First trimester prediction of the Small for Gestational Age fetus

Karagiannis, Georgios

Awarding institution:
King's College London

First trimester prediction of the Small for Gestational Age fetus

MD (Res)

Dr. Georgios Karagiannis

ACKNOWLEDGEMENTS

I would like to thank Professor Nicolaides for giving me the opportunity to be part of his research team and supporting me in producing this thesis. I would also like to thank Miss Poon, Mr. Akolekar and Mr. Kametas for giving me valuable assistance, support and education during my work in Harris Birthright Unit.

In addition, I would like to dedicate this thesis to my parents and sister and thank them for their unconditional love and support in pursuing my career aspirations.

ABSTRACT

Objective: In this thesis, we investigate the association between maternal factors, biophysical and biochemical markers and the delivery of small-for-gestational-age (SGA) neonates in the absence of preeclampsia (PE) at 11–13 weeks' gestation. We evaluate their performance as predictors of SGA, both in isolation and combined, in an effort to develop a model for the prediction of SGA in the first trimester of pregnancy.

Methods: Screening study in 1,536 SGA and 31,314 non-SGA pregnancies based on maternal characteristics, fetal nuchal translucency (NT) thickness, serum pregnancy-associated plasma protein-A (PAPP-A) and free β-human chorionic gonadotrophin (β-hCG). We also measured mean arterial pressure (MAP), uterine artery pulsatility index (PI) and performed case-control studies for measurement of maternal serum concentration of placental growth factor (PLGF), placental protein 13 (PP13), A Disintegrin And Metalloprotease (ADAM12), Soluble endoglin (sENG) and thyroid hormones (thyroid stimulating hormone (TSH), free triiodothyronine (FT3) and free thyroxine (FT4)). Regression analysis was used to develop a model for the prediction of SGA.

Results: In the SGA group, uterine artery PI and MAP were increased, serum PAPP-A, free β-hCG, PLGF, PP13, and ADAM12 and fetal NT were decreased while sENg and thyroid hormones were not significantly altered. At a false positive rate of 10%, the estimated detection rate by a combination of maternal factors and biophysical and biochemical markers at 11–13 weeks was 73% for SGA requiring delivery before 37 weeks and 46% for those delivering at term.

Conclusions: Half of the pregnancies with SGA neonates in the absence of PE could potentially be identified at 11–13 weeks.

PERSONAL CONTRIBUTION

I worked as a research fellow in Harris Birthright Fetal medicine Unit from August 2008 until August 2010. During this time I was extensively involved in clinical and research activities. My involvement is outlined below:

Data collection

- I performed first-trimester screening at King's Hospital. A proportion of patients that I assessed were included in the study population.
- I consented patients for inclusion in the studies, took their clinical history and recorded the data in an electronic database.
- I performed the first-trimester scan which involved measuring the ultrasound markers used in the studies (CRL, NT, uterine artery PI).
- I measured their weight, height and Blood pressure.
- I extracted serum blood for analysis of biochemical markers.
- Performed telephone follow-up of cases from which data was missing from our database.
- Performed systematic manual checking of paper patient notes in cases of missing data from the electronic files.

Laboratory work

- I worked in the unit laboratory where processing and storing of the serum samples was taking place.
- I centrifuged and extracted serum from fresh blood and stored it systematically including managing the logistics of appropriate transfer to the freezing facilities.
- I selectively extracted frozen samples from the stores, thawed them according to the recommended technique and run the analysis of various analytes in the DELFIA XPRESS analyzer (as described in the methods section).
- I collaborated with external laboratories for the analysis of analytes that we did not have the ability to process in-house.

Data processing and analysis

- Performed queries to retrieve data from Viewpoint and other clinical electronic databases in order to compile the final dataset.
- Performed formatting and ``clean up`` of raw data files so that they can be used for further analysis.
- In the publication: `` Reference range of birthweight with gestation and first-trimester prediction of small-for-gestation neonates`` the majority of the statistical analysis was performed by Miss L. Poon. I assisted in formatting and preparing the data files used for the analysis.
- In the publication ``Prediction of small-for-gestation neonates from biophysical and biochemical markers at 11-13 weeks`` I performed the descriptive statistics, parametric, non-parametric comparisons and regression analysis, in collaboration with Mr. R. Akolekar and under the guidance of Professor David Wright.
- In the publication ``Maternal thyroid function at eleven to thirteen weeks of gestation and subsequent delivery of small for gestational age neonates`` I performed the descriptive statistics, parametric and non-parametric comparisons, and regression analysis in collaboration with Miss N. Maiz.
- In the case of the analyte sENG which was not included in any published study prior to this thesis, I also performed the descriptive statistics, parametric and non-parametric comparisons, and regression analysis.

Literature review and authorship

- Performed literature search for identification, critical evaluation and summary of all topics discussed in this thesis:
    - Pathophysiological theories around FGR.
    - Screening for SGA/FGR.
    - Evidence around the use of biophysical and biochemical markers in other studies.
- I contributed significantly in the authorship of all publications that the chapters are based on. In addition, I was the sole author of any additional material seen in the thesis which does not appear in the publications.

CONTENTS

FIGURE LEGENDS

Figure 6.1 Receiver operating characteristics curves in the prediction of SGA neonates in the absence of PE by maternal factors only and by a combination of maternal factors, uterine artery PI, MAP, fetal NT, maternal serum PAPP-A, free β-hCG, PLGF, PP13, and ADAM.

TABLE LEGENDS

ADAM12 MoM and Δ- NT thickness, in pregnancies delivering non-SGA and SGA neonates

Table 7.1 Definitions of FGR from Delphi procedure

ABBREVIATIONS

11-13 weeks	11^{+0} – $13+^{6}$ weeks
AC	Abdominal circumference
ADAM12	A-Disintegrin and Metalloprotease-12
AGA	Appropriate for gestational age
Ang	Angiotensin
aOR	Adjusted odds ratio
ART	Artificial reproductive techniques
AUC	Area under the curve
AUROC	Area under the receiver operating characteristic
BMI	Body mass index
BP	Blood pressure
BW	Birth weight
CEMACE	Confidential enquiry into maternal deaths in the UK
CI	Confidence interval
CRL	Crown-rump length
DBP	Diastolic blood pressure
EFW	Estimated fetal weight
ELISA	Enzyme-linked immunosorbent assay
FGR	Fetal growth restriction
FPR	False positive rate
FT3	Free triiodothyronine
FT4	Free thyroxine
TSH	Thyroid stimulating hormone
GA	Gestational age
GH	Gestational Hypertension

HDL	High-density lipoprotein
HIC	Hypoxic ischaemic encephalopathy
HIF	Hypoxia-inducible factor
IGF	Insulin growth factor
IQR	Interquartile range
IVF	In vitro fertilization
IVH	intraventricular haemorrhages
KDR	Kinase domain receptor
LDL	Low-density lipoprotein
LH	Luteinizing hormone
LR	Likelihood ratio
MAP	Mean arterial pressure
MoM	Mean of median
NEC	Necrotizing enterocolitis
NK	Natural killer
NPV	Negative predictive value
NT	Nuchal translucency
OR	Odds ratio
PAPPA	Pregnancy associated plasma protein A
PCR	Polymerase chain reaction
PDGF	Platelet-derived growth factor
PE	Preeclampsia
Pg	Prostaglandin
PI	Pulsatility index
PLGF	Placental growth factor
PP13	Placental protein 13
PPV	Positive predictive value

RCOG	Royal college of obstetricians and gynaecologists
RDS	Respiratory distress syndrome
RI	Resistance index
ROC	Receiver operating characteristic
RR	Relative risk
SD	Standard deviation
sENG	Soluble Endoglin
SFH	Symphisio-fundal height
sFlt-1	Soluble fms-like tyrosine kinase -1
SGA	Small for gestational age
SLE	Systemic lupus erythematosus
ß-hCG	Beta human chorionic gonadotropin
TGF	Transforming growth factor
UA	Uterine artery
UAD	Uterine artery doppler
UAPI	Uterine artery pulsatility index
VEGF	Vascular endothelial growth factor
VLDL	Very low-density lipoprotein

CHAPTER 1. INTRODUCTION

1.1 OVERVIEW

Fetal growth is dependent on a complex and dynamic interaction between the mother, the placental unit, and the fetus itself. Parental genetics determine a theoretical optimum weight which is subsequently modulated by the endocrine and nutritional environments in utero (Cetin & Alvino 2009).

Fetal growth restriction is a term used to describe a pathological process which restricts the fetus from reaching its optimum genetic potential. Reduction in fetal growth has been associated with a plethora of different factors and has traditionally been considered under four main headings: maternal, fetal, environmental and placental (Table 1). The pathophysiological mechanisms that potentially explain the above associations are multiple and are still subject of research and debate but many share a common endpoint which is an inability of the placenta to provide adequate oxygen and nutrient transfer to the fetus (Lackman *et al*., 2001, Battalia & Regnault 2001, Cetin & Alvino 2009).

Growth restriction secondary to placental insufficiency is considered to be a major contributor to perinatal morbidity and mortality, being responsible for 50% of perinatal deaths occurring preterm and 20% at term (Kady & Gardosi 2005). In addition, it is well recognized that growth restricted fetuses carry increased risks of metabolic, cardiovascular and neurological diseases into adult life (Gluckman *et al*., 2008). It commonly presents in combination with the maternal syndrome of preeclampsia but can also be seen in the absence of maternal disease (Srinivas *et al.,* 2009). When

fetal growth restriction manifests early in pregnancy, marked abnormalities of placental function are typically evident. Usually this process takes a recognized course of progression with gradual changes in the blood flow pattern through the placenta and can be detected via Doppler ultrasound (Baschat 2005). In near-term FGR these abnormalities are far more subtle and may escape clinical detection (Miller *et al.,* 2008).

Small for gestational age is a descriptive term which denotes that a fetus weighs less than a defined standard of normality, usually a population average for a given gestation. The SGA group is used in an attempt to identify fetuses that are at increased risk of growth restriction, however, its sensitivity as a marker varies and studies have shown it can be as low as 30% (Alberry & Soothil 2007). Despite this poor correlation, SGA fetuses have been found to be at increased risk of adverse perinatal outcomes and their identification prenatally has been shown to improve fetal outcomes (Figueras & Gratacos 2014). The SGA group has been used traditionally as an outcome measure in studies because of its association with FGR and because it is easily available from birth records.

In this thesis we aim to investigate different modalities in an attempt to develop a screening tool for fetal growth restriction. Our study design is based on the use of the birth of an SGA neonate as an outcome measure but we also discuss the merits of using alternative definitions that might perform better as markers of FGR. We examine risk factors for placental disease from maternal history as well as a variety of biophysical markers, such as the measurement of Blood Pressure (BP), uterine artery Doppler (UAD), and biochemical markers, such as placental associated protein A (PAPP-A), placental growth factor (PLGF) or Placental protein 13 (PP13). Ultimately we use multivariate regression analysis of the above factors to develop a multifactorial screening model for SGA in the first trimester of pregnancy.

1.2 EPIDEMIOLOGY OF FETAL GROWTH RESTRICTION

1.2.1 Defining abnormal fetal growth

Abnormal fetal growth has traditionally been a great challenge to define both in clinical practice and research and hence a variety of different definitions are in use. Historically adverse fetal outcomes were observed in association with low birthweights and hence early definitions of abnormal weight considered a single weight cut-off independent of gestation (Table 1.2). This was usually the 2.5kg, 1.5kg or 1kg limit. This definition is now considered out-dated in favour of gestation-specific weight cut-offs since it allows differentiation of the effects of prematurity and fetal weight on perinatal morbidity and mortality (WHO *promoting optimal fetal development* 2006; Zhang *et al.*, 2010) . Commonly used cut-offs are that of the 3rd, 5th or 10th centiles of the birthweight distribution at every gestation.

Birthweight normograms have been derived from large cross-sectional population studies that include all births at all gestations without excluding high-risk pregnancies (Williams *et al.,* 1982; Alexander *et al.,* 1996; Zhang *et al.,* 2007), or can be derived by a selected “normal” population that excludes pregnancies with complications that are known to affect fetal growth (Ananth *et al.,* 2009). A fetus that falls below this specified centile is labeled as Small for Gestations Age (SGA) and carries increased risks of perinatal but also long-term morbidity and mortality.

Table 1.1 Common factors associated with SGA (Sankaran *et al.,* 2009)

Maternal factors	**Placental factors**
• Undernutrition • Maternal low birthweight • Low maternal weight gain • Maternal age <16 • Low socio-economic status • Parity • Medical disorders • Chronic hypertension • Pre-eclampsia • Systemic Lupus Erythematosus • Diabetes with vasculopathy • Renal disease	• Abnormal placentation • Chronic abruption • Chronic inflammatory conditions (villitis) • Single UA • Velamentous cord insertion • Placental haemangioma • Confined placental mosaicism
Fetal factors	**Environmental factors**
• Chromosomal anomalies • Genetic conditions • Congenital malformations • Intrauterine infections • Multiple pregnancy	• Drug use – smoking, alcohol, illicit drugs • High altitude • Irradiation • Placental factors

Table 1.2 Common definitions used in literature to define a reduced fetal weight

- Single weight cut-offs independent of gestation
  - Low birth weight - <2.5kg
  - Very low birth weight - <1.5kg
- Gestation specific centiles (<3rd, <5th,<10th centile)
  - Based on unselected populations
  - Based on selected populations
  - Based on ultrasound derived curves
    - Cross-sectional
    - longitudinal
- Customized centiles (Ultrasound derived gestation-specific centiles individually adjusted for maternal characteristics)

The SGA group will include constitutionally small fetuses that are theoretically at no higher risk of adverse outcomes than an Appropriate for Gestational Age (AGA) neonate and those that are pathologically growth restricted. At the same time, a proportion of true growth restricted fetuses might not drop below the centile cut-off but still suffer from the pathological process.

The inability of the previously mentioned definition to optimally distinguish pathological from physiological smallness led researchers into proposing optimized techniques for defining abnormal fetal growth. The following two main variations have emerged: the use of ultrasound-derived fetal growth curves as opposed to curves derived from birthweight data and the adjustment for maternal characteristics. Different studies have examined their use independently or combined in what is known as the “customized centile” (Gardosi *et al.,* 1995).

Ultrasound versus population derived centiles

Studies showed improved correlation between the ultrasound-defined SGA fetuses and perinatal morbidity and mortality (Figueras *et al.,* 2007) compared with population standards. This was initially attributed to the customization process, however, a recent large population-based study using strict inclusion criteria and excluding pathological pregnancies delivering preterm produced very similar growth curves with ultrasound based centiles (Poon *et al.,* 2012). Consequently, it is argued that exclusion of pathological preterm gestations either by strict selection or by using an ultrasound derived growth curve in order to define normal limits of birthweight appears to be the key in defining a true "normal" population. The fetuses that are classified as SGA by these methods have been shown to correlate better with perinatal morbidity and mortality compared with old population derived curves that have not excluded pathological preterm pregnancies (Groom *et al.,* 2007; Hutcheon *et a.l,* 2008; Poon *et al.,* 2012).

Adjustment for maternal characteristics

Birthweight varies with maternal characteristics (e.g. ethnicity, parity, parental height) and according to the "customized centile" school of thought, a birthweight centile that corrects for these characteristics is likely to differentiate better between physiological and pathological size (Gardosi *et al.,* 1992). The customized centile however also uses an ultrasound derived curve to define the optimum fetal weight for any gestation. The performance of the customized centile has been consistently shown to be superior to population derived methods, however, recent data attributes this effect to the exclusion of pathological preterm pregnancies and not to the adjustment for maternal characteristics (Hutcheon *et al.,* 2008; Poon *et al.,* 2012). The debate as to which technique performs best is still on-going.

In this thesis, we have used the weights from live births of a well-controlled normal population to define a birthweight standard for use in our prediction model.

An improved definition of FGR

The ideal definition of abnormal growth is the one that reflects pathology and has the ability to best predict adverse fetal outcome in the form of perinatal morbidity and mortality. A definition of fetal growth restriction relying solely on a deviation from a size norm can fall short in identifying truly affected fetuses (Zhang et al., 2010). Advances in the definition of FGR have been attempted by the integration of a variety of functional markers such as umbilical, uterine and middle cerebral artery blood flow measured by Doppler ultrasound or maternal serum biomarkers (Figueras & Gratacos, 2014). Our study design did not allow an opportunity for evaluating placental function in the third trimester and hence we have used as our primary outcome measure the birth of an SGA infant below the 5th centile adjusted for gestation as a proxy for FGR. In the final chapter of this thesis we discuss the potential value of using an improved definition of FGR, critically evaluate relevant studies and make suggestions about future study design.

1.2.2 Incidence of SGA and associated adverse outcomes

The incidence of SGA will depend on the definition used and traditionally ranges between 3%-10%.There is an established strong correlation between reduced fetal weight and adverse pregnancy outcomes and there is also an established correlation between SGA and later life complications (Table 1.3). Estimations describe a rate of around 30 million babies per year are born with intrauterine growth restriction in developing countries with developed countries expectedly having about 5 million. The highest incidence of SGA lies in Asia (75%), mainly South East Asia, followed by Africa (20%) and Latin America (5%) (De Onis *et al.,* 1998).

The association of placental insufficiency and antepartum stillbirth is well established but the degree of correlation varies considerably within the literature. The lancet reports a total number of 36300 stillbirths in high-income countries in 2008, corresponding to around 5/1000 total births. On analysing the causation of stillbirths in this high-income setting, 32% of stillbirths can be attributed to Fetal Growth Restriction (Lawn 2011). In the most recent CEMACE report (2009) 19% of stillbirths at any gestation are associated with reduced fetal growth, however, many authors believe that this number is a modest estimate and evidence suggests that a large proportion of stillbirths defined as “unexplained” are actually secondary to growth restriction (Vergani *et al.,* 2008; Korteweg *et al.,* 2008; Stillbirth Collaborative Research Network Writing Group 2011). In another study it is shown that by using a new classification for stillbirths taking into account fetal weight shows 43% of fetuses had a Birthweight below the 10th centile (Gardosi *et al.,* 2005), while another study examining stillbirths without any fetal anomalies found the birthweight was below the 10th percentile in 71.8% of those found at <32 weeks’ gestation (poon *et al.,* 2012). When examining predictors of stillbirth, prior delivery of a growth restricted infant is among the strongest risk factors for stillbirth, comparable to the history of prior stillbirth (Smith & Frett 2007).

Table 1.3 Perinatal complications of fetal growth restriction (Palloto& Kilbride, Clinical Obstetrics and Gynaecology 2006)

- Perinatal mortality higher among term and preterm fetuses with reduced growth
- Intrapartum hypoxia and distress
- Increased rates of Hypoxic Ischaemic Encephalopathy (HIC)
- Increased rates of seizures
- Increased effects of prematurity complications in preterm SGA
- Increased rates on Necrotizing Enterocolitis (NEC)
- Increased rates of Respiratory Distress Syndrome (RDS)
- Increased intraventricular hemorrhage
- Retinopathy of prematurity
- Increased neonatal complications
- Hypothermia
- Hypoglycaemia
- Polycythaemia
- Reduced immune function and more susceptible to sepsis
- Coagulopathy
- Bronchopulmonary dysplasia
- Late life complications
- reduced physical growth
- reduced neurodevelopmental capacity
- reduced school performance
- association with metabolic syndrome and type 2 diabetes
- association with cardiovascular disease

Besides the association with fetal death, SGA fetuses are at higher risk of a variety of perinatal complications both during labour and at the early neonatal period. Perinatal mortality in SGA infants is 10-20 times higher than that of an AGA infant (McIntire *et al.,* 1999; Revev *et al.,* 2003). SGA fetuses are more likely to experience Intrapartum distress resulting in Hypoxic injury. They have higher rates of meconium aspiration, lower Apgar scores on delivery and are more likely to have a low umbilical

cord PH (<7.0). Subsequently, SGA born neonates have higher rates of complications such as Hypoxic-ischaemic encephalopathy (HIE), Respiratory distress syndrome (RDS), Necrotizing Enterocolitis (NEC) and intraventricular hemorrhages (IVH). Prematurity and smallness have a cumulative effect on the risk of all perinatal complications (Regev *et al.,* 2004).

