 8

General discussion



The aim of the present thesis entitled 'Fetal sex dependency in pregnancy' was to evaluate fetal sex specific differences on placental, fetal and maternal level and to assess different definitions on fetal growth restriction and their associations with childhood outcomes.

All studies described in this thesis were embedded in The Generation R Study, a prospective cohort study from early pregnancy onwards in Rotterdam, The Netherlands (1, 2). Generation R aims to gain insight into environmental determinants of growth and development from fetal life until young adulthood and in maternal health during and after pregnancy. Eventually, the results will contribute to the development of strategies for optimizing health and healthcare for both pregnant women and their children.

The main findings of this thesis are summarized in **Table 8.1**. Below we discuss the underlying mechanisms, consequences for clinical practice and further recommendations following these findings.

Underlying mechanisms

The first and second aim of this thesis concerned fetal growth restriction (FGR) and its association with childhood outcomes and the search for a biomarker to retrospectively assess FGR. Fetal growth is a highly dynamic and multifactorial process which makes it difficult to study. Previous research has shown that a fetus does not have a stable growth trajectory from early pregnancy onwards (3). Fetal growth characteristics track moderately during pregnancy with stronger tracking coefficients present in later pregnancy. Although this low tracking of fetal growth during pregnancy could be due to measurement error in fetal ultrasound measurement, which is higher in early versus late pregnancy, it may also suggest unstable fetal growth trajectories. In this thesis we showed that FGR from the second trimester onwards is associated with adverse childhood health (**Chapter 3**). There may be critical periods of growth in pregnancy that influence the development of cardiovascular disease in later life. Since the highest development rates are during embryogenesis in the first trimester of pregnancy, previous research has focused on first trimester growth and cardiovascular outcomes during childhood (4). Smaller embryonic size was indeed associated with an increased risk of clustering of cardiovascular risk factors in childhood. This thesis however shows that not only first trimester growth or size is of importance but that also the growth trajectory during and after the second trimester of birth is highly relevant. Growth trajectories and hence FGR are hard to define without any internationally consensus. Therefore

Table 8.1 Overview main findings

Fetal sex		Fetal growth	
Male	**Female**	**SGA**	**FGR**
Fetal growth			
AC ↑ HC ↑ FL ↓ AC growth pattern = Different HC growth pattern FL growth pattern = Different EFW growth pattern	AC ↓ HC ↓ FL ↑ AC growth pattern = Different HC growth pattern FL growth pattern = Different EFW growth pattern		
Delivery outcomes			
		APGAR ↓ Admittance NICU ↑ Instrumental delivery ↑ Emergency CS ↑	APGAR = Admittance NICU = Instrumental delivery = Emergency CS =
Neonatal growth / cardiovascular health			
HC ↑ Body length ↓ Body weight ↑ / ↓ Different HC growth pattern Body length growth pattern = Different body weight growth pattern	HC ↓ Body length ↑ Body weight ↑ / ↓ Different HC growth pattern Body length growth pattern = Different body weight growth pattern	Accelerated growth ↑ BMI ↓ SBP = DBP = AOD ↓ LVM ↓ PWV =	Accelerated growth ↑ BMI ↓ SBP = DBP = AOD ↓ LVM ↓ PWV =
Biomarkers			
PlGF ↓ s-Flt1 ↓ PAI-2 ↓	PlGF ↑ s-Flt1 ↑ PAI-2 ↑		Fetal PlGF ↓
Maternal vascular adaptation			
PI-UtA 2nd trimester ↑ PI-UtA 3rd trimester ↑ Notching 3rd trimester ↑ Different bloodpressure patterns	PI-UtA 2nd trimester ↓ PI-UtA 3rd trimester ↓ Notching 3rd trimester ↓ Different blood pressure patterns		
Maternal pregnancy complication			
Pre-eclampsia (overall) ↑ Preterm pre-eclampsia ↓ Term pre-eclampsia ↑ Eclampsia ↑ PIH ↑ GD ↑	Pre-eclampsia (overall) ↓ Preterm pre-eclampsia ↑ Term pre-eclampsia ↓ Eclampsia ↓ PIH ↓ GD ↓		

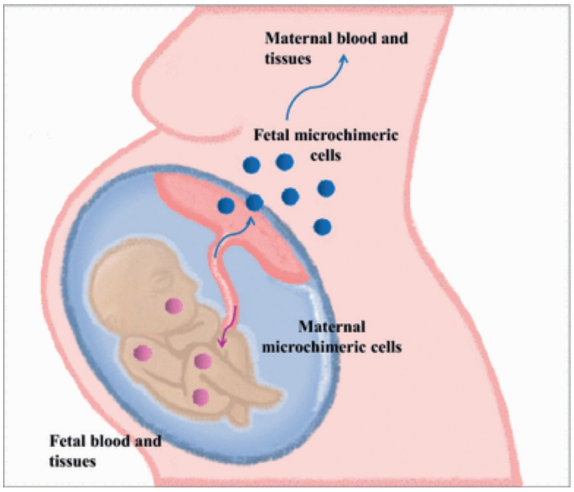
AC, abdominal circumference; AOD, aortic root diameter; BMI, body mass index; CS, cesarean section; DBP, diastolic blood pressure; FL, femur length; GD, gestational diabetes; HC, head circumference; LVM, left ventricular mass; NICU, neonatal intensive care unit; PAI-2, plasminogen activator inhibitor 2; PIH, pregnancy induced hypertension; PI-UtA, uterine artery pulsatility index; PlGF, placental growth factor; PWV, pulse wave velocity; SBP, systolic bloodpressure; s-Flt1, soluble fms like tyrosine kinase 1

in this thesis several cut-offs were used ranging from a decrease in growth of 30 to 50 percentiles between the second trimester of pregnancy and birth (**Chapter 3**). For some outcomes clear trends were available, in which a greater decrease in growth was associated with a greater effect on the outcome. Nevertheless results were already significant with the cut-off of 30 percentiles. Due to measurement error in fetal ultrasound measurement it is possible that a decrease of 30 percentiles is not a true decrease in growth but a result of this measurement error. Nevertheless significant associations with infant growth and childhood cardiovascular health were found, indicating that even mild FGR may have adverse effects on future health.

The third aim of this thesis was to evaluate fetal sex specific differences on a placental, fetal and maternal level. Fetal sex specific differences were found on all these three levels. Proposed mechanisms by which the presence of a male fetus could affect the risk of placenta mediated pregnancy complications include abnormal implantation, fetomaternal histoincompatibility as a result of an antigen present on the Y chromosome and altered hormonal levels (e.g. testosterone) (5-8). Either the maternal system is capable of detecting whether it is carrying a male or female fetus or the fetus itself manipulates the maternal system in such a way that it is beneficial for its own fetal sex specific development. Either way all suggest an intensive interplay between the placenta, the fetus and the mother. This interplay is demonstrated by the fact that maternal vascular function differs in relation to fetal sex (**Chapter 6**) (9). Corticotropin releasing hormone (CRH) induced dilation and basal blood flow are significantly enhanced in normotensive women pregnant with a male fetus relative to women pregnant with a female fetus. Moreover there are sex-specific differences in the production of proteins such as human chorionic gonadotropin leptin and testosterone from the fetoplacental unit which may have differential effects upon maternal physiology and possibly maternal vascular function. This thesis has shown that within the placental hormone production fetal sex specific differences exist, in which women carrying a female fetus have higher serum levels of PlGF, s-Flt1 and PAI-2 in the first trimester of pregnancy (**Chapter 3**). These placental biomarkers are part of the pathway between the association of fetal sex with the uterine artery pulsatility index, one of the parameters of maternal vascular adaptation to pregnancy (**Chapter 4**). Hence, one of the ways in which the fetus can communicate with its mother in a fetal sex specific way is via these placental biomarkers.

Another way of the maternal body to detect fetal sex besides levels of placental hormones is by fetal microchimerism (FM). Fetal microchimerism is characterized by the presence and persistence of fetal cells in maternal organs and in the circulation without any apparent graft-versus-host reaction or graft rejection as depicted in **Figure 8.1** (10-12).

Figure 8.1 Fetal microchimerism



Source: Cirello V et al, J Cancer Res Clin Oncol 2016. Printed with approval.

Fetal microchimerism is found to be a common event in pregnancy with the number of fetal cells progressively increasing during gestation with a peak at delivery and a decrease in the postpartum period (13-15). In healthy women, fetal cells are generally found in the mature cell compartment of the peripheral blood, hence in the vascular system already during pregnancy until decades after the delivery (10, 13, 16-18). Moreover fetal cells have also been found in endothelial progenitor cells (19). In current medical practice this is used in the Non Invasive Prenatal Test (NIPT), which aims to detect trisomy 21 (Down Syndrome), 13 (Patau Syndrome) and 18 (Edwards Syndrome), and in fetal Rhesus D typing in placental cells within the maternal circulation (20). Several hypotheses have been formulated to explain the role of microchimerism in women. It has been speculated that the presence of fetal cells may induce inflammatory responses with subsequent tissue damage (21). One example is the presence of syncytial aggregates in the lungs of women who experienced pre-eclampsia contributing

to the generalized endothelial dysfunction (ref PMID 23817495). Since there are fetal sex specific differences in maternal inflammatory response to pregnancy this is a very interesting theory. Moreover placentation is a highly immunological process. If due to inflammatory responses placentation occurs suboptimal this may have consequences for placenta mediated pregnancy outcomes which are indeed fetal sex specific (**Chapter 2**) (22). Many studies have reported a higher degree of placental inflammation in the presence of a male fetus. Histological examination has shown that male placentas obtained from pregnancies that led to spontaneous preterm births have more severe lesions of chronic inflammation than placentas from matched females (23). Mothers of male neonates born preterm have higher circulating levels of pro-inflammatory cytokines but lower levels of the anti-inflammatory cytokines IL-10 and G-CSF (24). IL-10 is of specific interest, since lower levels of IL-10 are associated with pregnancy loss which is in line with the higher rate of miscarriages in male embryos and thus the possibility of impaired placentation (**Chapter 2**) (25).

Some hypothesize that fetal microchimerism is merely a response to maternal microchimerism i.e. trafficking of cells from the mother to the fetus. The female fetus would react stronger to the presence of maternal cells compared to the male fetus (26). This hypothesis is in line with results found in this thesis, suggesting that female fetuses are more prone to adapting to environmental factors, at the expense of fetal growth (**Chapter 5**). Hence, female fetuses are more often born SGA (27-33). Moreover female placentas are protected against adverse effects such as hyperhomocysteinemia (**Chapter 3**). The downside of the fact that male fetuses are more focused on growth is that prenatal and prostnatal mortality in male fetuses and neonates is higher due to less capacity to adapt to their environment (8, 34-38).

Clinical practice and recommendations

This thesis makes clear that fetal sex is an underestimated but important factor in pregnancy. Not only for the fetus itself but also for the mother and possible pregnancy complications. Currently in research focused on pregnancy complications, placental biology and maternal adaptation to pregnancy fetal sex is not taken into account and results on male and female pregnancies are pooled. Future research should focus on physiological differences between pregnancies with either a male or female fetus before pathophysiological differences can be identified. To do so all studies regarding maternal adaptation to pregnancy, placental biology, fetal growth and pregnancy complications should stratify for fetal sex.