A growing body of research is revealing links of the consequences of fetal growth restriction in utero with later-life complications. It is hypothesized that the adaptations that take place as a result of the adverse intrauterine environment imprint permanent changes on the metabolic, cardiovascular and neurological programming of neonates and manifest as adult diseases (Gluckman *et al.,* 2008). More specifically smallness at birth and infancy is associated with increased rates of coronary heart disease, stroke, type 2 diabetes, adiposity, the metabolic syndrome and osteoporosis in later life and these results have been consistently replicated in studies and are independent of environmental risk factors in adults (Fall *et al.,* 1995; Phillips *et al.,* 1994; Bateson *et al.,* 2004; Cooper *et al.,* 1997; Barker *et al.,* 2012).

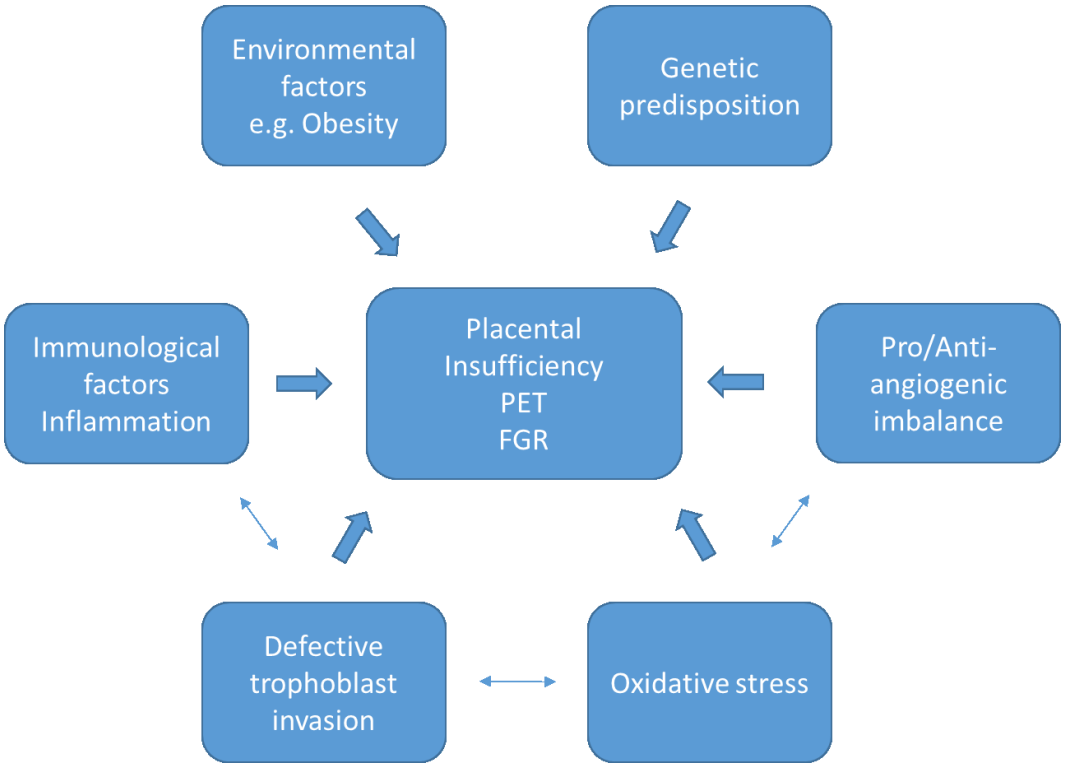
1.3 AETIOLOGY OF FETAL GROWTH RESTRICTION

The aetiological mechanism of growth restriction has traditionally been attributed to defective placentation during early stages of pregnancy. Classic histological findings have been described in a variety of studies such as shallow endovascular trophoblast invasion and inadequate uteroplacental artery remodeling and are seen both in SGA pregnancies and pre-eclampsia (PE). Hence the belief that both conditions share common pathophysiological pathways with different clinical manifestations.

The extent to which the two conditions overlap and the reasons why some pregnancies manifest the maternal syndrome while others develop isolated placental

insufficiency is still unclear. When considering the aetiological factors of growth restriction and pre-eclampsia the approaches vary with some studies examining isolated cases while others investigate the syndrome of placental insufficiency with varying degrees of overlap between PE and FGR. A multifactorial model for the pathogenesis of placental insufficiency appears to be the most representative way to explain the pathophysiology of the disease (Figure 1.1).

Figure 1.1 A simplified schematic representation of the factors that have been shown to contribute to the pathophysiology of placental disease resulting in PET and/or FGR. (Adapted from Pennington *et al.*, 2012).



The process of normal placentation will be summarized below in order to illustrate the different theories around the aetiology of inadequate placentation.

1.3.1 Normal placentation

From the first week after conception, the differentiating cells of the developing embryo form an outer layer of trophoblast cells which will eventually contribute to the fetal component of the placenta. Once trophoblast cells come in contact with the endometrium (apposition) the process of implantation begins. From the maternal interface uterine stroma and endometrial cells begin a cascade of changes such as influx of Natural Killer cells, increase mitotic activity and alteration in stroma cell morphology, collectively called decidualization in order to facilitate further implantation of the embryo (Eide *et al.,* 2005; Johnson *Essential reproduction* 2000). Decidualized endometrial cells will form part of the maternal component of the placenta. At the moment of contact metalloproteases (protease enzymes) dissolve the zona pellucida surrounding the trophoblast thus allowing the multiple adhesion molecules expressed on the surface of the trophoblast cells to adhere to the decidua (Cohen *et al.,* 2007). Besides dissolution of surrounding matrix trophoblast cells actively move through endometrial cells with the help of cytoskeletal microfilaments (Burrows *et al.,* 1996).

Initial exchange of nutrients between the developing embryo and mother occurs from lacunae, small clefts communicating with maternal endometrial sinusoids. These lacunae are the precursors of the intervillous space which facilitates circulation of maternal blood through the placenta. Two characteristics of the placenta define its ability to effectively exchange gasses and nutrients between fetus and mother. From the fetal interface, the development of chorionic villi allows for sufficient surface area

for such exchange to occur. Primary stem villi arise after day 13 or embryonic life and branch out into secondary and eventually tertiary villi that occupy the intervillous space and form the final placenta structure. From the maternal interface trophoblastic invasion of maternal blood vessels (haemochorial placentation) allows maternal blood to enter the intervillous space and bathe the chorionic villi with maternal blood. This process of invasion of maternal spiral arteries and their conversion from high resistance vessels to wider bore capacitance vessels is thought to be crucial in the correct development of the placental circulation and has been a particular focus of research. As will be discussed further inadequate invasion has been implicated in the development of placental insufficiency and all its clinical manifestations of preeclampsia and growth restriction. By 12 weeks the placenta has completed its architectural development and subsequently only increases in size while conversion of spiral arteries is thought to be complete between 16-18 weeks (Pijnenborg *et al.,* 1980).

Remodeling of spiral arteries

Uterine vasculature begins from the two uterine arteries traversing the lateral walls of the uterus in an upward direction with a small contribution from the ovarian arteries directed downwards. Each uterine artery supplies lateral branches that immediately enter the uterus and give off tortuous anterior and posterior arcuate divisions, which run circumferentially in the myometrium. Each arcuate artery throughout its course gives off numerous radial branches. Once these vessels reach the endometrial level, they branch into the basal arteries and spiral arteries, which support the specialized functions of each layer. The basal arteries are not responsive to hormones; they support the basal endometrial layer, which provides the proliferative cells for endometrial growth. The spiral arteries supply the endometrial layer and possess many unique characteristics allowing them to adapt to the changing environment of endometrial decidualization as well as trophoblastic invasion. In fact, current theories

on the mechanism of placentation indicate a two-stage process of physiologic remodeling of the spiral arteries during gestation involving a decidua-associated and a trophoblast-associated stage (Brosens *et al.,* 2011; Table 1.4).

Initial signs of the decidualization changes have been observed independently of cellular interactions with invasive cytotrophoblasts and include endothelial basophilia, expression of endothelial activation markers and vacuolization, followed by dilation, muscular hypertrophy, disorganization, and fibrinoid change (Craven *et al.,* 1998). It is thought that decidualized endometrium can modulate trophoblast function by altering expression of regulatory factors such as metalloproteinases, cytokines, surface integrins, and major histocompatibility complex molecules (Brosens *et al.,* 2002) .

The interstitial invasion of extravillous trophoblast proceeds from cytotrophoblast at the tips of the anchoring villi and can be seen in the decidual part of the spiral arteries from around 4 weeks. From 8 weeks of gestation onward, myometrial invasion ensues and peaks between 9 and 12 weeks (Aplin *et al.,* 1988). Interstitial trophoblast penetration proceeds with minimal tissue destruction and invading cells preferentially home toward the spiral arteries and encircle them to reach the arterial media (Kam *et al.,* 1999; Pijnenborg *et al.,* 1983). Invasion of the spiral arteries is evident in histological studies by amorphous acidophilic “fibrinoid” deposits in the arterial wall embedded with cytotrophoblastic cells. Finally, the complete absence of the musculo-elastic structure, enlarged funnel-shaped luminal diameter and re-endothelialization define a “physiologically changed” spiral artery or uteroplacental vessel (Pinjenborg *et al.,* 1983; Brosens, 1988).

Characteristics of normal trophoblastic invasion include invasion of junctional and myometrial segments of the spiral arteries and a higher invasion distribution in the central areas of the placental bed compared with peripheral areas (Matijevic *et al.,*

1995). The term “deep placentation” has been used to describe the observed complete transformation of the decidual and myometrial segments of approximately 100 spiral arteries (Brosens *et al.,* 2011).

Table 1.4 Steps of spiral artery remodeling (Brosens *et al.,* 2011)

1	Initial stage of decidua-associated remodeling,
2	intra-arterial trophoblast migration,
3	intramural invasion and trophoblast-associated remodeling,
4	Re-endothelialization and other maternal-induced changes.

1.3.2 Mechanisms of abnormal placentation

Histopathological examination of placenta beds was first performed in cases of pre-eclampsia with or without fetal growth restriction and was consistent with an absence of the previously described physiological changes of spiral artery transformation primarily in the junctional zone and myometrial layers (Brosens *et al.,* 1972). Junctional zone spiral arteries appeared to be of smaller caliber with a thicker lumen, muscular walls and more densely distributed (Starzyk *et al.,* 1997; Pijnenborg *et al.,* 1991). Hence a deduction was made that the defect lies in trophoblastic invasion. Further findings of atherosis of the non-invaded vessels with characteristics of necrosis, fibrin deposition and lipophage infiltration of the vessel wall were described (Robertson *et al.,* 1967; Brosens & Renaer 1972). Similar histological findings have been described also in placentas of pregnancies with growth restriction in the absence of preeclampsia (Sheppard & Bonnar 1981; Hustin *et al.,* 1983; De wolf *et al.,* 1980). Some studies have shown only partial transformation of the myometrial

spiral arteries in cases of growth restriction without preeclampsia (Khong *et al.,* 1986; Gerretsen *et al.,* 1981).

Several pathophysiological mechanisms have been associated with the above findings and include immune maladaptation, disturbed blood flow stress, impaired decidualization of myometrial spiral arteries or exaggerated inflammatory response among others (Chaiworapongsa *et al., 2014).* However, evidence suggests that impaired conversion of spiral arteries is present in a variety of different obstetric syndromes such as miscarriage, intrauterine death, placental abruption, premature rupture of membrane and preterm labour (Ball *et al.,* 2006*,* Brosens *et al.,* 2011, Khong *et al.,* 1987*)*. Hence inadequate spiral artery transformation cannot be deemed as the sole aetiological factor behind placental insufficiency but rather as a probable common step in a potentially complicated multi-step aetiological mechanism.

The greatest hindrance in determining causation is the fact that placental insufficiency has its pathogenic origins in the first trimester and as such causal relationships inferred from late-gestation decidual pathologies are inherently unreliable. An overview of the prevailing areas of investigation will be presented below.

Placental angiogenesis and its pathophysiology

Placental angiogenesis is a tightly controlled process that depends on many stimulating and inhibiting factors. The key signaling system that regulates proliferation and migration of endothelial cells forming the basis of any vessel are vascular endothelium growth factors (VEGF) and their receptors. The biological significance of the effect of such system is defined by the ratio of different isoforms present (Karamysheva 2008). In addition to VEGF, a variety of other signaling systems have been found to co-regulate angiogenesis. Such systems are tyrosine kinase receptor (Tie2) and its ligands the angiopoietins (Ang).

The VEGF family includes factors VEGF-A, B, C & D, and Placenta growth factor (PlGF). There are three VEGF family receptors, known as Flt-1, KDR, and Flt-4, as well as a soluble form of Flt-1(sFLT-1) (Huckle & Roche 2004; Thomas *et al.,* 2007; De Falco 2012). Immunohistochemical studies on the expression patterns of VEGF, PLGF, and their receptors in the placenta, as well as maternal serum levels throughout pregnancy, suggest an important role in villous angiogenesis (Cerdeira & Karumanchi 2012). Kaufmann *et al.,* showed a correlation between reducing expression of VEGF and increasing expression of PLGF with the transition from branching to non-branching angiogenesis of the placental villi (Kaufman *et al.,* 2003).

A variety of studies have associated the interrelationships of VEGF factors and receptors throughout different gestations with various physiological processes of placental implantation and development (Clark *et al.,* 1998; Helske *et al.,* 2001; Demir *et al.,* 2009) and as such, their role in placentation is beyond doubt significant. Other factors implicated in early angiogenesis include the angiopoietin family and its receptors Tie-2, PDGF, TGF-b and Endoglin (Eng) which is a co-receptor for transforming growth factor (TGF)-b 1 and 3 (Cheifetz *et al.,*1992). They are thought to be involved in the maturation and stabilization of primitive endothelial tubes (Kayisli *et al.,* 2006; Seval *et al.,* 2008; Dunk *et al.,* 2000).

At the same time, a variety of other hormones and pregnancy-specific growth factors have been evaluated and found to have pro-angiogenic properties. Zygmunt *et al.,* showed that physiological concentrations of β-hCG significantly increased in-vitro capillary formation and migration of endothelial cells (Zygmunt *et al.,* 2002). While in another study insulin-like growth factor-II (IGF-II) was found to have a potential role in promoting angiogenesis (Herr *et al.,* 2003)

Subsequently the focus of research extended in trying to detect and interpret differences between patterns of expression of such markers in pathological cases of preeclampsia and fetal growth restriction. sFLT-1 a factor with anti-angiogenic properties has been consistently shown to be increased in cases with preeclampsia while VEGF and PLGF, both vascular stabilizing factors, have been found to be reduced, hence supporting the theory of antagonism of VEGF and PlGF resulting in the appearance of hypertension (Buhimschi *et al.,* 2005; Levine *et al.,* 2005; Aggarwal *et al.,* 2006, Maynard *et al.,* 2008). Many of the imbalances between these factors are thought to be the causative factors of the vasculopathic syndrome of pre-eclampsia resulting in endothelial damage with all its consequences.

Concentrations of most markers appear to be maximally increased late in gestation as clinical symptoms develop. In the case of placental insufficiency without hypertension, there is the absence of endothelial damage and its clinical manifestations and hence investigation into these markers in normotensive growth restricted pregnancies has shown less definitive findings. Modest increase in serum concentrations of sFlt-1 have been found in normotensive growth restriction (Tsatsaris *et al.,* 2003), but equally, a difference was not demonstrated in another study (Shibata *et al.,* 2005). Decreased levels of oxygen tension were hypothesized to result in premature maturation of vessels and contribute to the abnormal development of villous trees (associated with non-branching angiogenesis) in another study (Mayhew *et al.,* 2004).

A natural consequence of the observations that changes in the serum/plasma concentrations of angiogenic and anti-angiogenic factors can be detected prior to the clinical recognition of the disease is that assays for such factors may be useful in the risk assessment for PE and FGR. Indeed, several studies have addressed this issue by investigating an array of different markers in isolation or in combination with other predictive markers such as clinical risk factors or Doppler velocimetry of uterine blood

flow. We review a variety of biochemical markers, their role in the pathophysiology of placental insufficiency and their value in the prediction of an SGA neonate further in this thesis.

Immune maladaptation

Interactions between the stromal and vascular components of the placenta and uterus do not take place in isolation but rather are subject to a superimposed layer of regulation by the maternal immune cells that populate the decidua. A dominant maternal-factor model used to explain preeclampsia is dysfunction of normal trophoblast immune privilege (Kaufman *et al.,* 2003). Two specific immune cells, macrophages, and Natural Killer cells have been particularly investigated for their role in trophoblast-decidua interactions.

Macrophages are the second most abundant leukocyte population within the human decidua (about 20% of total leukocytes) and are natural candidates for contributing to tissue remodeling at the maternal-fetal interface (Erlebacher 2013; Reister *et al.,* 1999). The observations of differential macrophage distributions between pregnancies suffering from placental complications such as preeclampsia and fetal growth restriction hint at interactions between trophoblast and macrophages (Hunt, 1990).

Few studies that examined third-trimester histological specimen have reported positive findings (Reister *et al.,* 1999; Lockwood *et al.,* 2006; Huang *et al.,* 2008; Schonkeren *et al.,* 2011) but others failed to detect significant differences (Reiger *et al.,* 2009; Redline *et al.,* 2001; Kim *et al.,* 2007). Therefore, further insight into the role of macrophages in decidual arteriole remodelling and placental insufficiency is needed to shed light to any possible causation links.

Natural Killer cells are the major maternal immune cell component of the decidua, comprising approximately 70% of the Leukocyte population. They are first seen in the secretory endometrium prior to implantation and are locally induced by chemokines, cytokines, and angiogenic factors to differentiate into cells with highly specialized pregnancy-specific functions (Koopman *et al.,* 2003; Manaster *et al.,* 2010, Wallace *et al.,* 2012). Studies have identified as potential roles of NK cells the facilitation of vascular changes necessary for maximizing maternal blood flow through the placenta (Hanna *et al.,* 2006) and there is strong evidence for their involvement in the aetiology of pre-eclampsia and placental insufficiency (Hiby *et al.,* 2004; Hiby *et al.,* 2012). Research in first trimester decidual specimens of pregnancies with high-resistance uterine artery Dopplers identified reduced NK cell apoptosis when compared with normal uterine resistance pregnancies (Fraser *et al.,* 2012).

Hypoxia and oxidative stress

Studies in the early stages of placental development indicate that the fetoplacental unit exists under a low oxygen tension environment which increases at the point of connection of the intervillous space with the maternal circulation. The result is a "physiological" ischaemic-reperfusion injury which has been documented in various studies (Jauniaux *et al.,* 2000). In cases of defective conversion of the maternal spiral arteries to low resistance vessels the increasing pressures of the arterial system cannot be accommodated and hence areas of the placenta are susceptible to similar hypoxia, reperfusion injury and oxidative stress above the physiological levels. A variety of studies have found evidence of oxidative stress and hypoxic reperfusion injury in cases of preeclampsia and growth restriction and also associated this with subsequent increased levels of apoptosis (Heazell *et al.,* 2008; Hung *et al.,* 2006). More promising findings were seen in a study that found Increased levels of biomarkers of oxidative stress in maternal urine from 12 weeks of gestation

suggesting that the finding is not a mere consequence of late placental hypoperfusion (Potdar *et al.,* 2008).

Apoptosis

Apoptosis has traditionally been described as programmed cell death and is a part of the normal regeneration process of the body. As a process, it possesses characteristics that facilitate its purpose, for example, the ability of effective removal of apoptotic cells without eliciting an inflammatory response via the action of phagocytes. Its role in physiological and pathological placentation has been extensively investigated however its precise role in the development of placental pathology and clinical conditions such as pre-eclampsia and FGR is yet to be determined (Heazell *et al.,* 2008). The reason for this is the inherent methodological difficulties in the histological investigation of samples from abnormal pregnancies that only become available after delivery (Heazell *et al.,* 2008; Fraser *et al.,* 2012).