When investigating fetal growth accurate pregnancy dating is crucial. It has been assumed that embryos follow the same growth pattern in early pregnancy, as illustrated by the current practice of pregnancy dating using crown rump length (CRL) (41, 42). Recent research, however, has shown that embryonic growth is dependent of many factors, including maternal age, lifestyle (smoking behavior, alcohol use, folic acid intake) and even embryonic sex (43). Therefore pregnancy dating on the basis of the last menstrual period (LMP) in a women who has a reliable and regular menstrual cycle is preferable. However, in about 40% of pregnancy the LMP can't be used since the date is not known, women have only recently stopped the use of oral contraceptives, or report to have irregular or prolonged menstrual cycles (44, 45). Even when the LMP is known and the cycle was regular, there may be subtle variations in true gestational age due to early or delayed ovulation, fertilization or implantation (44-48). Hence customized embryonic growth charts are necessary which take all these environmental factors into account to accurately date pregnancies in the first trimester of pregnancy.

In addition to challenges in pregnancy dating, different definitions are used to describe FGR. In many cases the term SGA is wrongfully used to define FGR. It is important to create awareness that both terms are not synonymous to each other. Where FGR describes a deviating or altered growth pattern, SGA merely describes the endpoint of fetal growth. Even when defining SGA multiple definitions are used, using different cut-offs of gestational age and fetal sex adjusted birth weights with or without the combination of other parameters like the fetal AC or the pulsatility index of the middle cerebral artery. It is highly important to create uniformity in order to compare studies on this topic. When interested in growth patterns instead of birth weight more research is needed to define a deviating growth pattern. In order to do so studies are required using multiple measurements to first acknowledge a normal growth pattern before defining a deviating growth pattern. For example by investigating fetal growth patterns of uncomplicated pregnancies of which the children have normal postpartum and childhood health outcomes. Moreover it is important to focus on adverse childhood outcomes, and if possible even adult outcomes, when defining this deviating growth pattern. The mechanisms underlying FGR and identification of the variables that have an adverse effect on fetal growth and therefore on future health provide possibilities to develop new strategies for identification of groups at risk and even prevention.

Due to the fact that FGR is mainly described by birth weight, potentially combined with other parameters, adverse growth patterns leading to a lower birth weight but within normal limits are now missed. The same birth weight may be the result of various fetal exposures and growth patterns. This thesis has shown that also these FGR fetuses who are born AGA constitute a vulnerable group with increased risks of accelerated growth and altered cardiovascular outcomes in childhood and possibly in their future lives.

In the most optimal situation FGR can be predicted and precautions can be made to prevent or reduce FGR or at least monitor fetal growth and intervene when necessary. Unfortunately methods for prediction and prevention of FGR are limited. The second best option is at least to acknowledge FGR after delivery. Since FGR doesn't always leads to low birth weight diagnosis FGR afterwards is challenging. Although this thesis showed that umbilical PlGF levels are associated with birth weight, fetal growth patterns and FGR more research is needed focused not solely on PlGF but also other biomarkers. Longitudinal research is needed to investigate whether a potential biomarker is not only able to retrospectively detect FGR but also to predict adverse health in infancy and childhood in order to identify those children at risk.

Increasingly placental biomarkers are of specific interest in the early identification or prediction of hypertensive pregnancy complications such as pre-eclampsia. Since the placenta also has a sex and this thesis has shown that at least some of these biomarkers are fetal sex specific it is plausible that also other biomarkers are different in male versus female pregnancies. This could lead to different test characteristics in which one biomarker has a higher sensitivity and/or specificity to identify a placenta mediated pregnancy complication in a female pregnancy compared with a male pregnancy or vice versa. This phenomenon should be studied more intensively before these biomarkers are used in clinical practice.

Interestingly, treatment of pregnancy-related disorders might also be fetal sex dependent. From previous research focused on non-pregnancy-related disorders (i.e. asthma) we know that symptomatology and the need for treatment during pregnancy can be fetal sex dependent. The presence of a female fetus is associated with increased inhaled corticosteroid requirements over pregnancy, decreased lung function, increased asthma symptoms and increased concentrations of systemic circulating monocytes compared to pregnancies with a male fetus (39, 40). The use of inhaled steroids by pregnant asthmatic women is beneficial for female fetal growth only and not for male

fetal growth (40). So not only requirement of treatment but also the effect of treatment is fetal sex dependent. Since asthma is an immune modulated disease and placentation is an immune modulated process this might imply that the treatment of placenta mediated pregnancy complications is fetal sex dependent as well. So far no studies have been performed to investigate whether treatment for pre-eclampsia (e.g. anti-hypertensive drugs) is sexual dimorphic. If so this phenomenon could lead to more patient tailored medical practice.

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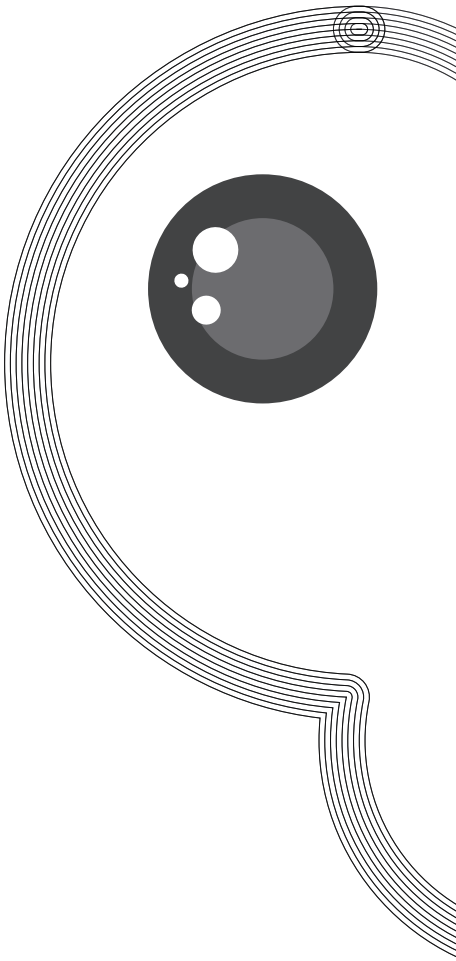
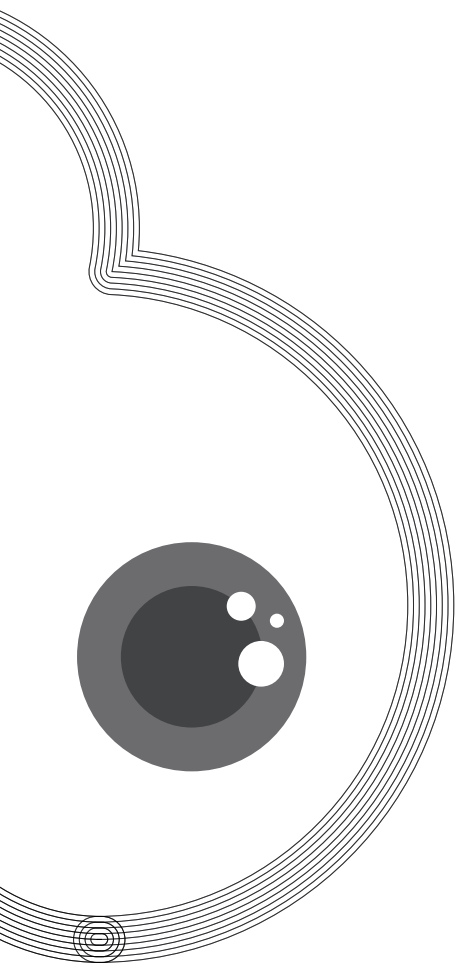


Chapter 9

Summary / Samenvatting



Summary

Part I describes the background and hypotheses for the studies presented in this thesis. Placentation and subsequent placental function play a central role in pregnancy course and outcome since the placenta forms the active interface between the maternal and fetal blood circulation. The placenta not only regulates maternal physiological changes during pregnancy but also fetal nutrient supply and fetal development. Impaired placentation leading to abnormal placental perfusion and placental dysfunction may be a key factor in the development of placental mediated pregnancy complications such as pre-eclampsia (PE) and small for gestational age (SGA). Pregnancy course on its turn is important for future maternal and fetal health. Since not only the fetus but also the placenta has a sex, sexual dimorphism in placental physiology and function and therefore placental mediated pregnancy complications have been described.

In **Part II** we evaluate fetal sex specific differences on a fetal level and on fetal growth. In **Chapter 2** we emphasize the difference between SGA and fetal growth restriction (FGR). We define SGA as a birth weight below the 5th percentile and FGR as a decrease of at least 40 percentiles between the second trimester estimated fetal weight (EFW) and birth weight. FGR occurs in approximately 10% of neonates born with an appropriate for gestational age (AGA) birth weight. Of all FGR fetuses, 90% is born AGA. Nevertheless FGR is, just as SGA, associated with accelerated growth at the age of two years and altered cardiovascular outcomes at six years. This study emphasizes that despite birth weight, a deviating growth curve is associated with adverse health in childhood and therefore possibly adulthood. In **Chapter 3** we assess the association between umbilical cord blood PlGF concentrations and fetal growth measured by birth weight, fetal growth pattern and FGR defined as described in **Chapter 2**. Lower PlGF levels are associated with a lower birth weight, with different fetal growth patterns and with FGR defined as a deviating growth curve. Therefore, cord blood PlGF might be a promising biomarker to determine deviations in fetal growth and FGR retrospectively enabling follow-up of these neonates in the postnatal period. In **Chapter 4** we show that already in the first trimester of pregnancy, male crown-rump-length (CRL) is larger as compared with female CRL. In the second and third trimester of pregnancy head and abdominal circumference are larger in male fetuses, while femur length is larger in female fetuses. Repeated measurements analyses show different growth patterns on head circumference and estimated fetal weight between male and female fetuses. Postnatally these different growth patterns persist. Body weight is higher in males until the age of 12 months, where after females have a higher body weight.

Part III focuses on the evaluation of fetal sex specific differences on a placental and maternal level. In **Chapter 5** we show that the concentrations of the placental biomarkers PlGF, soluble fms-like tyrosine kinase (s-Flt1) and plasminogen activator inhibitor (PAI-2) are higher in pregnancies with a female fetus as compared with a pregnancy with a male fetus. However, in pregnancies complicated with pre-eclampsia, spontaneous preterm birth (sPTB) or SGA these fetal sex specific differences are not observed. This suggests that other mechanisms causing these complications may dominate the effect of fetal sex. Furthermore interaction analyses are performed which show that the concentrations of PlGF, s-Flt1 and PAI-2 decrease in male placentas in the case of hyperhomocysteinemia but remain equal in female placentas. This suggests that female placentas are more protected against adverse effects and implies fetal sex dependent placental gene-environment interaction. **Chapter 6** evaluates fetal sex specific differences in maternal vascular adaptation to pregnancy as assessed by blood pressure, the pulsatility index of the uterine artery (PI-UtA) and presence of notching in the uterine artery. Differential blood pressure patterns are observed between pregnancies with a male or female fetus as well as between uncomplicated pregnancies and pregnancies complicated with either PE, sPTB and/or SGA. In pregnancies with a male fetus, PI-UtA and the occurrence of notching was higher as compared with pregnancies with a female fetus. In **Chapter 7** current literature on fetal sex specific differences in maternal pregnancy complications (i.e. gestational hypertension, pre-eclampsia, eclampsia, gestational diabetes, placental disruption and post-partum haemorrhage) are systematically reviewed and meta-analyzed. We show that most maternal pregnancy complications are associated with the presence of male fetus, with the exception of pre-term pre-eclampsia which is associated with the presence of a female fetus. This validates the hypothesis that carrying a male fetus is accompanied with a higher cardiovascular and metabolic load for the mother resulting in these pregnancy complications and possibly adverse health in later life.