Many studies have identified increased apoptotic markers in cases of preeclampsia and growth restriction hypothesizing that a tightly regulated process is necessary for maintaining the integrity of villous trophoblast (Levy & Nelson 2000, Leung *et al.,* 2001; Endo *et al.,* 2005; Athapathy *et al.,* 2003). Most of the hypothesized causative theories of placental insufficiency described above have been shown to be associated with increased apoptosis such as hypoxia and hypoxia re-oxygenation injury which has been shown to induce apoptosis in cultured villous trophoblast (Hung *et al.,* Levy *et al.,* 2000). Also, links have been identified with decreased immune tolerance (Abumare *et al.,* 2006; Sargent *et al.,* 2003) or angiogenic / anti-angiogenic interactions (Cockell *et al.,* 1997; Nevo *et al.,* 2006; Levine *et al.,* 2006; Guller *et al.,* 2007). However, the question still remains as to whether the changes that are being measured are secondary to an already damaged placental and not the cause of the damage itself.

Newer studies have started to look at the apoptotic process within the context of implantation in early pregnancy. Here apoptosis has been described as a prerequisite in the ability of the trophoblast to invade spiral arterioles and promote vascular remodelling. Various apoptotic markers have been identified in the decidua even prior to the presence of trophoblast in spiral vessels (Smith *et al.,* 2009). In a novel new study also described above, evidence showed a possible reduction in apoptosis mediated by NK cells in cases which were characterized as high risk for placental insufficiency via uterine artery Dopplers. Again the value and role of apoptosis in placental insufficiency remains to be determined.

Adhesion molecules and trophoblast invasion

The expression of cell adhesion molecules is necessary for trophoblast invasion because these molecules enable the trophoblast to adhere to the extracellular matrix, form colonies, and target cells in the vessel wall (Kaufmann & Castelluci 1997; Merviel *et al.,* 2001). Various cell adhesion molecules such as E-Cadherins or A-Integrins have been investigated for playing a role in the pathogenesis of placental insufficiency. Some studies report possible alterations to such molecules while others report negative data (Zhou *et al.,* 1997; Lyall *et al.,* 2001; Divers *et al.,* 1995).

In summary clinical and basic research data suggest that maladaptation and mal-invasion of uteroplacental arteries characteristic of growth restriction and pre-eclampsia can be a result of intrinsic (trophoblast-related) as well as extrinsic (decidual related) or most likely an interrelation of the two.

1.3.3 Pre-eclampsia vs. normotensive growth restriction

The theory about common origins of pre-eclampsia and fetal growth restriction is well accepted as various studies have linked the classical signs of inadequate conversion of maternal spiral arteries in placentas from both diseases (Kaufman *et al.,* 2003). The extent of overlap of these conditions and the characteristics that separate them has been subject of many studies.

In placental histopathological studies, growth restriction with or without Preeclampsia seems to share common characteristics such as significantly trimmed placenta and reduced intervillous space volume (Aviram *et al.,* 2010, Teasdale 1984)). Placentas with preeclampsia in the absence of growth restriction resembled those of age-matched normal controls with similar mean villous diameters, capillary volume densities and capillary/villus surface ratios (Burton *et al.,* 1996; Ansari *et al.,* 2003) hence supporting the hypothesis that late-onset PE is a maternal and not a placental disorder (Mayhew *et al.,* 2004). What unifies and what differentiates these two groups is a topic of on-going investigation with prevailing theories strongly implicating predisposition to the metabolic syndrome as the culprit for the development of the multi-organ endothelial dysfunction seen in preeclampsia.

The links between preeclampsia and cardiovascular risk factors have been well documented however there is some evidence that mothers who had growth restricted babies also have increased risks of ischaemic heart disease and later life hypertension (Smith *et al.,* 2001; Davey *et al.,* 1997; Kramer *et al.,* 1987). Such observations raise the possibility that there is an element of shared endothelial dysfunction. However in a large observational study of 39.000 pregnancies comparing risk factors of women having normotensive growth restriction versus pre-eclamptic growth restriction showed a disparity in the risk factors with the first being associated

with a previous history of SGA neonates and independent of previous pre-eclampsia and no link with metabolic syndrome (Villar *et al.,* 2006).

What is clinically evident and well documented is the distinct clinical manifestation of the two syndromes. Varying degrees of defective placentation resulting in hypoxia and ensuing oxidative stress, inflammation and endothelial activation in combination with a pre-existing susceptibility secondary to the maternal metabolic syndrome (adiposity, insulin resistance, hyperglycaemia, hyperlipidaemia, coagulopathies, and maternal inflammatory mediators) disrupts maternal endothelial function until the process crosses a clinical threshold and manifests as preeclampsia. The absence of maternal metabolic syndrome or other exogenous inflammatory triggers precludes the mother from developing appreciable maternal pathology (Ness *et al.,* 2005; Figure 1.3).

Evidence to support this includes a difference between neutrophil activation, a marker of inflammation in pre-eclampsia but not growth restriction (Von Dadelszen *et al.,* 1999) suggesting a reduced inflammatory state in the later. Also after pre-eclamptic pregnancies, metabolic syndrome markers tend to remain elevated, as does cardiovascular risk (Smith *et al.,* 2001). In the case of normotensive growth restriction, this effect is much less observed. Measured cholesterol, triglycerides, HDL, LDL, very-low-density lipoprotein (VLDL), or intermediate-density lipoprotein (IDL), do not appear to be elevated (Sattar *et al.,* 1999).

Figure 1.2 Schematic representation of a possible pathophysiological pathway in PET and FGR. Pre-pregnancy endothelial dysfunction results in hypoxia-driven dysregulation via Hypoxia Inducible Factor (HIF). HIF is associated with decreased levels of PLGF and increased levels of VEGF and TGF-β. Subsequent incomplete conversion of spiral arteries due to the anti-angiogenic unfavourable environment results in an exaggerated inflammatory response as measured by raised levels of the pro-inflammatory T lymphocyte (Th-1). A divergent pathway is proposed with women exhibiting specific metabolic phenotype manifest the clinical syndrome of PET and women without risk factors do not cross the threshold and do not exhibit the maternal symptoms but the fetus is still subject to the effects of the malfunctioning placenta and develops FGR. (From Ness and Sibai AJOG 2006)

When examining risk factors for pre-eclampsia versus normotensive growth restriction we see the first being much more common in obese women while the later shows a weaker association with raised BMI (Bernstein *et al.,* 1997). In the case of diabetes mellitus again we see a stronger association with pre-eclampsia and the link to SGA is mostly observed if the severity of diabetes has resulted in vasculopathy (Bradley *et al.,* 1988).

1.3.4 Genetic predisposition in placental insufficiency

Several epidemiological studies postulate an inherited component in the pathogenesis of placental insufficiency (Ghezzi *et al.,* 2003; Smith *et al.,* 2007). Women who themselves are small at delivery are at greater risk for having an SGA baby, whereas familial aggregation is well established for both PE and growth restriction (Hackman *et al.,* 1983; Magnus *et al.,* 1993). In a recent study investigating women having SGA babies or having PE in a genetically isolated population in the Netherlands it was found that more than three out of four could be traced back to a common single ancestor. In addition, it was found that those women that had PE and those that had SGA babies were more closely related than expected by chance (Berends *et al.,* 2008).

Another recent large epidemiological study showed that mothers born SGA are more likely to develop placental complications such as PE, abruption or stillbirth and in the case that both parents were born SGA the risk of the mother developing PE was increased 3-fold (Wilkstrom *et al.,* 2011). A recent systematic review showed that if a father is born SGA then there is a significant association with the delivery of an SGA offspring (Shah *et al.,* 2010).

1.3.5 Conclusion

In conclusion, we see that both pre-eclampsia and growth restriction share common pathophysiological findings that could point towards a single underlying mechanism which is modulated by different factors either genetic or acquired and results in the development of the different clinical manifestations. The quest to understand the basic pathogenesis, provide meaningful predictions or attempt possible therapeutic interventions requires research to move its focus at progressively earlier gestations where the signs of the disease can be absent or increasingly difficult to detect. In the following sections of the introduction, we examine closely the various techniques that have been used to identify women at risk of placental insufficiency either through risk stratification, ultrasound of the uterine arteries or analysis of maternal serum markers of defective placental function.

1.4 SCREENING FOR SMALL FOR GESTATIONAL AGE IN PREGNANCY

As mentioned previously the ultimate aim of identifying small fetuses in utero is because a proportion of them will suffer from growth restriction. Identifying the fetus that is likely to suffer from growth restriction antenatally allows the optimization of surveillance during pregnancy to ensure appropriate delivery in a manner that minimizes the risks of prematurity but at the same time minimizes the risk of intrauterine death (Mandruzzato *et al.,* 2008; Miller *et al.,* 2008).

The ideal screening protocol for SGA is an ever changing concept illustrating the difficulty involved in this task. Successful prediction is compounded by a variety of factors including the lack of defined thresholds for normality versus abnormality, the fact that the assessment of growth velocity (serial measurements) may be more valuable clinically than a single estimate of size and differences due to factors such

as maternal ethnicity (Bricker *et al.,* 2008; Altman *et al.,* 1989). If a fetus is identified as SGA a set of criteria is used to ascertain the diagnosis of true FGR. Common diagnostic criteria for FGR are presented in Table 1.5.

Table 1.5 Commonly used diagnostic criteria of fetal growth restriction

- Estimated fetal weight which is small for gestational age or certain biometric measurements being small for gestational age (most predictive being abdominal circumference).
- A reduction in growth velocity as demonstrated by a drop in "centiles" of the EFW or biometric parameters.
- Fetal and maternal blood flow abnormalities demonstrated via ultrasound doppler:
    - Increased resistance in the umbilical artery Doppler with progression to absent or reversed end diastolic flow.
    - Reduced resistance in the middle cerebral artery signifying a brain sparing effect of the hypoxic fetus.
- Reduced amniotic fluid.
- Reduced fetal movements or fetal biophysical profile.

Many times the diagnosis can only reliably be achieved postnatally by a form of a body mass index (BMI) known as the Ponderal Index. However, 50% of newborns identified as growth restricted by Ponderal Index had a birth weight above the 10th centile and 40% of newborns with a birth weight less than the 10th centile had a normal Ponderal Index (Weiner *et al.,* 1989). In cases of perinatal fetal death, for the purposes of assigning causation, an autopsy can demonstrate an elevated brain-weight/liver-weight ratio (Mitchell *et al.,* 2001).

Traditionally and in most clinical settings screening has been applied in the second or third trimester either in an unselected population or with an initial stratification of groups into low or high risk based on maternal characteristics applied early in pregnancy. Increasingly though, as more evidence emerges that the origin of obstetric pathology arises early in the first trimester in combination with the fact that most pregnant women engage with antenatal services in the first trimester as part of routine screening for chromosomal abnormalities we observe a consistent effort to employ screening modalities such as ultrasound or biochemical testing earlier in gestation (Zhang et al., 2010; Nicolaides 2011).

When considering population screening it is important to appraise the viability, effectiveness, and appropriateness of performing screening for that particular condition. A series of prerequisites known as Wilson's criteria is a commonly accepted evaluative method for this task. The criteria applied on screening for SGA in pregnancy are summarized in table 1.6. As can be seen four out of seven criteria are not met satisfactorily. However there are continuous efforts to improve on all four areas mentioned in the table and there are promising preliminary results in the areas of diagnosis SGA, managing the disease during pregnancy and applying early therapeutic interventions. Screening for SGA should move in parallel with advances in the other areas where criteria are partially met because as mentioned previously early prediction and the ability to diagnose or effectively treat are intertwined and early selection of a high-risk group will concurrently benefit the other areas as well.

In the following paragraphs, a review of the most relevant screening methods and their performance in predicting SGA will be performed with a focus on those applied in the first trimester.

Table 1.6 Wilson's criteria for screening applied to screening for SGA (Breeze & Lees 2007)

Criteria met
• The condition should be an important health problem
• The natural history of the condition should be understood
• There should be a recognisable latent or early symptomatic stage

Criteria not met or there is uncertainty
• There should be a test for the condition that is easy to perform and interpret and it is acceptable, accurate, reliable, sensitive and specific
• There should be an accepted management for the disease
• Treatment should be more effective if started early
• Diagnosis and treatment should be cost-effective

1.4.1 Risk stratification based on maternal characteristics

There are widely known predisposing factors for the likelihood of a fetus being SGA and the stratification of the pregnant population in terms of their risk of having an SGA pregnancy is an effective way of increasing the detection rate and positive predictive value of the screening method. The most relevant identified risk factors for SGA secondary to placental insufficiency will be discussed below.

Maternal Age

High maternal age has been consistently associated with the delivery of an SGA neonate. An Odds ratio (OR) of 1.28 for a birth weight <5th centile was reported in women aged >35 and 1.49 for women aged >40 (Jolly *et al.,* 2000a). In another study, the OR of a mother aged >35 delivering a neonate below the 10th centile was 3.1 (95%CI 1.9-5.4) (Odibo *et al.,* 2006). Young maternal age has not shown an association with SGA (Jolly *et al.,* 2000b). According to the latest RCOG guidance maternal age >40 years alone would put a woman in the high-risk category with serial growth scans recommended. However, there has been recent data to suggest that advanced maternal age might not be associated with stillbirth if congenital abnormalities are excluded (Stillbirth Collaborative Research Network 2011).

Parity and past obstetric history

Nulliparity confers a risk over having previous pregnancies of non-SGA babies with ORs of 1.31-2.1 (Shah *et al.,* 2010, Zhang *et al.,* 2010). Also in a large population study nulliparity has been associated with stillbirth which in line is highly associated with SGA (Gardosi *et al.,* 2013).

The association with previous SGA pregnancies and subsequent increased risk of SGA is well established (OR 3.9) (Wolfe *et al.,* 1987; Ananth *et al.,* 2007). Even stronger is the association with SGA and stillbirth which has been demonstrated both in the fact that women with as SGA pregnancy have an increased chance of a subsequent stillbirth (Surkan *et al.,* 2004, kleijer *et al.,* 2005) but also women with an initial stillbirth have increased risk of a subsequent SGA pregnancy (OR 6.4)(Bakewell *et al.,* 1997). More so this association shows a graded response i.e. the earlier SGA occurred in the first pregnancy, the higher the chances of stillbirth in a subsequent

pregnancy with SGA pregnancies below 33 weeks conferring a 6-13 fold increased risk (Rasmussen *et al.,* 2009).

Previous preeclampsia increases the risk of subsequent SGA. In a population cohort of 350.000 pregnancies, severe preeclampsia requiring preterm delivery (<37wks) had a much higher subsequent risk for SGA (aOR 11.22 (95%CI 7.4-16.9) and term preeclampsia having a much smaller association (aOR 1.53 (95%CI 1.24-1.87) (Wikström *et al.,* 2011).

In a recent meta-analysis, interpregnancy interval of fewer than 6 months was shown to increase the risk of subsequent SGA (aOR 1.26 (95%CI 1.18–1.33) but also a >60 month interval showed similar risk (aOR 1.29 (95%CI 1.20-1.39) (Conde-Agudelo *et al.,* 2006).

Maternal weight, height, and BMI

Maternal pre-pregnancy weight and height are shown to affect the birth weight of the neonate in a variety of studies (Kramer *et al.,* 1987; Niswander *et al.,* 1974). The extent that this effect can be described as “physiological” or “pathological” is rather controversial and stills a subject of scientific debate. In the case of using centiles with customization for maternal characteristics, we see an increased association with stillbirths and perinatal morbidity/mortality. However studies have attributed this effect not directly to the correction for these characteristics but to the inclusion of fetal rather than neonatal growth curves (Zhang *et al.,* 2007; Hutcheon *et al.,* 2008). In fact, studies examining maternal weight and stillbirth have shown a direct independent association and hence supporting the notion that reduced maternal weight can have a pathological component (Zhang *et al.,* 2010). In another large screening study of 70.000 pregnancies, adjustment for maternal characteristics did not improve detection of stillbirths (Poon *et al.,* 2012). The RCOG advocates the use of

customized centiles in its latest guidance for screening for SGA but recognizes as a minor risk factor maternal BMI < 10th centile, BMI 25-29.9th centile and BMI >30th centile (OR 1.2(95%CI 1.1-1.3), RR1.2(95%CI 1.2-1.3), RR1.5(95%CI 1.3-1.7).

A slightly less pertinent fact but one shown in several studies is the association between the mother being born SGA and the delivery of an SGA neonate. Odds ratios range between 2.6 and 4.7 (Hackman *et al.,* 1983; Magnus *et al.,* 1993; Shah *et al.,* 2010).

Paternal factors

Mostly paternal height and less so weight, have been shown to have a small effect on neonatal birth weight but since maternal height and weight have a much more substantial effect the value of paternal parameters is not widely used (Klebanoff *et al.,* 1998; Magnus *et al.,* 2001).

A strong association has been reported between the father being born SGA and offspring that are also SGA (Jaquet *et al.,* 2005). In a systematic review of 36 studies, the OR was found to be 3.47(95%CI 1.17-10.27) (Shah *et al.,* 2010). The integration of this parameter in routine screening is difficult due to the fact that most parents might not recall accurately whether born SGA or not.

Also in line with the previous discussed theories of an element of genetic predisposition in the origins of placental disease, changing partners alters the risk of subsequent SGA so that if a woman had a non-SGA pregnancy and subsequently changes partner the risk of SGA increases (aOR 1.19(95%CI 1.1-1.3)(Krulewitch *et al.,* 1997).

Maternal medical conditions

Chronic hypertension

Maternal Chronic hypertension increased the likelihood of an SGA neonate in a variety of studies by about 20%. McCowan *et al.,* reports an OR of 2.9 (95%CI 1.6-5.0) and Allen *et al.,* in a population study of 130.000 pregnancies showed an OR of 2.5 (95%CI 2.1-2.9) (McCowan *et al.,* 1996; Allen *et al.,* 2004; Zetterstorm *et al.,* 2006; Gilbert *et al.,* 2007; Chappell et al., 2008).

Diabetes

The risk of SGA is confined to those diabetic mothers that exhibit signs of microvascular disease (Langer *et al.,* 1989; Hare *et al.,* 1977). In a study comparing pregnancy outcomes in Type 1 diabetic mothers with microvascular disease (retinopathy, nephropathy, hypertension) Infants were more likely to be SGA (OR 6.0; 95%CI 1.54–23.33) and less likely to be macrosomic (OR 0.46; 95%CI 0.224–0.928) (Howart *et al.,* 2007).

Autoimmune disease

The prevalence of anticardiolipin antibodies in a low-risk obstetric population has been reported to be between 2.7% and 7% and besides the well-established association with fetal loss an association between anticardiolipin antibodies and low birth weight has also been reported (Lockwood *et al.,* 1989; Lynch 1994; Empson *et al.,* 2005). Another study also showed a considerable association (RR: 6.22 (95%CI 2.43–16.0)). Small studies have also shown an association with SLE and SGA which seems to be dependent on the severity of the disease (Clowse *et al.,* 2005; Yasmeen *et al.,* 2001).

Other maternal diseases have been associated with the delivery of SGA neonates. However their prevalence in the general pregnant population is small or their effect is very severity-dependent (i.e. only the very severe form, or uncontrolled disease) and hence their predictive value in screening for SGA in a low-risk population is limited. Among those are maternal renal disease, severe uncontrolled asthma, uncontrolled hyperthyroidism, cyanotic heart disease or cardiac failure.

Method of conception

Consistent evidence shows an association between artificial reproductive techniques (ART) and SGA (koudstaal *et al.,* 2000). Two meta-analysis studies have found increased ORs of 1.6(95%CI 1.3-2.0) and RR 1.45 (95%CI 1.04-2.00) in singleton pregnancies (McDonald *et al.,* 2009; Jackson *et al.,* 2004).

Maternal exposures

Smoking has a significant dose-dependent association with SGA,(Meyer *et al.,* 1972) with women smoking up to 10 cigarettes daily showing ORs of 1.54(1.39-1.7) and those above 10, 2.21(2.03-2.4)(Kramer *et al.,* 1999). In another recent study the prevalence of smokers in the first trimester of pregnancy was 10% and again an association was found with SGA (1.76 (95%CI 1.03- 3.02). In the same study mothers that had stopped smoking before pregnancy were not found at significantly higher risk (OR 1.06(95%CI 0.67-1.68) hence indicating that smoking cessation is a beneficial intervention (McCowan *et al.,* 2009).

Similar dose dependent effects have been shown with alcohol consumption (Jaddoe *et al.,* 20007) with <1 unit/day, OR for SGA = 1.1 (95% CI 1.00-1.13), 1-2 units a day,

OR = 1.62 (95% CI 1.26 - 2.09) 3-5 units a day, OR = 1.96 (95% CI 1.16-3.31) (Mills *et al.,* 1984).

Other exposures in pregnancy such as cocaine use or low fruit intake pre-pregnancy have been shown to be associated with SGA but their use in routine screening would be limited as not many mothers would disclose readily cocaine use and diet habits would be difficult to ascertain reliably within a setting of a routine antenatal appointment.

Ethnicity

Similar to maternal height and weight, ethnicity falls into the physiological vs pathological debate. Birthweight varies with ethnicity and non-caucasian women tend to exhibit lower average birth weight. The effect obviously is dependent on the population examined in mind. At the same time in the UK ethnic minorities appear to be at the highest risk of adverse pregnancy outcome including preeclampsia and having an SGA neonate as well as stillbirth (Akolekar *et al.,* 2011; Poon *et al.,* 2012, Gardosi *et al.,* 2013). First generation African and Pakistani women have the highest risk with RR 2.3 (95%CI 1.5-3.6) and 2.1 (95%CI 1.5-3.1) respectively (Gardosi *et al.,* 2013). This evidence together with evidence showing that adjustment for ethnicity does not improve prediction of stillbirth support the argument that ethnicity should not be corrected (Hutcheon *et al.,* 2011; Poon *et al.,* 2012).

1.4.2 Fetal size

Clinical examination

The most basic forms of screening using clinical examination during pregnancy as a surrogate for fetal size and the ones that have been traditionally used throughout obstetric practice are subjective abdominal palpation and the measurement of the symphysio-fundal height (SFH). The first has been consistently shown to be very inaccurate predictor with sensitivities of as low as 19% in low-risk populations and around 37–50% in high risk populations and is no longer recommended in routine practise (Bais *et al.,* 2004; Kean & liu 1996; RCOG Green-top guideline No. 31).

SFH measurement with the recommended standardized technique is believed to have a value in screening for SGA but with a great variation in predictive accuracy with significant Intra– and inter–observer variation and very prone to confounding factors such as maternal obesity, fetal lie, the presence of fibroids or polyhydramnios.

A large study reported a sensitivity of 27% and specificity of 88% (Bailey *et al.,* 1989; Persson *et al.,* 1986) with serial measurements improving slightly the accuracy. Nonetheless, the minimal cost involved and the opportunity to perform this test in routine antenatal visits makes it an appropriate adjunct to more accurate detection methods. In a Cochrane systematic review, no impact in perinatal outcome was detected with the use of SFH (Neilson 2000).

First-trimester crown-rump length

In a study comparing first trimester expected crown-rump length (CRL) based on maternal menstrual data and actual CRL, an association was found between small CRL in the first trimester and the delivery of an SGA neonate (Mook-Kanamori *et al.,*

2010). Similar results were found in a study examining pregnancies resulting from assisted reproduction techniques (ART) were the conception date is known showing that evidence of impaired growth can be detected from the first trimester of pregnancy (Bukowski *et al.,* 2007). However, this observation is difficult to incorporate into routine screening since dating the pregnancies by fetal CRL in the first trimester is standard clinical practice and hence the effect is artificially normalized.

Second or third-trimester biometry

The two prevailing techniques of assessing fetal size via ultrasound in the second and third trimesters are estimated fetal weight (EFW) and Abdominal circumference (AC). A measurement of either EFW or AC below the 10th centile for gestation produced sensitivities in the range of around 10% for the detection of an SGA neonate in a low-risk population and between 50-90% in a high-risk population (Chauhan *et al.,* 2006; Chang *et al.,* 1992). In a Cochrane meta-analysis of eight trials comparing routine ultrasound screening after 24 weeks with no screening found no overall evidence that routine screening in a low-risk population improved detection of an SGA neonate (RR 0.98% CI 0.74-1.28) or improved perinatal mortality (RR 0.94% CI 0.55-1.61) (Bricker *et al.,* 2008).

A reduction in growth velocity has also been proposed as a discriminating factor between FGR and constitutional SGA (Larsen *et al.,* 1990, Royston *et al.,* 1995). A study examining change in AC or EFW growth velocity in a high-risk population showed better correlation compared with single AC or EFW measurements and adverse perinatal outcome (Chang *et al.,* 1993).

The RCOG currently recommends ultrasound biometric screening with serial ultrasound measurements of customized EFW or AC from 26-28 weeks only in high-risk cases (Table :) (RCOG Green Top Guideline No.34).

1.4.3 Uterine artery Doppler as a screening tool for small for gestational age

Doppler ultrasound of the uterine arteries (UAD) has the ability to evaluate the uteroplacental circulation in a non-invasive way. The technique has shown increasing use in screening for PE with good predictive values in severe disease (Poon *et al.,* 2009). Given the common pathophysiological findings of PE and FGR, its use has extended to the evaluation of uterine artery resistance in placental insufficiency in the absence of PE.

The technique of evaluating the uterine vessels involves insonation of the lateral walls of the cervix and uterus either through the transvaginal route or the transabdominal route. Visualization of the vessels is aided by the use of colour flow mapping and sampling the blood flow via pulsed Doppler (Jurkovic *et al.,* 1991). The resistance can be calculated by using a variety of indexes. The most commonly used indices are the pulsatility and resistant index (PI and RI) which showed the highest predictive value (Cnossen *et al.,* 2008, Gomez *et al.,* 2008). The technique has been standardized in the screening of PE (Poon *et al.,* 2009) and can be performed both in the second or the first trimester. Screening performance in the setting of PE has been shown to be optimal with the use of the lowest uterine artery PI (UAPI) (Poon *et al.,* 2009; Napolitano *et al.,* 2010). UAPI shows a progressive decrease from the first trimester until the late stages of pregnancy (Gomez *et al.,* 2008). Notching of the uterine arteries is considered a sign of increased resistance however its value as a predictive marker in the first trimester has been shown to be poor, especially due to high false positive results (Melchiorre *et al.,* 2009).

Pulsatility index =(peak systolic velocity - minimum diastolic velocity)/Mean velocity

Resistance index = (peak systolic velocity - minimum diastolic velocity)/Peak systolic velocity

Second trimester screening

A multitude of studies has shown a degree of association of high uterine blood flow resistance with the delivery of an SGA neonate. There is a lot of heterogeneity in the use of resistance indices and definitions of SGA. In general, the prediction is stronger in severe/preterm SGA and when screening is limited in high-risk populations. Data from a systematic review summarizing all studies in the literature showed very heterogeneous results. In a low risk setting sensitivities ranged between 12-53% while specificities for all SGA ranged between 83-95% and for severe SGA 26-65% and 88-91%. When only a high-risk population was screened prediction was improved with ranges from 29-89% and 68-92% and for severe SGA 64-82% and 80-92% (Cnossen *et al.,* 2008). Current RCOG guidelines advise against universal UAD screening in the second trimester but support UAD use in high-risk patients (RCOG Green Top Guideline No. 34).

First-trimester screening

Studies have demonstrated that uterine artery Doppler indices reflect the degree of trophoblastic invasion in the first trimester (Prefumo *et al.,* 2004). The observation that trophoblast invasion was maximal in the first trimester justifies the evaluation of uterine artery Doppler findings in the first trimester of pregnancy (Caniggia *et al*.,

2000). Studies that have assessed the value of uterine Doppler in the prediction of SGA in the first trimester of pregnancy are summarized in Table 1.7.

Table 1.7 Studies examining first-trimester uterine resistance in prediction of SGA in low-risk populations

Author	SGA	Controls	Cut-off	Sensitivity	False +ve	Definition SGA
Melchiorre *et al.,* 2009	377	2445	RI>95th	16.7%	4.9%	<10th
Poon *et al.,* 2008	296	609	MoMs	17.6%	10%	<5th customized
Pilalis *et al.,* 2007	35	893	RI>2.52	22.8	Not shown	<5th
Dugoff *et al.,* 2005	12	1008	RI>0.82	16%	5%	<10th
Gomez *et al.,* 2005	37	999	RI>95th	24%	4.9%	<5th
Martin *et al.,* 2001	290	3045	mPI>2.35	11.7%	4.4%	<10th
Severe SGA (<32 wks)						
Martin *et al.,* 2001	18[1]	3045	PI>2.35	27.8%	5.0%	<10th

Few studies exist in the literature but they seem to have relatively homogeneous definitions of SGA being either the 5th or 10th centile. At the same time, all studies have used either RI or PI as a measure of resistance. Comparable results can be seen across all studies with sensitivities ranging between 12-24% for false positive rates between 5 -10%. All the studies have examined low-risk obstetric populations. Martin *et al.* stratified further SGA by severity (<32wks) and showed improved prediction in the high-risk group.

The value of uterine artery Doppler as a screening tool in isolation is low. However, it is a test which has been directly linked to the pathological process of impaired

placentation and its value in combination with other predictive parameters is to be determined.

1.4.4 Mean arterial pressure

Chronic hypertension in pregnancy is a recognized risk factor for the delivery of an SGA neonate. Measurement of blood pressure at the booking visit is routine practice in most established antenatal surveillance protocols. At the same time, many studies have examined the use of blood pressure (BP) measurement as a screening test for the subsequent development of hypertensive disorders in pregnancy and have been analysed in a recent systematic review (Cnossen *et al.,* 2008). Evidence shows that women destined to develop PE can exhibit higher blood pressure readings as early as in the first-trimester of pregnancy (Moutquin *et al.*, 1985; Higgins *et al.*, 1997). By definition when examining pregnancies that resulted in the delivery of SGA neonates in the absence of hypertensive disorders of pregnancy we exclude those that subsequently developed hypertension. However, the use of BP screening in a systematic way with the use of Mean Arterial Pressure (MAP) readings has been successfully incorporated in routine screening for PE in the first trimester of pregnancy in some tertiary centers (Poon *et al.,* 2011). No study so far has examined the value of MAP in screening for SGA but the incorporation of this measurement into a predictive algorithm for SGA in the first trimester might have a value through detecting either cases of previously undiagnosed chronic hypertension or cases that did not subsequently exceed the diagnostic threshold for the diagnosis of gestational hypertension.

1.4.5 Nuchal translucency

Few studies have raised the possibility for an association between fetal nuchal translucency thickness (NT) and birth weight; however, the available published data regarding this possible association is limited (Kelecki *et al.,* 2005). A negative correlation was found with Δ-NT and the delivery of SGA neonate in a recent study with aOR of 0.57(95%CI 0.38-0.86) (Papastefanou *et al.,* 2012). The proposed mechanism for the association of Δ-NT with fetal growth in a small population of diabetic pregnancies was that hyperglycemia, which is a causal factor for large for dates neonates causes augmented capillary permeability, resulting in increased collection of nuchal fluid. Given the availability of data collected from routine first trimester screening the presumed association warrants further investigation.

1.4.6 Biochemical markers in screening for small for gestational age

A variety of biochemical markers detected in maternal blood has been evaluated in the prediction of an SGA neonate (Table 1.8). The majority of these markers have been seen in parallel with research on PE and others are already in use in screening for aneuploidies. Many have been extensively evaluated in the second and third trimesters and less so in the first trimester. By the nature of placental insufficiency in the vast majority of times as disease progresses the biochemical response gets accentuated. Hence many studies have demonstrated marked differences between biomarkers in the third trimester. However since the focus of research effort has moved towards the assessment of placental insufficiency in the first trimester, the following review of the relevant biomarkers will focus on those markers that have potential value in early assessment.

Table 1.8 Biomarkers for predicting small for gestational age identified in the literature (Conde-Agudelo *et al*: Novel biomarkers for predicting intrauterine growth restriction: a systematic review and meta-analysis, BJOG 2013)

Angiogenesis-related biomarkers Placental growth factor Soluble fms-like tyrosine kinase-1 Soluble endoglin Vascular endothelial growth factor Angiopoietin **Endothelial function/oxidative stress-related biomarkers** Homocysteine Leptin Asymmetric dimethylarginine Soluble vascular cell adhesion molecule-1 Soluble intercellular adhesion molecule-1 Isoprostanes 8-oxo-7,8-dihydro-2′-deoxyguanosine Fibronectin Lactate dehydrogenase Pentraxin 3 Interferon-c Interleukin-1 receptor antagonist Interleukin-12 Eotaxin Regulated on activation, normal T-cell expressed and secreted (RANTES) C-reactive protein Folate	**Placental proteins/hormone-related biomarkers** Insulin-like growth factor binding protein-1 and -3 A disintegrin and metalloprotease-12 Placental protein-13 Activin A Placental growth hormone Pregnancy-specific b-1-glycoprotein Annexin A5 Hepatocyte growth factor **Others** Urinary albumin:creatinine ratio Vitamin D Thyroid function tests (thyroid-stimulating hormone, free thyroxine, free triiodothyronine) Metabolomics Genetic biomarkers

Placenta proteins / Hormones

Pregnancy Associated plasma protein A (PAPP-A)

PAPP-A is a protease that facilitates the release of free insulin-like growth factor (IGF) (Lawrence *et al.,* 1999). IGFs are believed to play an important role in the regulation of trophoblast invasion. Low PAPP-A is presumed to be a reflection of reduced IGFs and has been associated with poor placental growth and placental-related conditions (van Kleffens *et al.,* 1998; Irwin *et al.,* 1999; Conover *et al.,* 2004). Associations have been found with low PAPP-A and spontaneous fetal loss, stillbirth, severe pregnancy-induced hypertension (PIH), preeclampsia, and SGA infant. In a systematic review of screening studies performed in the first trimester combined predictive value for SGA <5th centile was a level of PAPP-A < 1st centile with positive LR of 4.36(95%CI 3.27-5.80) and negative LR 0.97(95%CI 0.96-0.98). In a large screening study with 33000 controls and 1300 SGA pregnancies, the sensitivity of screening with low PAPP-A was 12.2% for an FPR of 9.5% (Dugoff *et al.,* 2004). In conclusion, the value of PAPP-A in isolation for predicting SGA is low but this marker might have a value in combined screening. RCOG guidance considers a PAPP-A level of <0.415 MoM (5th centile) at first trimester screening a major risk factor (OR 2.7) and recommends further monitoring with growth scans in the 3rd trimester. Studies examining the value of PAPP-A in the prediction of SGA in the first trimester are summarized in table 1.9.

Table 1.9 Studies examining PAPP-A in the first trimester

year	Authors	n SGA	n controls	Lev SGA	Lev contr.	P value	Def SGA	comment
2004	Krantz	270	6276	<5th centile MoM			<10th	DR 9.7% FP 4.9%
2008	Spencer	104	240	0.78MoM	0.98MoM	P<0.001	<5th gender	
2007	Pihl	36	108	0.64 MoM	1.02 MoM	P<0.001	<5th gender	
2007	Pilalis	35	878		1.19MoM			DR17.1% PPV 13.3%
2004	Duggoff	1300	33395	<5th cent			<5th	DR 12.2% FP 9.5%
2003	Tul	51	1136	0.76MoM	1.02MoM	P<0.002	<10th	
2003	Kwik & Morris	105	722	<0.5MoM	>0.5MoM	P<0.001	<10th	DR 32.7% FP 11%
2000	Ong	171	4297	0.0900	1.05			

Beta Chorionic Gonadotrophin

β-hCG in addition to its function in the regulation of steroidogenesis during early gestation promotes trophoblast cell motility (Zygmut *et al.,* 1998). β-hCG /LH receptors are abundantly expressed in uterine endothelial cells implicating a possible functional responsiveness of these cells to β-hCG and a possible role of β-hCG as an angiogenic factor (Lei *et al.,* 1992; Toth *et al.,* 1994). Some studies have associated hypoxic cytotrophoblasts with increased β-hCG production. In screening for SGA β-

hCG has shown small predictive value with a meta-analysis of 5 studies performed in the first trimester showing a β-hCG > 2MoM having a positive LR 1.74(1.48-2.04) and negative LR 0.95(0.93-0.96) (Morris *et al.,* 2008). β-hCG is widely performed as part of screening for aneuploidies and might have a value as part of a combined screening protocol.

Placental protein 13 (PP13)

Placental protein 13 (PP13) is a 32-kd dimer protein and is a placental product thought to have a role in implantation and maternal spiral artery remodelling (Sammar *et al.,* 2005; Burger *et al.,* 2004). Levels measured in the first trimester have been found to be reduced in women with early preeclampsia (Nicolaides *et al.,* 2006). Two studies have examined the value of PP13 in the first trimester in women delivering SGA neonates and are summarized in table 1.10. In a study comparing 42 SGA pregnancies with 290 controls for a FPR of 10% the sensitivity for PP13 was 33% and OR 4.3 4.3 (95% CI, 2.1-9.1) (Chafez *et al.,* 2007). In another study, no significant difference was found between SGA and controls and the authors in similar populations and with similarly defined SGA group (<5th) (Cowans *et al.,* 2008). The value of PP13 in the first trimester in the prediction of SGA remains to be confirmed.

Table 1.10 Studies examining PP13 in the first trimester

year	Authors	n SGA	n controls	Lev SGA	Lev contr.	P value	Def SGA	comment
2008	Cowans	488	364	1.058MoM	1.096MoM	ns	<5th	
2007	Chafez	42	290	0.6MoM	1.0MoM	P<0.001	<5th	DR 33%, FP 10%

A-Disintegrin and Metalloprotease-12 (ADAM12)

A-disintegrin and metalloprotease (ADAM12) is a placenta-derived glycoprotein that has been shown to have regulating properties upon the bioavailability of insulin-like growth factor which in turn promotes placental growth (Shi *et al.,* 2000). ADAM-12 has been shown to be decreased in the first trimester in both PE and SGA pregnancies (Spencer *et al.,* 2008; Cowans *et al.,* 2007). Studies examining ADAM12 as a first-trimester marker of SGA are summarized in table 1.11. A study examining 296 SGA pregnancies detected a significant difference in the levels of ADMA12 in the first trimester (SGA 0.84 vs Control 1.01 p<0.001) (Poon *et al.,* 2008) but when used in a multivariate predictive model did not confer additional value in the screening performance. Its value in a larger population sample will be examined in further chapters of this thesis.

Table 1.11 Studies examining ADAM12 in the first trimester

year	Authors	n SGA	n controls	Lev SGA	Lev contr.	P value	Def SGA
2010	Matwejew	8	452	0.92 MoM	1.03 MoM	ns	<10th
2008	Poon	296	570	0.848 MoM	1.011 MoM	P<0.001	<5th Gardosi
2007	Cowans	523	414	0.885 MoM	1.0 MoM	P<0.00001	<5th
2007	Pihl	36	108	0.74 MoM	0.97 MoM	P<0.004	<5th gender

Angiogenic factors

An imbalance between angiogenic and anti-angiogenic factors has been implicated as a mechanism of disease in PE and for delivery of a small-for-gestational-age neonate (Shibata *et al.,* 2005; Tidwell *et al.,* 2001).

Placental Growth factor

Placental growth factor (PGF) is a member of the VEGF (vascular endothelial growth factor) subfamily and is believed to be a key molecule in angiogenesis of the placental trophoblast (Luttun *et al.,* 2002). Low levels of PLGF have been associated with PE but also low maternal serum concentration of PLGF has been reported in pregnancies complicated by the birth of SGA neonates in the absence of PE. Studies that have investigated the levels of PLGF between normal pregnancies and those delivering SGA neonates in the first trimester are summarized in table 1.12. The largest study examining PLGF in the first trimester for the prediction of SGA compared 296 neonates born with a BW <5th centile with 609 controls found that PLGF was reduced in the SGA group and the use of this marker in isolation had a sensitivity of 14.5% for FPR 10% (Poon *et al.,* 2008).