In **Chapter 8** we discuss the general conclusions, underlying mechanisms and implications of the studies in this thesis. We conclude that fetal sex specific differences are found in pregnancy on a placental, maternal and fetal level. Possible underlying mechanisms all suggest an intensive interplay between the placenta, the fetus and the mother. Since currently fetal sex is not taken into account in research, we recommend that all studies regarding maternal adaptation to pregnancy, placental biology, fetal growth, pregnancy complications and pharmacological therapies should stratify for fetal sex.

Furthermore it is of high significance to realize that SGA is not synonymous to FGR and that FGR not resulting in low birthweight can have the same effect on childhood health as SGA. Hence, detecting a deviating growth pattern during or after pregnancy is of importance.

Samenvatting

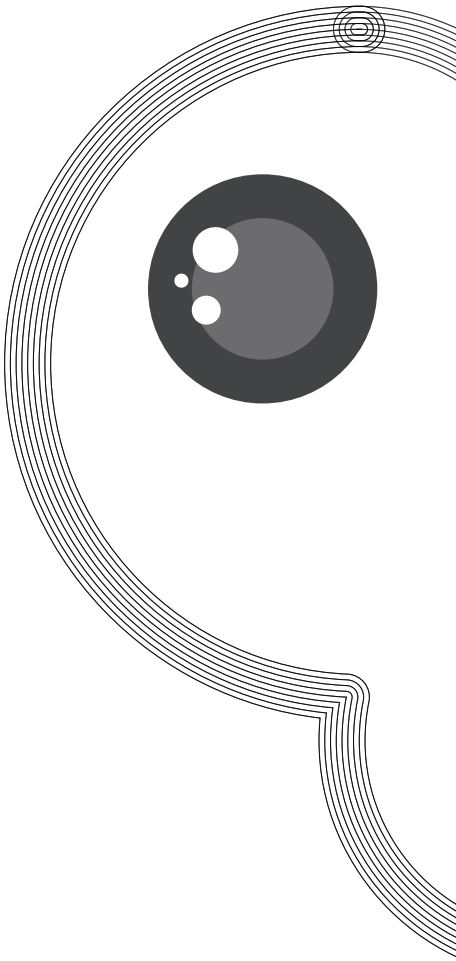
Deel I beschrijft de achtergrond en de hypothese voor de studies die gepresenteerd worden in dit proefschrift. De moederkoek, ofwel placenta, vormt de schakel tussen de bloedcirculatie van moeder en kind en is daarom erg belangrijk voor het zwangerschapsbeloop. De placenta reguleert niet alleen de normale en noodzakelijke lichamelijke veranderingen tijdens de zwangerschap in de moeder maar is ook verantwoordelijk voor de aanvoer van voedingsstoffen en daarmee de ontwikkeling van het ongeboren kind. Een minder goed aangelegde placenta kan als gevolg hebben dat deze minder goed doorbloed is en daardoor ook minder goed functioneert. Dit zou een sleutelrol kunnen spelen in het ontstaan van enkele zwangerschapscomplicaties zoals zwangerschapsvergiftiging (ofwel pre-eclampsie) of een kindje met een te laag geboortegewicht. Daarnaast is het zwangerschapsbeloop ook belangrijk voor de gezondheid van de moeder ná de bevalling. Aangezien niet alleen het ongeboren kind maar ook de placenta een geslacht heeft, zijn eerder geslachts-specifieke verschillen in placentafunctie en daarmee zwangerschapscomplicaties beschreven.

In **Deel II** kijken we naar geslachts-specifieke verschillen voor het kind en naar de groei van het ongeboren kind. In **Hoofdstuk 2** benadrukken we het verschil tussen een te laag geboortegewicht en een afbuigende groeicurve. Pasgeborenen met een te laag geboortegewicht kunnen gedurende de zwangerschap altijd al klein zijn geweest. Het is echter ook mogelijk dat ze eerst normaal van gewicht waren en vervolgens door bepaalde factoren, zoals roken of ziekte van de moeder, afbuigen in groei met als gevolg een te laag geboortegewicht. Op deze manier is het mogelijk dat pasgeborenen met hetzelfde geboortegewicht toch een ander groeipatroon hadden tijdens de zwangerschap. Van alle pasgeborenen met een afbuigende groeicurve wordt 90% desondanks geboren met een normaal geboortegewicht. Echter, deze kinderen hebben net als de kinderen met een te laag geboortegewicht inhaalgroei op de leeftijd van twee jaar en veranderde hart- en vaatuitkomsten op de leeftijd van zes jaar. Deze studie benadrukt dat ondanks het geboortegewicht, een afbuigende groeicurve gerelateerd is aan gezondheidsuitkomsten in de kinderleeftijd en daarmee mogelijk ook op de volwassen leeftijd. In **Hoofdstuk 3** hebben we gekeken of een bepaalde biomarker (placental growth factor [PlGF]) uit het navelstrengbloed de kinderen met een afbuigende groeicurve kon identificeren. Lage concentraties PlGF bleken gerelateerd te zijn aan een lager geboortegewicht, met een ander groeipatroon en met een afbuigende groeicurve. Daarom zou navelstreng-PlGF een potentiële biomarker kunnen zijn om veranderingen vast te stellen in

groeipatroon en achteraf, ten tijde van de geboorte, wat follow-up van deze kinderen mogelijk maakt. In **Hoofdstuk 4** tonen we aan dat al in het eerste trimester van de zwangerschap, de kop-stuit-lengte groter is bij jongetjes ten opzichte van meisjes. Dit is van belang aangezien deze kop-stuit-lengte bij alle vrouwen in Nederland wordt gemeten bij de termijnecho en waarmee de uitgerekende datum wordt berekend. In het tweede en derde trimester van de zwangerschap is de hoofd- en buikomtrek groter bij jongens, terwijl het bovenbeen langer is bij meisjes. Dit zijn belangrijke echometingen voor het meten van de groei van het ongeboren kind. Daarnaast is ook het groeipatroon van de hoofdomtrek en het geschatte gewicht anders bij jongens dan bij meisjes. Na de geboorte bleven deze verschillen bestaan. Het gewicht van jongens is namelijk hoger tot de leeftijd van 12 maanden; daarna is het gewicht van meisjes hoger.