Table 1.12 Studies examining PLGF in the first trimester

year	Authors	n SGA	n contr.	Lev SGA	Lev contr.	P value	Def SGA	comment
2010	Cowans	8	452	0.56MoM	0.99MoM	P<00.1	<10th	
2010	Steinbergh	34	318	53pg/ml	56pg/ml	P<0.78 NS	<10th gender	Levels adjusted
2008	Poon L.	296	609	0.900MoM	0.991MoM	P<0.001	<5th Gardosi	
2008	Erez O	145	201	30pg/ml	34.5pg/ml	P=0.005	<10th	
2008	Romero.	56	46	No value	No value	P<0.0009	<10th	
2004	Thadhani	40	80	21pg/ml	63pg/ml	P<0.05	<10th gender	
2001	Ong.	137	400	0.99MoM	1.57MoM	P<0.001	<5th	Conflicting result

Soluble fms-like tyrosine kinase 1 (sFlt1) and Soluble Endoglin

Soluble fms-like tyrosine kinase 1 (sFlt1) and soluble endoglin (sENG) are both anti-angiogenic factors. It has been hypothesized that increased levels might be reflective of the endothelial dysfunction seen in impaired placentation. Several studies have reported that in PE the maternal plasma concentration of sFLT1 and sEng is increased (Stepan *et al.,* 2007; Masuyama *et al.,* 2007; Foidart *et al.,* 2010). Few studies have shown increased levels of these angiogenic factors in the second or third trimester in cases of SGA without PE and are summarized in table 1.13. There is very little data on the behaviour of these markers in the first trimester in cases of

SGA. In one study a very small difference was demonstrated for sFLT1 but not sENG (Erez *et al.,* 2008). Also in a recent study, no difference in first trimester levels of sFLT1 was detected between cases of PE and controls (Akolekar *et al.,* 2010). In the following chapters of this thesis, we will examine the levels of sENG in SGA pregnancies in the first trimester.

Table 1.13 Studies examining sENG in the first and second trimesters

year	Authors	n SGA	n contr.	Lev SGA	Lev contr.	P value	Def SGA
First Trimester							
2008	Erez O	145	201	7.1ng/ml	7.2ng/ml		<10th Gest
2008	Romero.	56	46	No value	No value	P<0.0001	<10th Gest
Second or third trimester							
2007	Stepan H.	11	15	25.9ng/ml	5.3ng/ml	P<0.001	<5th + UAPI+oligoh or UAPI
2008	jeyabalan	10	10	19.9ng/ml	17.5ng/ml	P=0.002	Late asymmetric FGR
2008	Erez O	145	201	6ng/ml	5.9ng/ml		<10th Gest
2008	Savvidou M	15	40	7.5ng/ml	6.5ng/ml	P<0.05	23w gest SGA <5th for Gest and Sex
2008	Romero.	56	46	No value	No value	P<0.0001	<10th Gest

Table 1.14 Studies examining sFLT-1 in the first trimester

year	Authors	n SGA	n controls	Lev SGA	Lev contr.	P value	Def SGA
First Trimester							
2008	Erez O	145	201	1615pg/ml	1788pg/ml	P=0.005	<10th for Gest
Second or third trimester							
2005	Shibata e.	13	16	2912pg/ml	2472pg/ml	P=0.99	<10th sex gender gest
2005	Savvidou	17	42	555pg/ml	463pg/ml		<5th gest sex
2007	Stepan H.	13	38	879.3pg/ml	451pg/ml	P<0.05	<5th ?customized + oligo or UAPI
2007	Wallner W	16	15	4479pg/ml	2199pg/ml	P<0.0086	<10th gest
2008	Erez O	145	201	1799pg/ml	1687pg/ml	P=0.005	<10th for Gest
2008	Romero	56	46	No value	Na value	P=0.147	<10th gest

Thyroid stimulating Hormone, Free Triiodothyronine, and Free Thyroxine

In-vitro studies reported that thyroid hormone receptors are expressed in extravillous human trophoblast and thyroid hormones upregulate proliferation and the invasive potential of this trophoblast (Barber *et al.,* 2005; Oki *et al.,* 2004). The addition of thyroid hormones to an organ culture system of human placental tissue from early pregnancy stimulated the production of several placental hormones, including progesterone, human chorionic Gonadotrophin, and estradiol (Maruo *et al.,* 1991). There is also contradictory evidence that clinical and subclinical hypothyroidism is associated with increased risk for both PE and birth of SGA neonates in the absence of PE (Leung *et al.,* 1993; Levine *et al.,* 2009; Sahu *et al.,* 2010, Blazer *et al.,* 2003; Casey *et al.,* 2007; Allan *et al.,* 2000). In a recent study it has been reported that in pregnancies that develop PE, maternal serum thyroid stimulating hormone (TSH) at 11-13 weeks' gestation was higher and free thyroxine (FT4) was lower than in

normotensive controls (Ashoor *et al.,* 2010). A summary of the studies that has investigated the association of thyroid hormones with birthweight is presented in table 1.15. The prevalence of maternal thyroid hypofunction at 11-13 weeks' of gestation and any potential value in first-trimester screening will be investigated further in subsequent chapters.

Table 1.15 Studies examining thyroid hormones and birthweight

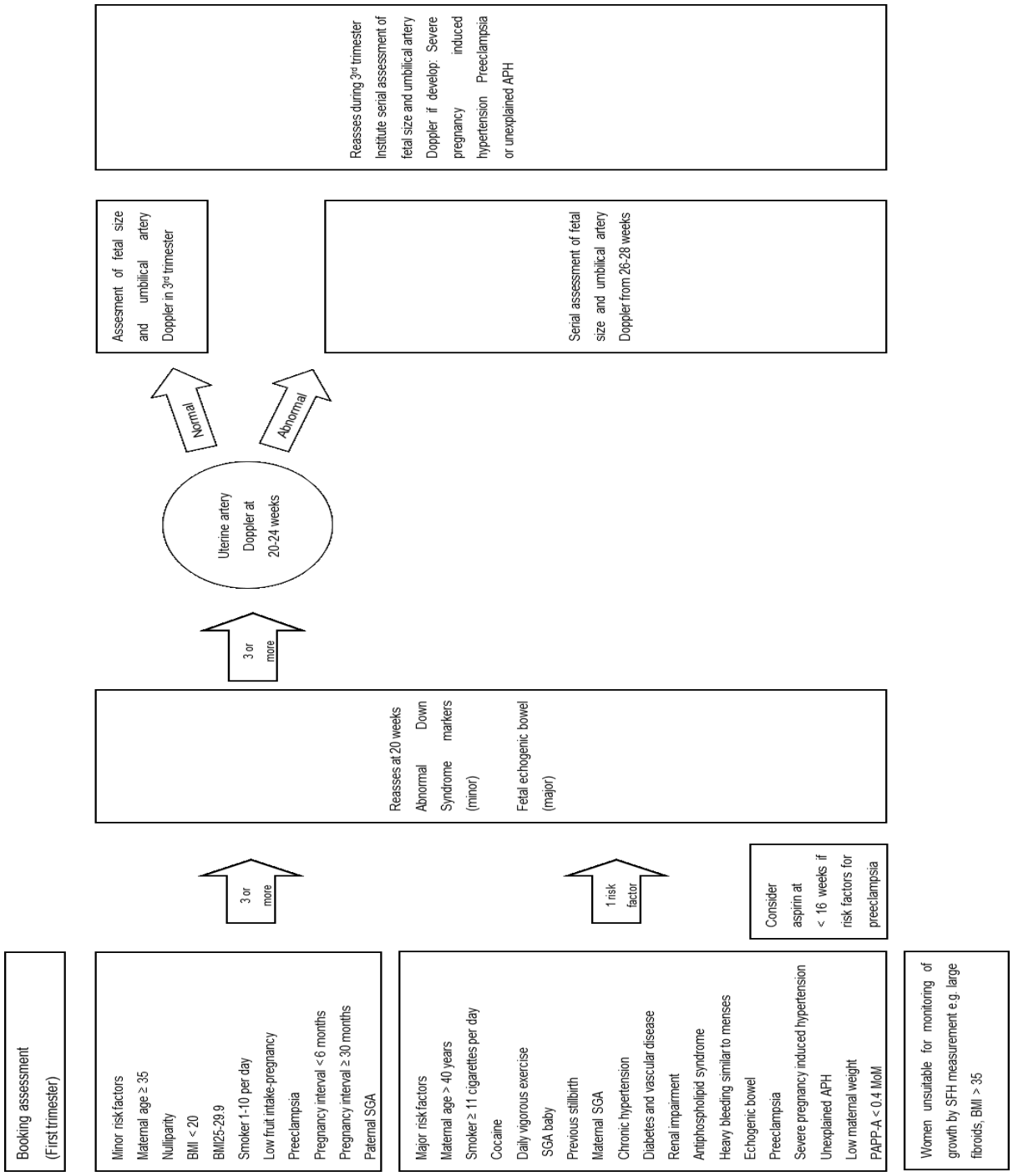
Author	**Normal (TSH)**	**Overt (TSH)**	**Subclinical (TSH)**	**Gestation**	**Outcome measure**	**SGA normal**	**SGA overt**	**p**	**SGA subclinical**	**p**
Allan *et al.,* 2000	9194 (<6mU/L)	37 (>10mU/L)	172 (6-9.99mU/L)	15-18w	Mean BW	3448 g	3498 g	ns	3451g	ns
Idris *et al.,* 2005	127 (<5.5mU/L)	40 (>5.5mU/I)		1st presentation	Median BW	3380 g			3450g	ns
Blazer *et al.,* 2003	139	-	222	-	Mean BW	3454 g			3295g	0.001
Casey *et al.,* 2007	16011 (0.08-2.99mU/L)		598 (>3mU/L)	Before 20w	BW<1000g	0.6%			0.7%	0.83
					BW<2500g	6%			7%	0.24
Titoria *et al.,* 2009	552	29	41	13 – 26 w	SGA <10th centile	4%	13.8 %	0.01	2.4%	ns

1.4.7 Combined screening protocols

The endpoint of antenatal screening is the reduction in stillbirths secondary to FGR along with a reduction in perinatal morbidity. In isolation screening by maternal risk factors, Doppler ultrasonography, or maternal serum analytes has not proven sufficient in the prediction of adverse pregnancy outcomes to permit routine clinical use, many investigators have attempted to improve the predictive value by combining these modalities (Imdad *et al.,* 2011; Zhang *et al.,* 2010, McCowan *et al.,* 2010). The combination of uterine artery Doppler and maternal serum markers has been shown in case-control and cohort studies to have an improved predictive ability for the SGA neonate, although predictive values are still poor (Filipi *et al.,* 2011; Stampalija *et al.,* 2010). Use of combination testing in the 2nd trimester appears to predict adverse outcome related to placental insufficiency more effectively than 1st trimester screening (Dane *et al.,* 2010).

Current Royal College of Obstetricians and Gynaecologists guidance on screening for SGA in pregnancy recommends that all women should be assessed at booking for risk factors for an SGA fetus. Those with a major risk factor should be referred for serial ultrasound growth measurements and Dopplers from 26-28 weeks of gestation while those with three or more minor risk factors should be referred for uterine artery Doppler at 20-24 weeks of gestation with a view to being stratified into the high-risk category depending on the uterine Doppler results. The risk factor and diagram of management of these recommendations are presented in figure 1.4.

Figure 1.4 RCOG recommended protocol for screening for SGA in pregnancy



There is no published data on the performance of this screening strategy. What is apparent, however, is the need for an improvement in detection of placental disease. Increasing evidence illustrates the strong link between SGA and stillbirths and according to National Statistics, the stillbirth rates in the United Kingdom are among the highest in high-income countries with little improvement since the 90s (Office of National Statistics 2011; Flenady *et al.,* 2011). In a recent study across 19 maternity units in the UK in a 2-year period, it was found that the overall detection rate of SGA neonates (BW<10th centile) was only 31%. This is within a setting of nationally coordinated maternity care provision with uniform guidelines. Furthermore, when examined a cohort of 195 stillbirths within the studied population which were thought to be secondary to FGR only 17.9% of them were detected antenatally (Gardosi *et al.,* 2013). In an earlier confidential inquiry into stillbirths in 2007 only 21% of cases that were thought to be due to FGR were detected antenatally (Confidential inquiry into stillbirths with FGR 2007, Perinatal institute).

Screening in the first trimester

Early detection of a high-risk group has multiple aims. Firstly, it allows targeted maternal and fetal surveillance with the more focused allocation of resources. Early recognition of SGA could improve maternal and perinatal outcome by administration of medication for fetal lung maturation and early delivery (Alfirevic *et al.,* 1995; Figueras *et al.,* 2010). Secondly, it allows the possibility of preventative interventions at a time that they are more likely to have a therapeutic effect (i.e. the time of trophoblast invasion). Low-dose aspirin is an example of a preventative intervention that could be targeted to those with evidence of abnormal placentation (Askie 2007). Not many studies have applied a combined protocol for the prediction of SGA in the absence of PE. The available studies in the literature are summarized in table 1.16. In a study by Poon *et al.,* examining 296 cases of SGA (<5th centile) and 609 controls,

the use of maternal risk factors, PLGF and PAPP-A in the first trimester detected 36.8% for a FPR of 10%.

Table 1.16 First trimester combined screening studies

Author	Year	n SGA	n Control	Markers	Sensitivity	FPR	Def SGA	Comment
Papastefanou	2012	261	4236	PAPP-A β-hCG NT	55%	20%	<5th	
Poon	2008	296	609	Maternal history + PLGF + PAPP-A	36.8%	10%	<5th Gardosi	
Pihl	2008	36	108	PAPP-A + β-hCG	33%	10%	<5th Gender	Only caucasian
Pilalis	2007	35	878	PAPP-A + UAPI	Not given	Not given	<5th	AUC: 0.689

1.5 THERAPEUTIC AND PREVENTIVE INTERVENTIONS

Although multiple therapeutic strategies have been tested to promote intrauterine growth and decrease perinatal morbidity and mortality limited, if any, success has been achieved in this area. In the developed world the single most successful intervention for the reduction of FGR secondary to placental insufficiency is smoking cessation (Lumley *et al.,* 2009). In developing countries, balanced nutritional supplements in undernourished women and magnesium and folate supplementation (in some studies) decrease the rate of SGA newborns (Glumezoglu *et al.,* 2003). Aspirin administration for the prevention of defective placentation has shown promising preliminary results.

1.5.1 Timing of delivery and steroid administration

The two principle therapeutic interventions in SGA pregnancies are the administration of prenatal steroids and delivery. The administration of a completed course of steroids is recommended until 34 weeks' gestation and shows the strongest therapeutic effect in cases of preterm iatrogenic delivery (RR 0.77(0.67-0.89) (Baschat *et al.,* 2005; Morris *et al.,* 2013). The timing of delivery in early-onset FGR still remains challenging because gestational age has an overriding effect on the neonatal outcome until the late second trimester and randomized trials of specific delivery triggers are lacking (Baschat 2011). Despite the uncertainties in the optimum management of the SGA fetus when considering the benefits of screening as a whole data support that early antenatal detection improves outcome. In a study from Sweden awareness of SGA before delivery, in combination with a structured program of surveillance for those identified as SGA, was found to be related to a four-fold lowered risk of adverse fetal outcome (Lindqvist *et al.,* 2005). Also in support of an early risk stratification protocol a systematic review of 12 randomized, controlled trials of Doppler ultrasonography of the umbilical artery in high-risk pregnancies reported that, in the Doppler group, there was a 52% reduction in the number of caesarean section rates for fetal distress (24%-69%). Furthermore, the clinical action guided by Doppler ultrasonography reduced the odds of perinatal death by 38% (15%-55% (Alfirevic *et al.,* 1995).

1.5.2 Aspirin

The hypothesis that antiplatelet agents might prevent placental-related complications such as PE and FGR holds considerable interest for the last 30 years (Bujold *et al.,* 2010). Its use is hypothesized to prevent failure of physiological spiral artery transformation and thus impaired placentation. In a randomized study of periconseptual aspirin administration in ART patients it was found that in the aspirin group arcuate artery PI at 6 weeks' gestation and UA PI at 18 weeks' gestation were

significantly lower compared to the placebo group suggesting that early aspirin administration might have a beneficial role in improving early placental blood flow (Haapsamo *et al.,* 2008). Increasing data are emerging from meta-analysis of randomized controlled trials that the use of early aspirin administration before 16wks reduces the rates of PE and SGA (Bujold *et al.,* 2010, Roberge *et al.,* 2012). In a recent meta-analysis of 42 studies, low dose aspirin started before 16 weeks' was found to significantly reduce the risk of SGA 0.46(0-33-0.64)(Roberge *et al.,* 2013). Given the current emerging evidence the benefits of early detection of pregnancies at risk of impaired placentation becomes increasingly relevant.

1.5.3 Smoking cessation

Smoking cessation during pregnancy improves fetal outcomes (Lumley *et al.,* 2009; Batech *et al.,* 2013). Early screening besides taking into account this parameter as a risk factor for SGA allows the opportunity for mothers to engage earlier with antenatal services and perhaps increase the opportunity to implement behavioural interventions and support in order to promote smoking cessation.

1.5.4 Nutritional supplements

In a systematic review of trials, micronutrient supplementation showed a modest increase in mean birth weight and a reduction in the incidence of SGA neonates (RR 0.68, 95% CI 0.56–0.84) (Haider *et al.,* 2006). Most of the included studies were done in developing countries with high fertility rates, low maternal body mass index (BMI), a high prevalence of iron deficiency anaemia and frequent subclinical micronutrient deficiencies and this makes it uncertain whether this effect will be replicated in the setting of developed countries. Although maternal nutrient supplementation has been attempted in suspected SGA/FGR (including intra–amniotic administration of

nutrients), there is not enough evidence to evaluate its effects. (Say *et al.,* 2003, RCOG Green Top guideline no.34).

1.6 CONCLUSION

We have seen that placental insufficiency and the ensuing growth restriction is a prevailing problem in modern obstetric care. Despite technological advances in fetal monitoring and detection of novel markers of placental function the fundamental aim or reducing the number of stillbirths still remains a challenge. Closer examination of the aetiology of stillbirths puts fetal growth restriction as the number one preventable causative factor. A higher understanding of the normal and abnormal processes underlying placental implantation and the ever increasing realization that the first trimester of pregnancy is the window of opportunity for both risk stratifying pregnancies or applying novel therapeutic techniques makes the need for an accurate and reliable screening system a priority. In the following chapters of this thesis we evaluate the most promising current modalities in an attempt to develop a combined predictive algorithm for the delivery of an SGA neonate in the first trimester of pregnancy.

1.7 HYPOTHESIS AND AIMS

Hypothesis:

We hypothesize that it is possible to predict the birth of small for gestational age neonates from the first trimester of pregnancy by using a combination of maternal characteristics, biochemical and biophysical factors.

Aims

- To develop a reference range of birthweight with gestation for an inner city multi-ethnic London population.
- To investigate maternal risk factors associated with the delivery of SGA neonates and to develop a screening algorithm based on multivariate analysis of those maternal risk factors.
- To examine the associations of biophysical markers such as fetal nuchal translucency, mean arterial maternal blood pressure and uterine artery pulsatility index with the birth of an SGA neonate and to examine their value in screening for SGA in the first trimester of pregnancy.
- To examine the association of biochemical markers detected in maternal blood in the first trimester of pregnancy with the birth of an SGA neonate and evaluate their performance in the prediction of the birth of an SGA neonate.
- To evaluate the combined performance of the above maternal risk factors, biophysical and biochemical markers for the prediction of SGA neonates and develop a predictive algorithm that can be applied in the first trimester of pregnancy.
- To compare the estimated performance of the combined predictive algorithm with existing screening strategies for the detection of SGA fetuses antenatally.

CHAPTER 2. PATIENTS AND METHODS

2.1 STUDY POPULATION

The data for this study were derived from prospective screening for adverse obstetric outcomes in women attending for their routine first hospital visit in pregnancy between March 2006 and September 2009. In this visit, which is held at 11^{+0} to 13^{+6} weeks of gestation, we record maternal characteristics and medical history and perform combined screening for aneuploidies by measurement of the fetal CRL and NT thickness and maternal serum PAPP-A and free β-hCG (Robinson & Fleming 1975; Snijders *et al.,* 1998; Kagan *et al.,* 2008). We also measured the maternal MAP by automated devices (Poon LC *et al.,* 2010) and used transabdominal colour Doppler ultrasound to visualise the left and right uterine artery, measure the PI in each vessel and calculate the mean PI (Placencia *et al.,* 2007). At the same time, we collected samples of serum and plasma which were stored at -80 ° C for subsequent biochemical analysis.