Deel III kijkt naar geslachts-specifieke verschillen in de placenta en in de moeder. In **Hoofdstuk 5** wordt aangetoond dat de concentraties van de bepaalde biomarkers die gemeten worden om de placentafunctie te bepalen hoger zijn in meisjes-zwangerschappen in vergelijking met jongens-zwangerschappen. Echter, in geval van zwangerschapsvergiftiging, vroeggeboorte of een te laag geboortegewicht zijn deze verschillen niet meer te zien. Dit wekt de suggestie dat er andere mechanismen zijn die deze complicaties veroorzaken en het effect van het geslacht overschaduwen. **Hoofdstuk 6** onderzoekt geslachts-specifieke verschillen in de vasculaire aanpassing van moeder tot de zwangerschap, gemeten door middel van de bloeddruk en de weerstand in de baarmoederslagader (PI-UtA). Vrouwen zwanger van een jongen hebben een ander bloeddrukpatroon tijdens de zwangerschap in vergelijking met vrouwen die zwanger zijn van een meisje. In jongens-zwangerschappen is de PI-UtA hoger in vergelijking met meisjes-zwangerschappen. In **Hoofdstuk 7** is samengevat en geanalyseerd wat er tot op heden bekend is over geslachts-specifieke verschillen in zwangerschapscomplicaties voor de moeder (i.e. zwangerschaps-hypertensie, zwangerschapsvergiftiging, zwangerschapssuiker, loslating van de placenta en ruim bloedverlies na de bevalling). We laten zien dat de meeste zwangerschapscomplicaties voorkomen bij jongens-zwangerschappen, met als uitzondering de vroege zwangerschapsvergiftiging welke juist vaker voorkomt bij meisjes-zwangerschappen. Dit bevestigt de veronderstelling dat jongens-zwangerschappen een grotere belasting zijn voor het vaatsysteem en de stofwisseling van de moeder wat deze zwangerschapscomplicaties als gevolg heeft.

In **Hoofdstuk 8** bespreken we de algemene conclusies, de onderliggende mechanismes hiervan en de gevolgen. We concludeerden dat geslachts-specifieke verschillen in de zwangerschap worden gevonden voor zowel de placenta, de moeder als het ongeboren kind. Mogelijke onderliggende mechanismen wijzen allemaal in de richting van intensieve wisselwerking tussen de placenta, de moeder en het ongeboren kind. Aangezien op dit moment het geslacht van het ongeboren kind niet wordt meegenomen in onderzoek, adviseren wij dat in al het onderzoek gericht op de aanpassing van moeder tot de zwangerschap, placentafunctie, groei en zwangerschapscomplicaties resultaten apart worden weergegeven voor jongens- en meisjeszwangerschappen. Verder is van groot belang te bedenken dat een laag geboortegewicht niet per se gelijk is aan een afbuigende groeicurve maar dat een afbuigende groeicurve wel dezelfde gevolgen voor de gezondheid in de kinderleeftijd kan hebben. Daarom is het belangrijk om een afbuigende groeicurve tijdens of na de zwangerschap vast te stellen.



Chapter 10

Authors and affiliations
List of abbreviations
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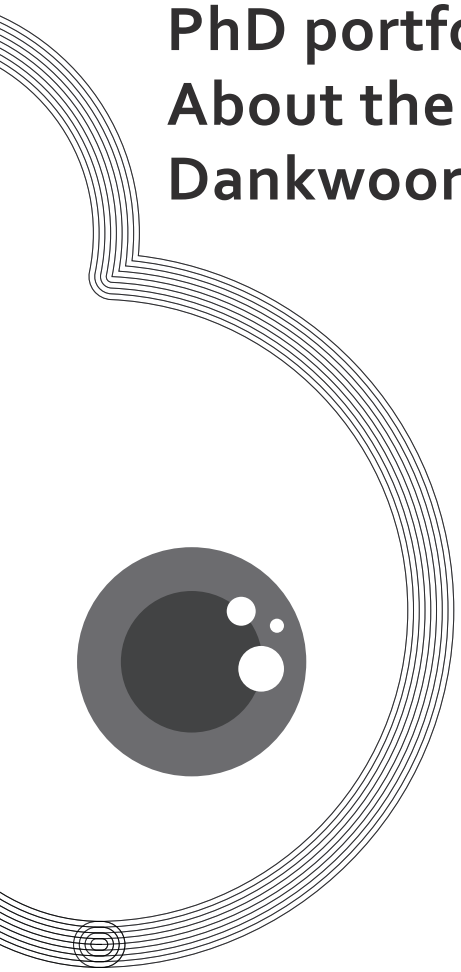
Dankwoord



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List of abbreviations

AC	Abdominal circumference
AGA	Appropriate for gestational age
ANOVA	Analysis of variance
AOD	Aortic root diameter
BMI	Body mass index
BPD	Biparietal diameter
CI	Confidence interval
CpG	Cytosine-guanine
CRL	Crown rump length
CS	Cesarean section
DBP	Diastolic blood pressure
DM	Diabetes mellitus
DNA	Desoxyrinonucleinezuur
DOHaD	Developmental Origion of Health And Disease
EFW	Estimated fetal weight
FGR	Fetal growth restriction
FL	Femur length
FM	Fetal microchimerism
GA	Gestational age
G-CSF	Granulocyte colony-stimulating factor
GD	Gestational diabetes
HC	Head circumference
hCG	Human chorionic gonadotrophin hormone
IUFD	Intra uterine fetal death
IL	Interleukin
IVSTD	Intraventricular septum thickness in diastole
LBW	Low birth weight
LGA	Large for gestational age
LMP	Last menstrual period
LVIDD	Left ventricular internal diameter in diastole
LVM	Left ventricular mass
LVPWTD	Left ventricular posterior wall thickness in diastole
MoM	Multiple of the median
mRNA	Messenger DNA
NA	Not applicable
NICU	Neonatal intensive care unit

NIPT	Non invasive prenatal test
NO	Nitric oxide
NS	Not significant
OR	Odds ratio
PA	Placental abruption
PAI-2	Plasminogen activator inhibitor 2
PE	Pre-eclampsia
PIH	Pregnancy induced hypertension
PI	Pulsatility index
PI-UtA	Pulsatility index of the uterine artery
PlGF	Placental growth factor
PPH	Post partum haemorrhage
PTB	Preterm birth
PWV	Pulse wave velocity
RI-UtA	Resistance index of the uterine artery
SAS	Statistical Analysis System
SBP	Systolic blood pressure
SD	Standard deviation
SDS	Standard deviation score
s-Flt1	Soluble fms-like tyrosine kinase 1
SGA	Small for gestational age
SLE	Systemic lupus erythematosus
SPSS	Statistical Package Social Sciences
sPTB	Spontaneous preterm birth
tHcy	Total homocystein
UtA	Uterine artery
VEGF	Vascular endothelial growth factor
Wks	Weeks
Yrs	Years

List of publications

Z.A. Brown, Y.V. Louwers, S. Lie Fong, O. Valkenburg, E. Birnie, F.H. de Jong, B.C.J.M. Fauser, J.S.E. Laven
The phenotype of polycystic ovary syndrome (PCOS) ameliorates with aging.
Fertility and Sterility 2011;96:1259-65

Z. A. Brown, S. Schalekamp – Timmermans, H.W. Tiemeier, A. Hofman, V.W.V. Jaddoe, E.A.P. Steegers
Fetal sex specific differences in human placentation: a prospective cohort study.
Placenta 2014;35:359-64

Z.A. Broere – Brown, S. Schalekamp – Timmermans, A. Hofman. V.W.V. Jaddoe, E.A.P. Steegers
Fetal sex dependency of maternal vascular adaptation to pregnancy.
BJOG 2016;123:1087-95

Z.A. Broere – Brown, E. Baan, S. Schalekamp – Timmermans, B.O. Verburg, A. Hofman, V.W.V. Jaddoe, E.A.P. Steegers
Sex-specific differences in fetal and infant growth patterns.
Biology of Sex Differences 2016;7:65-73

Z.A. Broere – Brown, S. Schalekamp – Timmermans, V.W.V. Jaddoe, E.A.P. Steegers
The discrepancy between small for gestational age and fetal growth restriction on childhood outcomes.
Submitted

Z.A. Broere – Brown, S. Schalekamp – Timmermans, V.W.V.Jaddoe, E.A.P. Steegers
Placenta growth factor (PlGF) cord blood levels and fetal growth.
Fetal Diagnosis and Therapy 2017, in press

Z.A. Broere – Brown, L. Benschop, M.J. Tielemans, S. Schalekamp - Timmermans, T.Muka, R. Gonçalves, W. Bramer, J. Schoufour, T. Voortman, E.A.P. Steegers, O. Franco Duran
Fetal sex and maternal pregnancy outcomes: a systematic review and meta-analysis
Submitted

Z.A. Broere – Brown, L. Benschop, R. Goncalves, J. Schoufour, M.J. Tielemans, T. Voortman, S. Schalekamp – Timmermans, E.A.P. Steegers, O. Franco Duran
Fetal sex and fetal and neonatal pregnancy complications: a systematic review and meta-analysis
In preparation

L. Benschop, **Z.A. Broere – Brown**, S. Schalekamp – Timmermans, V.W.V. Jaddoe, E.A.P. Steegers, J.M. Roberts, R.E. Gandley
Placental growth factor and cardiovascular adaptation during and six years after pregnancy
Submitted

PhD Portfolio

Name PhD student:	Z.A. Broere - Brown
Research school:	NIHES
Erasmus MC Department:	Obstetrics and Gynecology
PhD period:	2012 - 2017
Promotores:	Prof. Dr. E.A.P. Steegers and Prof. Dr. V.W.V. Jaddoe
Co-promotor:	Dr. S. Schalekamp – Timmermans

	Year	Workload (ECTS)
1. PhD training		
General courses		
Master's Degree Health Sciences, specialisation Clinical Epidemiology, NIHES, Erasmus University Rotterdam, the Netherlands	2013 - 2015	70
Erasmus Summer Programme:		
Principles of Research in Medicine	2013	0,7
Clinical Decision Analysis	2013	0,7
Methods of Public Health Research	2013	0,7
Markers and Prognostic Research	2013	0,7
The Practice of Epidemiologic Analysis	2013	0,7
Health economics	2013	0,7
Logistis Regression	2014	1,4
Introduction to Bayesian Methods in Clinical and Epidemiological Research	2014	1,4
Causal inference	2014	0,7
Social Epidemiology	2014	0,7
Core curriculum:		
Study design	2013	4,3
Biostatistical Methods I: Basic Principles	2013	4,3

Clinical Epidemiology	2015	5,7
Methodologic Topics in Epidemiologic Research	2014	1,4
Biostatistical Methods II: Classical regression models	2014	4,3
Advanced courses		
Women's Health	2013	0,9
Courses for the Quantitative Researcher	2013	1,4
Epidemiology of Infectious Diseases	2014	1,4
Repeated Measurements	2014	1,4
Missing Values in Clinical Research	2014	0,7
General academic skills		
Biomedical English Writing and Communication	2014	3,0
Seminars and Workshops		
Generation R research meetings	2012-2016	4,0
Generation R Maternal and Child Health meetings	2012-2016	4,0
(Inter)national conferences		
IPPIC Colaborators Meeting, Birmingham, Groot-Brittannie	2016	0,3
Nederlandse Vereniging Obstetrie en Gynaecologie. Gynaecongres Eindhoven. *Oral presentation.*	2016	0,3
RCOG onderzoeksdag / Wladimiroff Symposium, Erasmus MC, Rotterdam. *Oral presentation.*	2016	0,3
Sophia Onderzoeksdag, Rotterdam. *Oral presentation.*	2016	0,3
SRI 63rd Annual Scientific Meeting Montreal, Canada. *Poster presentations.*	2016	1,4
Seminar Groote Schuur Hospital, Kaapstad, Zuid-Afrika. *Oral presentation.*	2015	0,3