During the study period, first-trimester combined screening for aneuploidies was carried out in 36,743 singleton pregnancies. We excluded 3,893 cases because they had missing outcome data (n = 2,005), the pregnancies resulted in miscarriage, termination or the birth of babies with major defects (n =1,136), or they were complicated by PE as defined by the International Society for the study of Hypertension in Pregnancy (n = 752) (Davey and MacGillivray, 1988). In the remaining 32,850 cases there were 1,536(4.7%) SGA and 31,314 (95.3%) non-SGA pregnancies.

2.2 ETHICAL COMMITTEE APPROVAL

Written informed consent was obtained from the women agreeing to participate in the study, which was approved by King's College Hospital Ethics Committee (ref: 02-03-033). An information leaflet was provided to all patients outlining all basic details (Table 2.1).

Table 2.1 Patient information leaflet

Patient information leaflet

Early prediction of women at risk of preeclampsia and fetal growth restriction

We would like to invite you to take part in a research study. Before you decide whether to do so it is important for you to understand why the research is being done and what it will involve. Please take time to read this leaflet. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. Thank you for reading this.

What is the purpose of the study?

We are looking for new ways through scientific research to improve the care of pregnant women and their unborn babies. As part of this work, we are inviting all women that attend for the 11-13 weeks scan to participate in a large study on preeclampsia (high blood pressure of pregnancy) and fetal growth restriction (poor fetal growth).

Preeclampsia and fetal growth restriction are two important complications of pregnancy which can have serious implications for mother and baby. These problems can affect any pregnant woman, irrespective of previous healthy pregnancies and irrespective of how healthy the mother is.

Our aim is to try and identify the women who are at high risk of developing these problems and to do so as early in pregnancy as possible.

Why have I been chosen?

All pregnant women attending for the 11-13 weeks scan are welcome to take part in this study.

Do I have to take part?

It is up to you to decide whether you would like to take part. If you decide to take part you will be given this information sheet to keep and will be asked to sign a consent form. Once you have decided to take part you are still free to withdraw at any time without giving any reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

What will happen to me if I take part?

The study consists of three components which are done at the time of the 11-13 week scan:

1. Maternal blood markers

This involves us saving some of the blood that we take from you as part of the test to determine the risk for Down's syndrome. Since new tests may become available in the future we feel it would be prudent to store some of your blood sample for future studies.

2. Measurement of blood flow from the mother to the placenta

During your visit, we will use ultrasound to examine your baby. We will also use ultrasound to look at the vessels that supply blood to the uterus and the placenta. This extra scan takes a couple of minutes to do. It is not uncomfortable and does not carry any risks to you or your baby.

3. Blood pressure measurement

During your visit, we will measure your blood pressure. Usually, this measurement is taken from your left arm. We are trying to find out if it is better to use the reading from the left or the right arm or the average one from both arms. We would take blood pressure measurements from both of your arms simultaneously.

What are the possible benefits of taking part?

If we find that you have high blood pressure we will arrange for any follow-up tests and monitoring that would be necessary. This will have a direct benefit for you. In addition, the information we get from the study may help us to help you and/or other women in the future.

What are the possible disadvantages and risks of taking part?

The blood pressure measurement may also be uncomfortable because of the inflation of the cuffs. If you find this examination intolerable please let us know, we will stop immediately.

Will my taking part in this study be kept confidential?
Yes. All the information about your participation in this study will be kept confidential.
What if I want to complain?

If you have a concern about any aspect of this study, you can ask to speak with one of the researchers who will do their best to answer your questions. By agreeing to take part in the study you do not lose any legal rights. If you remain unhappy and wish to complain formally, you can do this through the NHS Complaints Procedure. Details can be obtained from the hospital (Contact: Mr. Tim Hiles on 0207 346 3983).

What will happen to the results of the research study?

It is hoped that the results will be published in medical journals and perhaps also in the press. You may request a copy of any published documents in relation to the study. You will not be identified in any of these reports.
Who is organising and funding the research?

This research is carried out by the team of Professor Kypros Nicolaides and it is funded by the Fetal Medicine Foundation (which is a registered charity).

Table 2.2 Consent form

Consent form

Research Study: Early identification of preeclampsia and fetal growth restriction

We are requesting your permission to participate in the research study that essentially involves the following:

1. Maternal blood analysis
2. Measurement of blood flow to the placenta
3. Measurement of blood pressure

We hope that you find it worthwhile to take part in this study. If you should decide to participate, please sign the consent below. We would ask you to sign three copies of this form, one for your own records, one for our research, and one for your medical notes. Thank you.

1. I confirm that I have read and understand the information sheet for the above study and have had the opportunity to ask questions.
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without affecting my medical care or legal rights.
• I agree / disagree to have a sample of my blood taken for current testing and storage for future tests

• I agree / disagree to the measurement of flow in the uterine arteries

• I agree / disagree to the measurement of my blood pressure.

Patient's name:
ID number:
Date: .
Patient's signature: .
Doctor's name: .
Doctor's signature:

2.3 RECORDING OF INFORMATION

Women attending for their routine 11-13 weeks scan were asked to supply the following information during a medical interview and were documented electronically via computer software.

- Age
- Race (Caucasian, African, South Asian and Mixed),
- Cigarette smoking during pregnancy,
- Method of conception (spontaneous or assisted conception by either ovulation induction alone or in vitro fertilisation)
- Medical history (including chronic hypertension, diabetes mellitus, anti-phospholipid syndrome, thrombophilia and sickle cell disease)

- Medication (including anti-hypertensive, anti-depressant, anti-epileptic, aspirin, steroids, betamimetics, insulin and thyroxine), parity (parous, nulliparous with no previous pregnancies, nulliparous with miscarriage or termination before 24 weeks)
- Previous pregnancy with PE (yes or no) and family history of PE (mother, sister or both).
- Previous delivery of an SGA neonate (only SGA, only non-SGA or both SGA and non-SGA)

The maternal characteristics of each of the outcome groups are shown in Table 2.3.

2.4 BIOPHYSICAL MEASUREMENTS

The following measurements were made to all women included in the study

- Maternal weight and height and subsequent calculation of the body mass index (BMI) in Kg/m2,
- Stabilised measurement of MAP
- Fetal crown-rump length in mm
- Fetal Nuchal translucency in mm
- Uterine artery PI of the two arteries

Table 2.3 Maternal characteristics in the unaffected and in those delivering small for gestational age (SGA) neonates

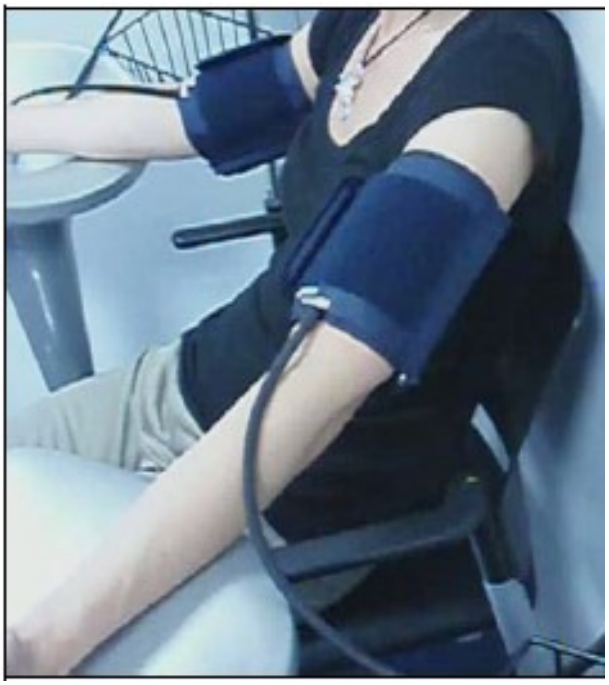
Variables	**Unaffected (n=31.314)**	**SGA (n=1.536)**
Maternal age in yrs, median (IQR)	32.3 (28.0-36.0)	31.4 (26.3 – 35.7)‡
Weight in kg, median (IQR)	66.0 (59.0-75.0)	61.1 (55.0-77.0) ‡
Height in cm, median (IQR)	165.0(160-169.0)	161.8(157.0-166.0) ‡
Racial origin		
Causacian, n (%)	22.898 (73.1)	867 (56.4) ‡
African, n (%)	5.635 (18.0)	416(27.1) ‡
South Asian, n (%)	1.290 (4.1)	140 (9.1) ‡
East Asian, n (%)	600(1.9)	51(3.3) †
Mixed, n (%)	891(2.8)	62(4.0)*
Parity		
Nulliparous, n (%)	14.746(47.1)	952(62) ‡
Parous with previous non-SGA neonate, n (%)	15.302(48.9)	409(26.6) ‡
Parous with previous SGA and non-SGA neonate, n (%)	586(1.9)	62(4.0) ‡
Parous with previous SGA neonate, n (%)	680(2.2)	113(7.4) ‡
Cigarette smoker	2.483(7.8)	257(16.7) ‡
Conception		
Spontaneous, n (%)	30.163(96.3)	1,455(94.7)
Assisted conception, n (%)	1.151(3.7)	81(5.3) *
Chronic hypertension, n (%)	297(0.9)	25(1.6) *
Pre-pregnancy diabetes mellitus, n (%)	235(0.8)	10(0.7)

Comparisons between the SGA and the unaffected groups were by Chi-square or Fisher exact test for categorical variables and Mann-Whitney-U test for continuous variables: * $p < 0.05$, † $p < 0.001$, ‡ $p < 0.0001$.

2.4.1 Blood pressure

The blood pressure was taken by validated automated devices (3BTO-A2, Microlife, Taipei, Taiwan; Reinders *et al.*, 2005) that were calibrated before and at regular intervals during the study (every 1,000 inflations). The recordings were made by doctors who had received appropriate training on the use of these machines. Women were allowed to rest for 15–30 minutes and the BP measurements were taken in a quiet room with a temperature of between 20°C and 24°C. The women were in the seating position, with their arms supported at the level of the heart and either a small (< 22 cm), normal (22-32 cm) or large (33-42 cm) adult cuff was used depending on the mid-arm circumference (Pickering *et al.*, 2005). The BP was measured in both arms simultaneously (Figure 2.1) and a series of recordings was made at 1-minute intervals until variations between consecutive readings fell within 10 mmHg in systolic BP and 6 mmHg in diastolic BP in both arms (National Heart Foundation of Australia, 2004). When this point of stability was reached, we calculated the MAP of each arm as the average of the last two stable measurements and as recommended we took the arm with the highest final MAP and its measurements of systolic BP and diastolic BP for the subsequent analysis of results (National Heart Foundation of Australia, 2004).

Figure 2.1 Simultaneous measurement of blood pressure in both arms



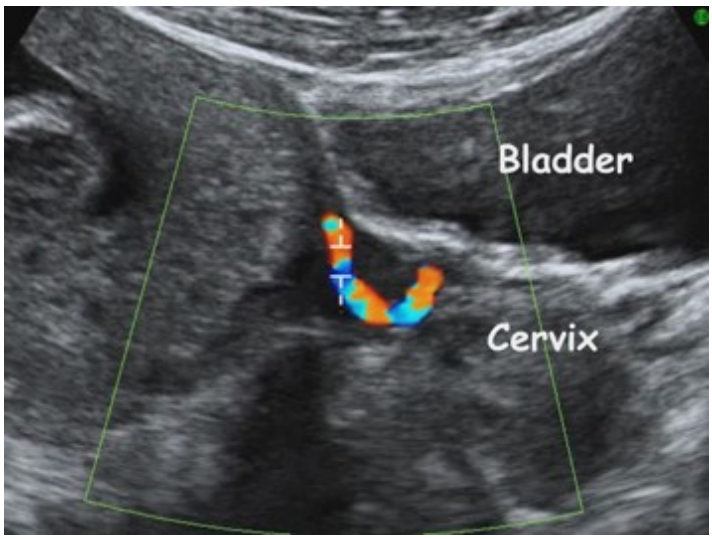
2.4.2 Fetal CRL and NT measurements

The measurement of fetal CRL and NT was performed as part of routine screening for chromosomal abnormalities in the first trimester of pregnancy. All screening was performed by Fetal Medicine Foundation-accredited operators following the recommended technique for CRL and NT measurements (http://www.fetalmedicine.com).

2.4.3 Uterine artery Doppler

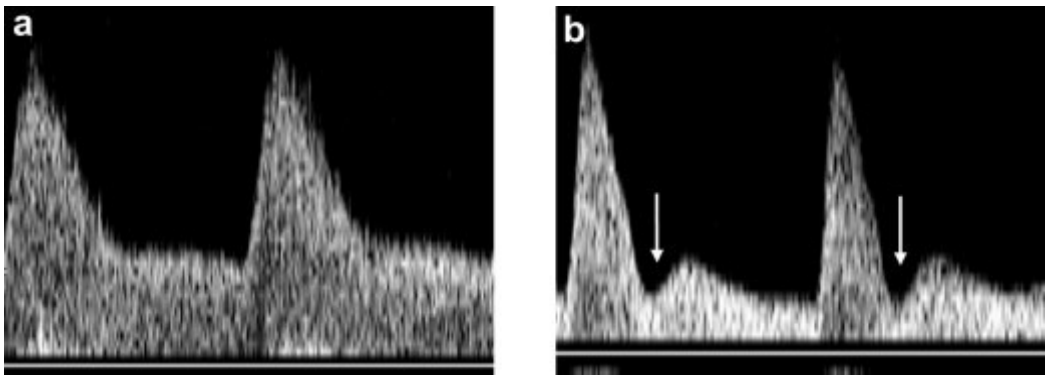
Uterine artery pulsatility index (PI) was measured by transabdominal ultrasound by obtaining a sagittal section of the uterus, cervical canal and identifying the internal cervical os. Subsequently, colour flow mapping was used to identify the uterine arteries by insonating the lateral walls of the uterus.

Figure 2.2 Image of sampling of the uterine artery via the transabdominal route



Pulsed wave Doppler was used to sample the uterine flow with the gate set at 2 mm to cover the whole vessel and an insonation angle of less than 30º. When three similar consecutive waveforms were obtained, the uterine artery PI was measured from the left and right arteries (Figure 2.3). The lowest PI value was used as it has been shown to be the most predictive of PET in a previous study (Poon *et al.,* 2009).

Figure 2.3 Uterine artery waveforms (a. normal resistance, b. increased resistance with notching)



All sonographers that participated in the study had obtained the Fetal Medicine Foundation Certificate of competence in obstetric Doppler imaging (http://www.fetalmedicine.com). The results of the Doppler studies were not given to the women or their doctors and did not influence the subsequent management of the pregnancies.

2.5 BIOCHEMICAL MEASUREMENTS

The following biochemical markers from maternal blood were evaluated for predicting an SGA neonate in the first trimester.

- PAPP-A
- Free β-hCG
- PLGF
- PP13
- ADAM12
- sENG
- Free T3,T4,TSH

All women taking part in our study had a blood sample taken for the analysis of PAPP-A and β-hCG which is part of the routine screening for chromosomal abnormalities. At the same time, Samples of serum and plasma were stored at -80C for subsequent biochemical analysis.

In the case of PLGF, PP13, ADAM12, FT3, FT4 and TSH these markers were examined in case-controlled studies. The cases were drawn from the screening study population on the basis of availability of stored serum. The non-SGA cases were from singleton pregnancies with no history of thyroid disease, which did not develop PE and resulted in the live birth of phenotypically normal neonates with birth weight above the 5th percentile for gestational age. The control cases were matched to the SGA cases for storage time. Data from the case controlled studies of PLGF, PP13 and ADAM 12 were included in previous publications (Poon *et al.,* 2008a, Akolekar *et al.,* 2009; Poon *et al.,* 2008b), while data on sENG, TSH, FT3, FT4 has not been published before. In this study, we combine all available data to develop an integrated algorithm for the early prediction of SGA in the absence of PE.

2.5.1 PAPP-A & β-hCG

Maternal serum pregnancy-associated plasma protein-A (PAPP-A) and β-hCG were measured using the DELFIA XPRESS analyzer (PerkinElmer Life and Analytical Sciences, Waltham, USA). In the case of PAPP-A, the variation of the DELFIA XPRESS PAPP-A assay was determined in 20 runs with two replicates using this DELFIA XPRESS system. The calibration curve of the first run was used as a reference curve during the 14-day-period. The intra-assay and inter-assay variations were 1.2% and 2.1%, respectively, at a PAPP-A concentration of 462 mU/L, 1.4% and 2.3% at 2124 mU/L and 1.3% and 2.5% at 5543 mU/L.

2.5.2 PLGF

Duplicate serum sample of 100 µL was used to measure PlGF concentration by a quantitative enzyme-linked immunoassay (ELISA) technique using Quantikine human PlGF immunoassay (R&D systems Europe Ltd., Abingdon, UK). The assays were performed on an automated ELISA processor (Dade-Behring BEP 2000, Liederbach, Germany). Absorbance readings were taken on a VICTOR3 plate reader (PerkinElmer Life and Analytical Sciences, Turku, Finland) and PlGF concentrations were determined using Multi-Calc software (PerkinElmer Life and Analytical Sciences, Turku, Finland). The lower limit of detection of the assay was 7 pg/mL and the between batch imprecision was 8.3% at a PlGF concentration of 48 pg/mL, 5.6% at 342 pg/mL and 5.1% at 722 pg/mL. Samples whose coefficient of variation of the duplicates exceeded 15% were reanalysed

2.5.3 PP13

Serum PP13 was measured in maternal serum samples (25 µL/well in duplicate by DELFIA ® (Dissociation-Enhanced Lanthanide Fluorescent Immunoassay) using research reagents (Perkin Elmer Life and Analytical Sciences, Turku, Finland). The concentration of PP13 measured was directly proportional to the fluorescence measured on a time-resolved fluorometer at 615 nm. The coefficient of variation (CV) was 4.1% at a PP13 concentration of 16.6 pg/mL, 2.0% at 60.4 pg/mL and 2.7% at 136.2 pg/mL. Samples with duplicate CVs greater than 10% were reanalysed.

2.5.4 ADAM12

A single serum sample of 25 microliters was used to measure ADAM12 concentration by a heterogeneous time-resolved fluorescent immunoassay, where ADAM12 concentration was directly proportional to the fluorescence measured on a time-

resolved fluorometer at 615 nm (DELFIA/AutoDELFIA ADAM12 research kit, PerkinElmer Life and Analytical Sciences,Turku, Finland). Fresh aliquots of ADAM12 quality control samples of 77.6, 294.4, and 736.2 pg/mL concentration were measured in duplicate at the beginning and at the end of each run. The mean coefficients of variation were 5.4%, 2.8%, and 3.2%, respectively.

2.5.5 Soluble Endoglin

Plasma sEng and serum PlGF were measured by enzyme-linked immunoassay technique using DuoSet® human sENG and Quantikine® human PlGF immunoassay (R&D Systems Europe Ltd., Abington, UK). The lower limits of detection of the assays were 5 pg/mL for sEng and 7 pg/mL for PlGF. Samples whose coefficient of variation of the duplicates exceeded 15% were re-analyzed.

2.5.6 FT3, FT4, TSH

The maternal serum concentrations of FT3, FT4 and TSH were measured by immunoassay using direct, chemiluminometric technology (Siemens Advia Centaur assays, Siemens Healthcare Diagnostics Ltd, Surrey, UK). The minimum detectable concentrations of FT3, FT4 and TSH were 0.3 pmol/L, 1.3 pmol/L and 0.003 mIU/L, respectively. The inta-assay coefficients of variation were 3.08%, 2.35% and 2.47% at FT3 concentrations of 2.9 pmol/L, 6.6 pmol/L and 14.2 pmol/L, respectively; 4.69%, 2.31% and 2.22% at FT4 concentrations of 6.1 pmol/L, 13.9 pmol/L and 39.9 pmol/L, respectively; 2,48%, 2.44% and 2.41% at TSH concentrations of 0.74 mIU/L, 5.65 mIU/L and 18.98 mIU/L, respectively.

2.6 OUTCOME MEASURES

Data on pregnancy outcome were obtained from the maternity computerised records or the general medical practitioners of the women and were recorded in our database. The neonate was considered to be SGA if the birth weight was less than the 5th percentile for gestation at delivery, using a reference range derived from our population (Poon *et al.,* 2010). Neonates with birth weight at or above the 5th percentile were classified as non-SGA. We excluded pregnancies with major fetal abnormalities, those ending in termination, miscarriage or fetal death before 24 weeks, those with PE and cases with no pregnancy follow-up. We further stratified SGA cases between those delivering before and after 37 weeks of gestation.

The severity of SGA is reflected in the gestation at delivery with most of the preterm SGA neonates being delivered due to iatrogenic reasons. The obstetric records of all women with an SGA pregnancy were examined to determine if there was no concomitant gestational hypertension or Preeclampsia. Similarly, for quality control, we examined the records of 500 randomly selected cases without pregnancy-associated hypertension.

2.7 STATISTICAL ANALYSIS

The distribution of birth weight was made Gaussian after logarithmic10 transformation. Regression analysis was used to determine the association of birth

weight with gestation at delivery (GA) and to establish a reference range with gestation (Figure 3.1, chapter 3).

Baseline data for the outcome groups were summarised by the median and inter-quartile range (IQR) and number (%) in Table 3.2 and comparisons between outcome groups were by Chi-square or Fisher exact test for categorical variables and by Mann–Whitney U test for continuous variables, both with posthoc Bonferroni correction (critical statistical significance *p<0.0167). Multivariate logistic regression analysis was used to determine the factors amongst the maternal characteristics with significant contributions in predicting SGA.

The patient-specific risk for the SGA group is calculated from the formula: odds / (1+odds), where odds = eY. Y is derived from logistic regression analysis of factors amongst the maternal characteristics, medical and obstetric history in predicting pregnancies delivering an SGA neonate. The performance of screening for SGA by maternal characteristics was estimated by receiver operating characteristic (ROC) curves (chapter 3).

In each patient in the SGA and non-SGA groups above biophysical and biochemical markers were measured as described, comparisons between the groups were conducted by X^2 or Fisher's exact test for categorical variables and by Mann-Whitney U test for continuous variables and Gaussian distributions were fitted. These fitted distributions define the likelihood ratios for the screening tests that can be combined with the prior risk to produce a posterior risk. The performance of screening by the above markers was compared by the areas under the ROC curves (AUROC) (Zweig and Campbell, 1993) (Chapters 3,4,5&6)

Subsequently the biophysical and biochemical markers were simulated for 500,000 pregnancies from the SGA populations and the non-SGA distributions. For the

markers this involved sampling from the fitted multivariate Gaussian distributions. For the prior risks, this involved drawing samples, with replacement, 500,000 records from the screening samples of SGA and non-SGA pregnancies. These records were then used to define maternal factors related a priori risks for SGA that were multiplied by the likelihood ratios of the biophysical and biochemical markers to derive the a-posterior risks in simulated samples of 500,000 SGA and 500,000 non-SGA pregnancies. The a priori and a posteriori risks in the SGA and non-SGA groups were used to calculate the detection rates at fixed false positive rates of 5 and 10%. The process of sampling with replacement from the SGA and non-SGA screening data means that the modelled screening performance reflects the screening population. The samples of 500,000 were chosen to make the error resulting from the simulation negligible. (Chapter 6)

The statistical software package SPSS 16.0 (SPSS Inc., Chicago, Ill., USA) was used for data analyses. Monte-Carlo simulations were programmed in R (The R Foundation for Statistical Computing, R version 2.11.0, ISBN 3–900051–070–0, http://www.rproject.org).

CHAPTER 3. DEFINING SMALL FOR GESTATIONAL AGE

ABSTRACT

Objective: *Firstly, to establish a reference range of birth weight with gestation at delivery and to identify maternal characteristics significantly associated with birth weight.*

Method: *In 33,602 women with singleton pregnancies at 11 weeks to 13 weeks 6 days maternal demographic characteristics and medical history were recorded. Regression analysis was used to determine the association of birth weight with gestation at delivery and to establish a reference range with gestation. Logistic regression analysis was used to determine if maternal factors contribute significantly in predicting SGA in the absence of preeclampsia.*

Results: *Birth weight increased with maternal weight and height; it was higher in parous than in nulliparous women and in those with a medical history of pre-pregnancy diabetes mellitus, and it was lower in cigarette smokers, in all racial groups other than in Caucasian women, and in those with a medical history of chronic hypertension and in those who previously delivered SGA neonates.*

Conclusion: *Prediction of the birth of SGA neonates in the absence of preeclampsia can be provided in the first trimester of pregnancy by a combination of maternal characteristics.*

This chapter is based on:

Poon LC, Karagiannis G, Staboulidou I, Shafiei A, Nicolaides KH: Reference range of birthweight with gestation and first-trimester prediction of small-for-gestation neonates. Prenat Diagn 2011.

3.1 INTRODUCTION

Birth weight is affected by gestational age at delivery and several maternal characteristics, including racial origin, age, body mass index, parity and cigarette smoking (Gardosi et al., 1995a; Wen et al., 1990; Clausson et al., 1998; Gardosi, 2006).

The aims of this study in a population of more than 30,000 singleton pregnancies attending for routine care at 11-13 weeks were to establish a reference range of birth weight with gestation at delivery and to identify maternal characteristics that were significantly associated with birth weight.

3.2 PATIENTS AND METHODS

This was a prospective screening study for adverse obstetric outcomes in women attending for their routine first hospital visit in pregnancy at 11-13 weeks of gestation. See Chapter 2 for a description of the population, data collection, and outcome measures.

Patients were asked questions on maternal age, racial origin (Caucasian, African, South Asian, East Asian and mixed), method of conception (spontaneous or assisted conception requiring the use of ovulation drugs), cigarette smoking during pregnancy (yes or no), history of chronic hypertension (yes or no), history of type 1 or 2 diabetes mellitus (yes or no) and obstetric history including parity (parous or nulliparous if no previous pregnancies at or after 24 weeks) and birth weight of previous neonates (only SGA, only non-SGA, or mixture of SGA and non-SGA). The answers were documented by a doctor and the maternal weight and height were measured.

During the study period (March 2006 to September 2009) first-trimester combined screening for aneuploidies was carried out in 36,743 singleton pregnancies. We excluded 3,141 (8.5%) cases because they had missing outcome data (n=2,005), or the pregnancies resulted in miscarriage before 24 weeks of gestation (n=431), they were terminated or they resulted in the birth of babies with major defects (n=649), they were terminated for maternal psychosocial indications (n=56) (n=1,136). Statistical analysis was performed in the remaining 33,602 pregnancies.

3.2.1 Statistical analysis

The distribution of birth weight was made Gaussian after logarithmic10 transformation. Regression analysis was used to determine the association of birth weight with gestation at delivery (GA) and to establish a reference range with gestation. The neonate was considered to be SGA if the birth weight was less than the 5th percentile for GA. Multivariate logistic regression analysis was used to determine the factors amongst the maternal characteristics with significant contributions in predicting SGA. The performance of screening was estimated by receiver operating characteristic (ROC) curves.

The statistical software package SPSS 15.0 (SPSS Inc.,Chicago, IL) and Medcalc (Medcalc Software, Mariakerke, Belgium) were used for the data analyses.

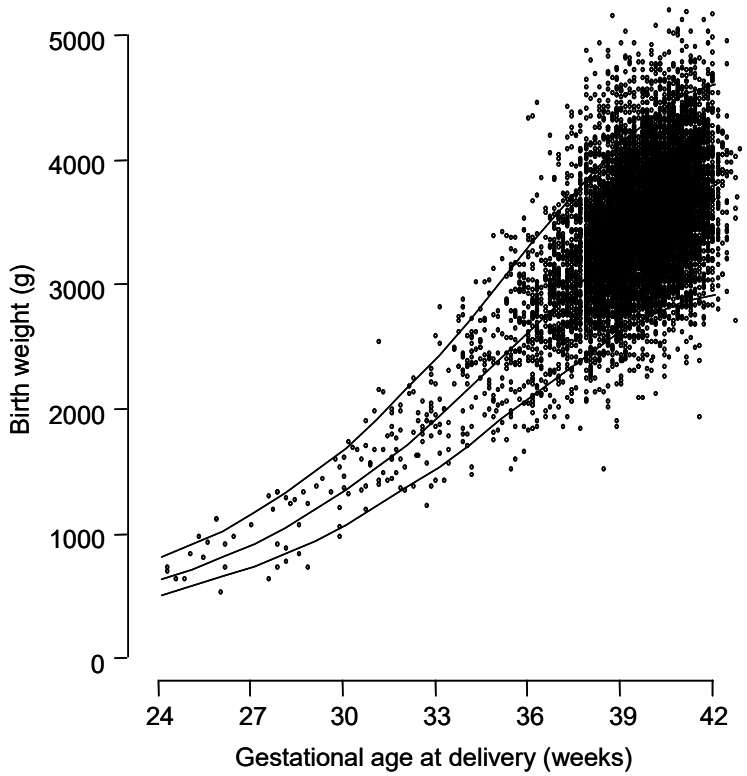
3.3 RESULTS

3.3.1 Birth weight corrected for gestation at delivery

In the total population of 33,602 pregnancies there was a significant association between birth weight and gestation at delivery (Figure 3.1):

Expected $\log_{10}$ birth weight = -0.63294048133589 + 0.187282402775684 x (GA) - 0.00207767621420371 x $(GA)^2$; R^2=0.574, SD=0.058051, p<0.0001.

Figure 3.1 Relationship between birth weight and gestational age at delivery with 95th, 50th and 5th percentiles.



3.3.2 Birth weight and maternal characteristics

Multivariate linear regression analysis demonstrated that for $\log_{10}$ birth weight significant independent contributions were provided by GA, weight , height, smoking status, parity, racial origin, medical history of chronic hypertension and pre-pregnancy diabetes mellitus (R^2=0.625, SD=0.054439, p<0.0001; Table 3.1).

3.3.3 Prediction of small for gestational age neonates

In 752 (2.2%) of the 33,602 pregnancies, there was preeclampsia and these were excluded from further analysis. In 1,536 (4.7%) of the 32,850 pregnancies, the neonatal birth weight corrected for GA was below the 5th percentile. The maternal characteristics of the SGA and unaffected pregnancies are shown in Table 3.2.

Comparisons between the SGA and the unaffected groups were by Chi-square or Fisher exact test for categorical variables and Mann-Whitney-U test for continuous variables: *p<0.05, $^{\dagger}p$<0.001, $^{\ddagger}p$<0.0001.

The risk for SGA was calculated from the formula: odds / (1+odds) where odds= e^{Y}. Y was derived from multivariate logistic regression analysis of maternal factors (Table 3.3).

Table 3.1 Linear regression analysis for the prediction of log_{10} birth weight by gestational age at delivery (GA) and maternal characteristics and medical history.

Independent variable	*b*	SE	*p*
Intercept	-0.935219	0.042717	<0.0001
GA	0.186853	0.002072	<0.0001
$(GA)^2$	-0.002078	0.000028	<0.0001
Weight	0.003726	0.000622	<0.0001
$(Weight)^2$	-0.000030	0.000008	<0.0001
$(Weight)^3$	8.820640E-08	2.926274E-08	0.003
Height	0.000965	0.000048	<0.0001
Age	0.001466	0.000441	0.0009
$(Age)^2$	-0.000026	0.000007	0.0002
Parous	0.016986	0.000631	<0.0001
Smoking	-0.024867	0.001116	<0.0001
Racial origin			
Caucasian	0		
African	-0.021769	0.000809	<0.0001
South Asian	-0.017824	0.001503	<0.0001
East Asian	-0.005543	0.002176	0.011
Mixed	-0.009063	0.001788	<0.0001
Chronic hypertension	-0.020995	0.002834	<0.0001
Diabetes	0.031430	0.003438	<0.0001
Assisted conception	-0.004015	0.001581	0.011

Table 3.2 Maternal characteristics in the unaffected and in those delivering small for gestational age (SGA) neonates.

Variables	Unaffected (n=31,314)	SGA (n=1,536)
Maternal age in yrs, median (IQR)	32.3 (28.0-36.0)	31.4 (26.3-35.7)‡
Weight in Kg, median (IQR)	66.0 (59.0-75.0)	61.1 (55.0-70.0)‡
Height in cm, median (IQR)	165.0 (160.0-169.0)	161.8 (157.0-166.0)‡
Racial origin		
Caucasian, n (%)	22,898 (73.1)	867 (56.4)‡
African, n (%)	5,635 (18.0)	416 (27.1)‡
South Asian, n (%)	1,290 (4.1)	140 (9.1)‡
East Asian, n (%)	600 (1.9)	51 (3.3)†
Mixed, n (%)	891 (2.8)	62 (4.0)*
Parity		
Nulliparous, n (%)	14,746 (47.1)	952 (62.0)‡
Parous with previous non-SGA neonate, n (%)	15,302 (48.9)	409 (26.6)‡
Parous with previous SGA and non-SGA neonate, n (%)	586 (1.9)	62 (4.0)‡
Parous with previous SGA neonate, n (%)	680 (2.2)	113 (7.4)‡
Cigarette smoker, n (%)	2,483 (7.8)	257 (16.7)‡
Conception		
Spontaneous, n (%)	30,163 (96.3)	1,455 (94.7)
Assisted conception, n (%)	1,151 (3.7)	81 (5.3)*
Chronic hypertension, n (%)	297 (0.9)	25 (1.6)*
Pre-pregnancy diabetes mellitus, n (%)	235 (0.8)	10 (0.7)
Comparisons between the SGA and the unaffected groups were by Chi-square or Fisher exact test for categorical variables and Mann-Whitney-U test for continuous variables: $*$ $p < 0.05$, † $p < 0.001$, ‡ $p < 0.0001$		

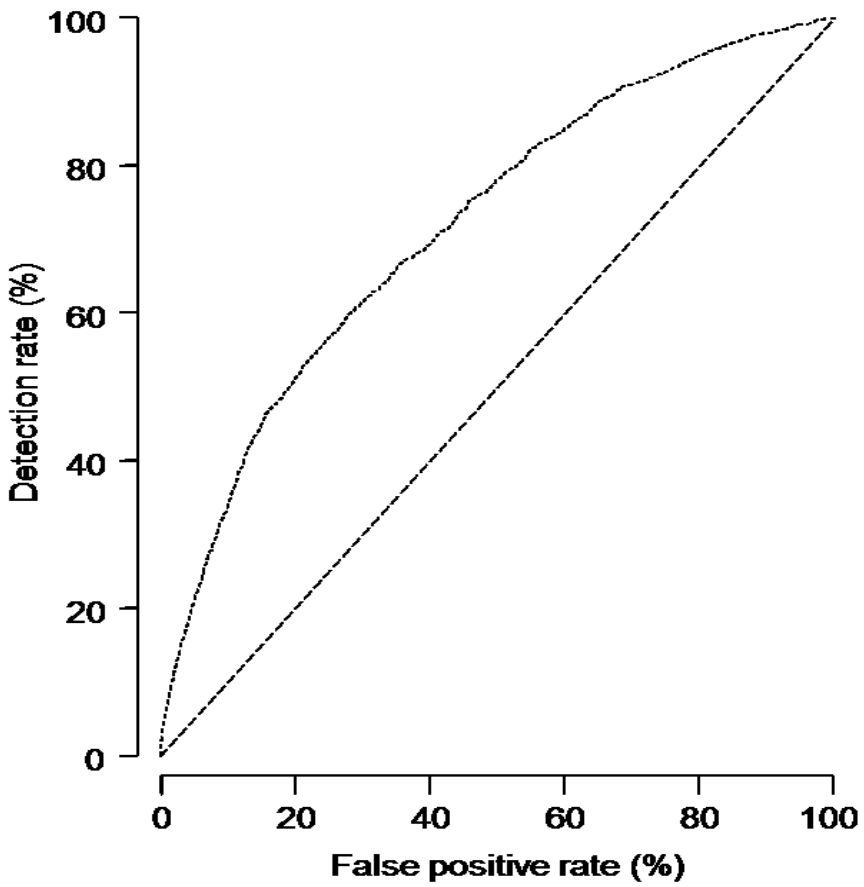
Table 3.3 Logistic regression analysis for the prediction of small for gestational age by maternal factors

Independent variable	**OR**		
Age (per year)	**1.659**	**95%CI**	**P**
(Age)2	0.983	1.152-2.389	0.006
(Age)3	1.000	0.971-0.994	0.004
Weight (per kg)	0.781	1.000-1.000	0.002
(Weight)2	1.003	0.696-0.876	<0.0001
Weight)3	1.000	1.001-1.004	0.001
Height (per cm)	0.968	1.000-1.000	0.005
Racial origin		0.960-0.977	<0.0001
Caucasian	1		<0.0001
African	2.292	2.010-2.615	
South Asian	2.129	1.742-2.602	<0.0001
East Asian	1.456	1.742-2.602	<0.0001
Mixed	1.702	1.070-1.982	0.017
Cigarette smoking	2.736	1.296-2.237	<0.0001
Assisted conception	1.481	2.350-3.186	<0.0001
History of chronic hypertension	1.683	1.160-1.893	0.002
Parity		1.087-2.605	<0.0001
Nuliparous (reference)	1		
Parous with previous SGA	1.823		
Parous with previous SGA and non-SGA	1.054	1.464-2.270	<0.0001
Parous with previous non-SGA	0.392	0.795-1.398	0.715
R2	0.095	0.346-0.444	<0.0001

Performance of screening

The area under receiver operating curve (AUROC) for the detection of SGA in screening by maternal factors is illustrated in Figure 3.2. The AUROC was 0.719 (95% CI 0.706-0.732). The detection rates were 21.0% and 34.0% at false positive rates of 5% and 10%, respectively

Figure 3.2 Receiver operating characteristics curve of maternal factors in the prediction of small for gestational age.



3.4 DISCUSSION

This study has established a reference range of birth weight for gestation in a large heterogeneous inner-city population of singleton pregnancies in which gestational age was determined by an ultrasound scan in early pregnancy. Birth weight is significantly influenced by maternal characteristics such as racial origin, weight, height, parity, cigarette smoking and medical history of chronic hypertension and pre-pregnancy diabetes mellitus.

Birth weight increased with maternal weight and height, it was higher in parous than in nulliparous women and in those with a medical history of pre-pregnancy diabetes mellitus, and it was lower in cigarette smokers, in all racial groups other than in Caucasian women and in those with a medical history of chronic hypertension. The risk for SGA decreased with maternal weight and height and increased with maternal age and in cigarette smokers, nulliparous women, in women of all racial groups other than Caucasians, in those with a medical history of chronic hypertension and in women who had assisted conception. The associations between birth weight and maternal characteristics such as age, weight, parity, racial origin, and cigarette smoking have been extensively reported (Gardosi *et al.*, 1995; Wen *et al.*, 1990; Clausson *et al.*, 1998; Gardosi, 2006). It is recognized that it is necessary to adjust the birth weight for these maternal variables to establish appropriate growth standards in order to define growth abnormalities (Gardosi *et al.*, 1992; Gardosi *et al.*, 1995).

In our screening study of over 30,000 singleton pregnancies for SGA in the absence of preeclampsia we chose 11-13 weeks as the gestation for screening because this is often the first hospital visit of pregnant women at which combined sonographic and biochemical testing for chromosomal and other major defects is carried out (Snijders *et al.*, 1998; Kagan *et al.*, 2008). At this visit, maternal characteristics are recorded,

an ultrasound scan is carried out to confirm the gestation, screen for major defects and measure fetal NT, and maternal blood is taken for the measurement of free ß-hCG and PAPP-A. Combining maternal characteristics with sonographic and maternal serum biochemical markers provides effective early screening for both chromosomal abnormalities and the development of preeclampsia with a detection rate of about 90% at a false positive rate of 5% (Poon *et al.*, 2009).