DOHaD 9th World Congress, Kaapstad, Zuid-Afrika. *Oral presentations.*	2015	1,4
Bi-annual symposium 'Developmental Origins of Health and Disease', Utrecht.	2015	0,3
ISSHP 13th World Congress, New Orleans, USA. *Oral presentation.*	2014	1,4
Sophia Onderzoeksdag, Rotterdam. *Poster presentation.*	2014	0,3
Nederlandse Werkgroep Pre-eclampsie (NEDWEP) bijeenkomst, Groningen. *Oral presentation.*	2014	0,3
Nederlandse Vereniging Obstetrie en Gynaecologie. Gynaecongres Leeuwarden. *Oral presentations.*	2014	0,3
SGI 61th Annual Scientific Meeting Florence, Italy. *Poster presentations.*	2014	1,4
RCOG onderzoeksdag / Wladimiroff Symposium, Erasmus MC, Rotterdam. *Oral presentation.*	2014	0,3
ISSHP European Congress, Tromso, Norway. *Poster presentation.*	2013	1,4
RCOG onderzoeksdag / Wladimiroff Symposium, Erasmus MC, Rotterdam. *Oral presentation.*	2013	0,3
Developmental Origins of Health and Disease (DOHaD), Rotterdam, the Netherlands	2012	0,7

Awards	
Juriy Wladimiroff Onderzoeksprijs 2016	2016
Juriy Wladimiroff Onderzoeksprijs 2014	2014
ISSHP award for best posterpresentation in the category 'clinical research' 2013	2013

Reviewing papers		
Review paper for PLoS ONE (1)	2014	0,2
Review papers for European Journal of Epidemiology (3)	2015-2016	0,6
Review paper for Reproduction, Fertility and Development (1)	2015	0,2

Review paper for Journal of Reproductive Immunology (1)	2016	0,2

2. Teaching

Tutor for first year medical students		1,0
Supervising Master's thesis		
Esme Baan, Medical Student, Erasmus MC, the Netherlands.	2014	2,0
Project title: Fetal sex specific differences in fetal growth.		
Abigail Maduro, Medical Student, Erasmus MC, the Netherlands.	2015	2,0
Project title: GSTP1 SNP and the placental syndrome.		
Eni Manoku, Medical Student, Erasmus MC, the Netherlands.	2015	1,0
Project title: Maternal vascular adaptation to pregnancy in a multiple pregnancy		
Romy Goncalves, Medical Student, Erasmus MC, the Netherlands.	2016	2,0
Project title: Maternal vascular adaptation to pregnancy in singleton versus twin pregnancies.		

About the author

Zoe Anne Broere - Brown was born on July 11th 1984 in Southampton (UK) and grew up in Tilburg. In 2003, she finished her secondary school (VWO) at the Theresia Lyceum. In the same year she started medical school at the Erasmus Medical Center in Rotterdam and obtained her medical degree with honor in 2011. After her graduation, she worked as a resident not in training at the department of Obstetrics and Gynecology in the Maasstad Ziekenhuis in Rotterdam (dr.A. Verhoeff). In 2012 she started her PhD project at the Department of Obstetrics and Gynecology at the Erasmus Medical Center University Hospital and the Generation R Study under the supervision of professor Steegers, professor Jaddoe and dr. Schalekamp – Timmermans. The studies performed during her PhD are described in this thesis. During her PhD project she graduated from the Research Master 'Clinical Epidemiology'. Since april 2017 she is a gynecologist in training and returned to the Maasstad Ziekenhuis in Rotterdam (dr. P. Timmers).

Zoe Anne Broere – Brown werd geboren op 11 juli 1984 in Southampton (GB) en groeide op in Tilburg. In 2003 slaagde zij voor haar VWO op het Theresia Lyceum. In hetzelfde jaar startte zij met haar studie geneeskunde aan het Erasmus Medisch Centrum en behaalde haar arts-examen cum laude in 2011. Na haar afstuderen heeft ze een jaar als ANIOS gewerkt op de afdeling Gynaecologie en Obstetrie in het Maasstad Ziekenhuis in Rotterdam (dr.A. Verhoeff). In 2012 begon zij met haar promotietraject op de afdeling Verloskunde en Vrouwenziekten in het Erasmus Medisch Centrum onder begeleiding van professor Steegers, professor Jaddoe en dr. Schalekamp – Timmermans. De onderzoeken die tijdens haar promotie-traject zijn verricht zijn beschreven in dit proefschrift. Tijdens haar promotie-traject behaalde zij de onderzoeks master 'Clinical Epidemiology'. Per april 2017 is zij gynaecoloog in opleiding en is teruggekeerd naar het Maasstad Ziekenhuis te Rotterdam (dr. P. Timmers).

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En dan is het moment aangebroken om het laatste hoofdstuk van je proefschrift te schrijven. Een hoofdstuk wat niet alleen een proefschrift afsluit maar een periode van bijna 5 jaar. Ik maak daarom graag van de gelegenheid gebruik om hier de mensen te bedanken die voor mij het verschil hebben gemaakt in mijn promotietraject.

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De leden van de **kleine commissie,** bestaande uit Prof.dr. D. Tibboel, Prof.dr. I.K.M. Reiss en Prof.dr. E. Lopriore. Dank voor tijd en de moeite die u heeft genomen om mijn proefschrift kritisch door te nemen en te beoordelen. In het bijzonder dank aan Prof.dr. D. Tibboel voor het optreden als secretaris van de kleine commissie.

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