There is currently no effective method of early screening for SGA in the absence of preeclampsia. This study demonstrated that a combination of maternal demographic characteristics and medical history can detect 34% of pregnancies that subsequently deliver SGA neonates, at a false positive rate of 10%. When considering the AUROC value of 0.719 it is evident that the detection capabilities of maternal characteristics used in isolation would be a poor test for SGA. The extent to which a substantial increase in the performance of screening for SGA can be achieved by a combination of maternal factors with a series of biochemical and biophysical parameters will be investigated in the subsequent chapters of this thesis.

CHAPTER 4. BIOPHYSICAL MARKERS AT 11-13 WEEKS IN THE PREDICTION OF FETAL GROWTH RESTRICTION

ABSTRACT

Objective: To develop a model for prediction of small-for-gestational age (SGA) neonates in the absence of preeclampsia (PE) based on maternal factors and biophysical markers at 11-13 weeks' gestation.

Method: Screening study in 1,536 SGA and 31,314 non-SGA pregnancies based on maternal characteristics, fetal nuchal translucency (NT) thickness, mean arterial pressure (MAP) and uterine artery pulsatility index (PI). Regression analysis was used to develop a model for the prediction of SGA.

Results: In the SGA group, uterine artery PI and MAP were increased and fetal NT was decreased. By using a combination of all biophysical markers in addition to maternal factors the detection rate for SGA was 25.5% for FPR of 5% and 37.7% for FPR of 10%. Screening performance with all biophysical markers for SGA< 37 weeks was 33.7% for FPR of 5% and 46.8% for FPR of 10%.

Conclusion: Prediction of the birth of SGA neonates in the absence of preeclampsia can be provided in the first trimester of pregnancy by a combination of maternal characteristics, fetal nuchal translucency (NT) thickness, mean arterial pressure (MAP) and uterine artery pulsatility index (UAPI).

This chapter is based on:

Karagiannis G, Akolekar R, Sarquis R, Wright D, Nicolaides KH. Prediction of small-for-gestation neonates from biophysical and biochemical markers at 11-13 weeks. Fetal Diagn Ther. 2011;29(2):148-54

4.1 INTRODUCTION

Histological studies reported that in pregnancies complicated by preeclampsia (PE) and SGA without PE there is evidence of impaired placentation characterized by inadequate trophoblastic invasion of the maternal spiral arteries (Sheppard *et al.,* 1981; Khong *et al.,* 1986; Pijnenborg *et al.,* 2006). PE, which is commonly associated with SGA, can now be predicted effectively at 11-13 weeks' gestation by algorithms combining maternal characteristics with uterine artery pulsatility index (PI) and mean arterial pressure (MAP) (Akolekar *et al.,* 2010). Screening for PE can be performed concurrently with screening for chromosomal abnormalities with the use of NT and maternal characteristics.

Markers available from screening for chromosomal abnormalities and pre-eclampsia likely hold value in the prediction of SGA in the absence of PE. Already promising evidence exists for the use of uterine artery Doppler studies in the prediction of SGA from the first trimester (Dugoff *et al.,* 2005; Poon *et al.,* 2008). MAP has not been evaluated in the prediction of SGA without PE as yet and one study has raised the possibility for an association between fetal nuchal translucency thickness (NT) and birth weight; however, the available published data regarding this possible association is limited (Kelecki *et al.,* 2005).

We performed a screening study for the evaluation of biophysical markers obtained in the first trimester of pregnancy for the prediction of the delivery of an SGA neonate.

4.2 PATIENTS AND METHODS

This was a prospective screening study for adverse obstetric outcomes in women attending for their routine first hospital visit in pregnancy at 11-13 weeks' of gestation. See Chapter 2 for a description of the population, data collection, and outcome measures.

Maternal history was recorded on a computerized system during the consultation. BP was measured by automated devices (poon *et al.,* 2010) and transabdominal colour Doppler ultrasound was used to visualise the left and right uterine artery, measure the PI in each vessel and calculate the mean PI as previously described (chapter 2). The performance of screening for SGA by combinations of disease-specific maternal factor-derived a priori risk with UAPI, MAP and NT was determined.

In the case of uterine artery PI and MAP, we used data from the screened population in which these biophysical measurements were recorded.

4.2.1 Statistical analysis

In each patient in the SGA and non-SGA groups the measured uterine artery PI and MAP were converted to multiples of the expected normal median (MoM) corrected for fetal CRL, maternal age, weight, smoking, parity, racial origin and method of conception as described in previous studies (Akolekar *et al.,* 2010, Kagan *et al.,* 2008).

The measured NT was expressed as a difference from the expected normal mean for gestation (Δ value) (Wright *et al.,* 2008).

For MAP Multiple regression analysis of the unaffected group in previous studies demonstrated that for log_{10} MAP, significant independent contributions were provided by fetal crown-rump length (CRL), maternal body mass index (BMI), age, smoking and racial origin (Poon *et al.,* 2010). The formula below was used in each patient to derive the expected log_{10} MAP and then expressed the observed values as multiples of the expected median (MoM).

Log_{10} expected MAP = 1.861313 - 0.000178 x CRL in mm + 0.002572 x BMI in Kg/m2 + 0.000426 x Age in years + (-0.008440 if smoking, 0 if no smoking) + (-0.004583 if African race, -0.004449 if South Asian race, -0.010079 if East Asian race, -0.006255 if Mixed); R2=0.112, p<0.0001.

For uterine artery PI multivariate regression analysis in the unaffected group demonstrated that for mean log_{10} uterine artery PI significant independent contributions were provided by fetal CRL, maternal BMI, age and racial origin. In each patient, we used the formula below to derive the expected log uterine artery PI and then expressed the observed value as a MoM of the expected value.

Log_{10} expected uterine artery PI (mean) = 0.404278 - 0.002080 x CRL in mm – 0.001826 x BMI in Kg/m2 – 0.000735 x Age in years + (0.021656 if African race, 0.019438 if Mixed, 0 if other racial origins); R2=0.028, *p*<0.0001.

Comparisons between the SGA and non-SGA groups were conducted by x^2 or Fisher's exact test for categorical variables and by Mann-Whitney U test for continuous variables.

The Mean MoM values for the unaffected and SGA groups are summarized in table 4.1

Subsequently the biophysical and biochemical markers were simulated for 500,000 pregnancies from the SGA populations and the non-SGA distributions as described in chapter 2. The a priori and a posteriori risks in the SGA and non-SGA groups were used to calculate the detection rates at fixed false positive rates of 5 and 10%.

4.3 RESULTS

Uterine artery PI was measured in 19,957 Non-SGA pregnancies and 1,133 SGA pregnancies and was found to be significantly increased in all three SGA groups. The difference was greater for the SGA<37 group.

MAP was measured in 12,854 non-SGA and 661 SGA pregnancies and was found to be significantly increased in the all-SGA and SGA ≥ 37 groups.

NT was measured in the whole screened population (31,314 non-SGA and 1,133 non-SGA) and was found to be decreased in all three SGA groups but was only significant in the all-SGA and SGA ≥ 37 groups.

Table 4.2 Median and interquartile range (IQR) of uterine artery PI, MAP and NT thickness in the non-SGA group and in those delivering SGA neonates

Biophysical markers	Non-SGA		All SGA		SGA < 37wks		SGA ≥ 37wks	
	n	MoM	n	MoM	n	MoM	n	MoM
Uterine artery PI	19,957	1.02 (0.84–1.22)	1,133	1.14 (0.90–1.34)*	126	1.27 (0.96–1.53)*	1,007	1.12 (0.89–1.32)*
MAP	12,854	1.00 (0.95–1.06)	661	1.01 (0.96–1.07)*	68	1.00 (0.96–1.07)	593	1.02 (0.96–1.07)*
Δ NT	31,314	0.12 (–0.08–0.34)	1,536	0.10 (–0.12–0.30)*	163	0.11 (–0.09–0.31)	1,373	0.10 (–0.12–0.30)*

Comparisons between the SGA and the non-SGA groups by Mann-Whitney U test.
Significance level * $p < 0.00001$, † $p < 0.01$, ‡ $p < 0.025$.

Figure 4.1 Box plot with median, interquartile range and 95% CI for uterine artery PI (MoM) in the non-SGA group and in those delivering SGA neonates

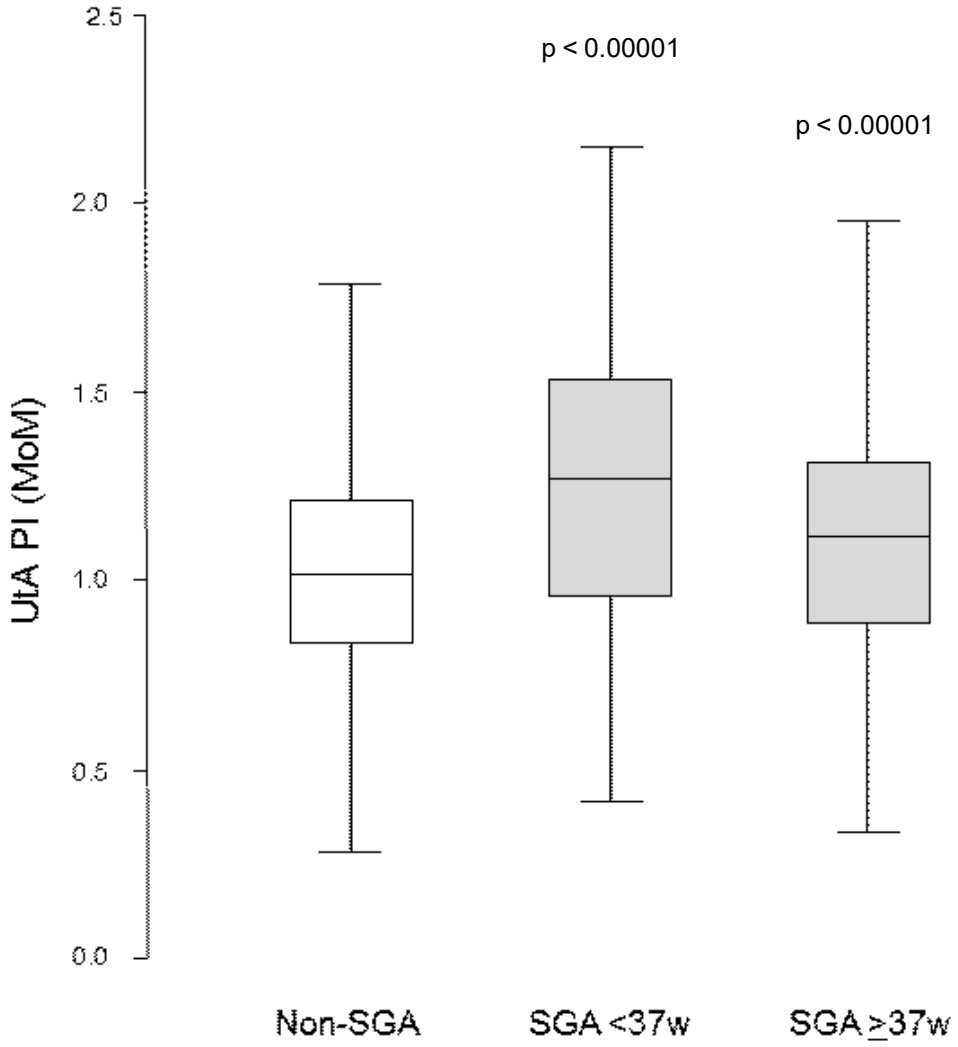


Figure 4.2 Box plot with median, interquartile range and 95% CI for mean arterial pressure (MoM) in the non-SGA group and in those delivering SGA neonates

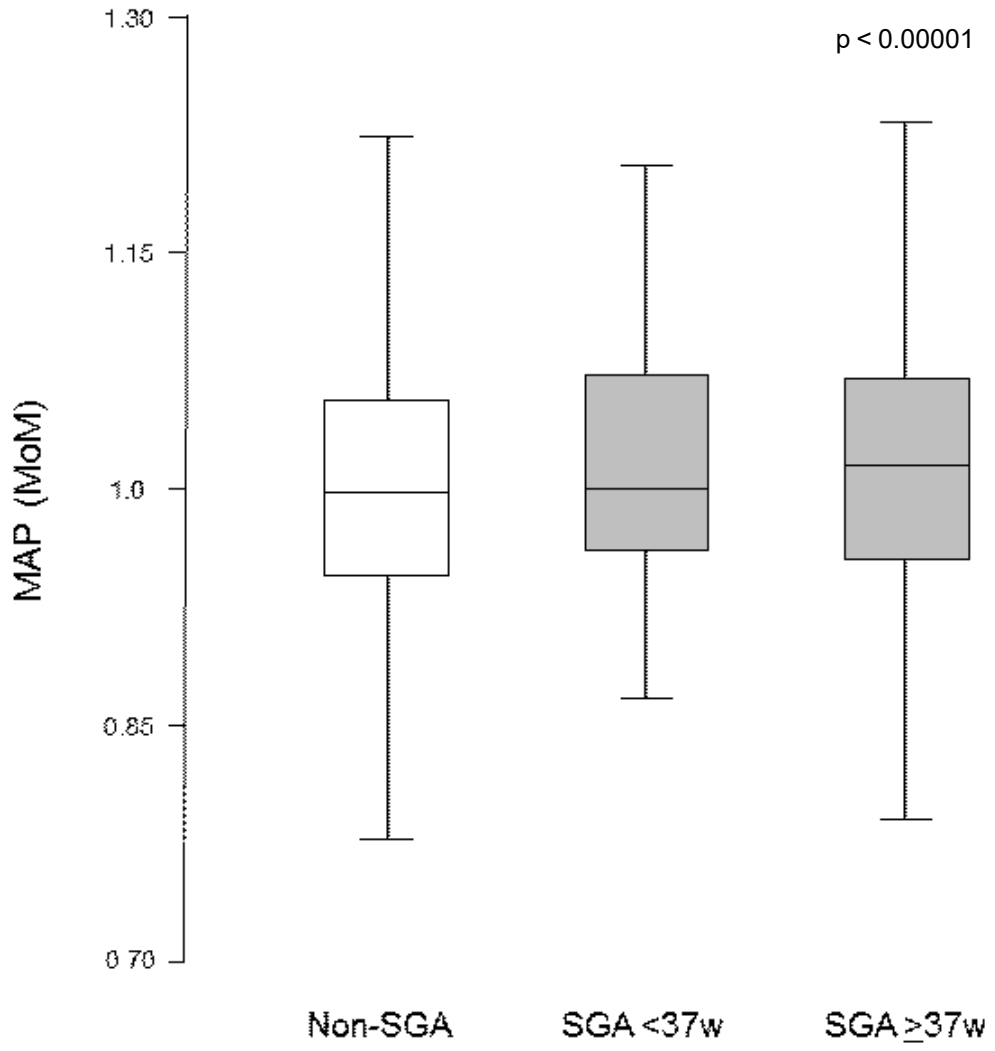
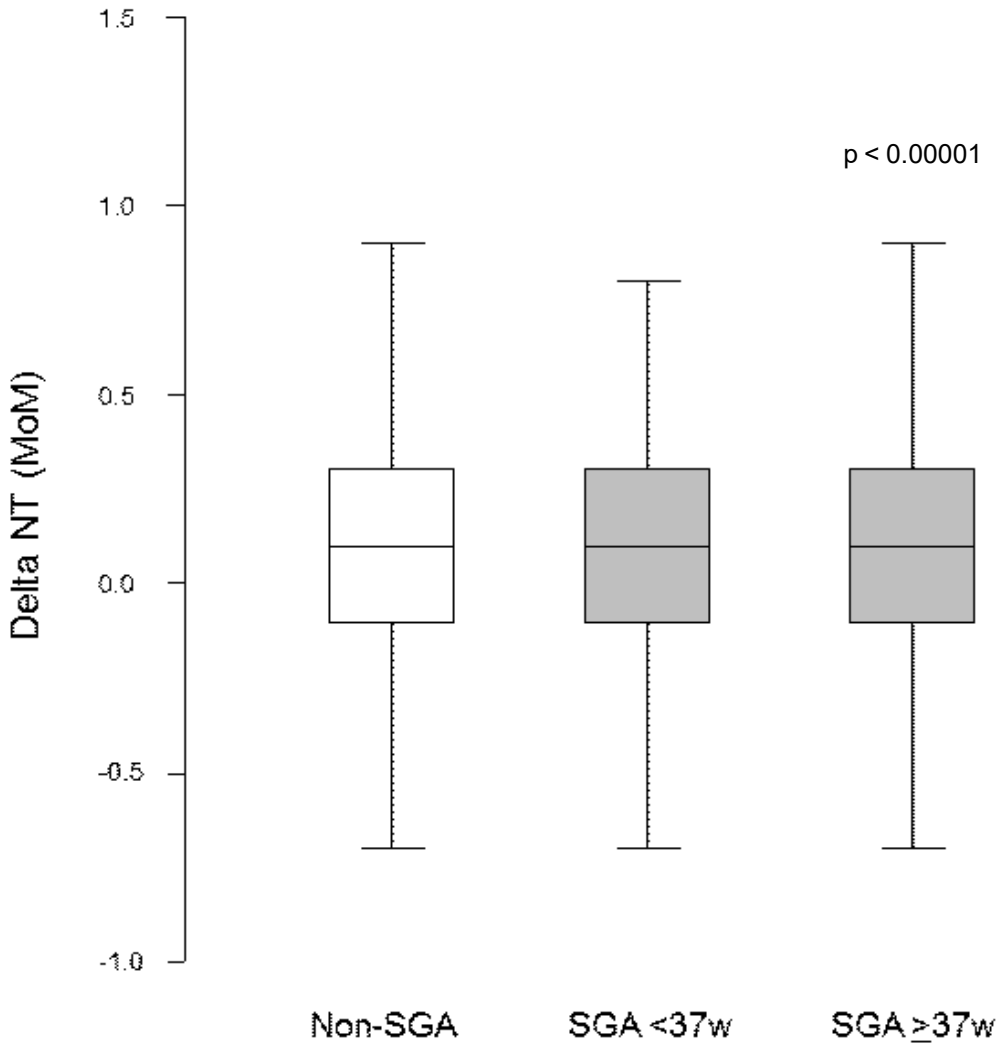


Figure 4.3 Box plot with median, interquartile range and 95% CI forΔ-NT in the non-SGA group and in those delivering SGA neonates



4.3.1 Performance of screening

The detection rates for Maternal factors plus uterine artery PI were 23.9% for a FPR of 5% and 35.9% for FPT of 10%. For Maternal factors plus MAP was 22.5% and 34.5% and for NT was 21.8% and 33.9% respectively. By using all biophysical markers in addition to maternal factors the detection rate increases to 25.5% for FPR of 5% and 37.7% for FPR of 10%. The results were also analysed by stratifying SGA to <37 weeks and ≥ 37weeks. The detection rate of maternal factors plus all biochemical factors for SGA< 37 weeks was 33.7% for FPR of 5% and 46.8% for FPR of 10%. The breakdown of screening performance is summarized in table 4.3.

Table 4.3 Performance of screening for delivery of SGA neonates by maternal factors only, maternal factors with uterine artery pulsatility index, mean arterial pressure and fetal nuchal translucency thickness.

Method of screening	ALL SGA		SGA<37 weeks		SGA >37 weeks	
	DR for FPR 5%	DR for FPR 10%	DR for FPR 5%	DR for FPR 10%	DR for FPR 5%	DR for FPR 10%
Maternal factors	21.0	34.0	23.3	35	20.8	33.9
Maternal factors plus						
Uterine artery PI	23.9	35.9	32	44.5	23.1	34.9
MAP	22.5	34.5	22.6	34.6	22.6	34.6
NT	21.8	33.9	22.5	34.5	22.0	33.6
Biophysical markers	25.5	37.7	33.7	46.8	24.3	36.8

DR = Detection rate; FPR = false positive rate

4.4 DISCUSSION

The data confirm the results of previous studies and indicate that, as in the case of PE, in pregnancies with SGA in the absence of PE there is evidence of increased placental resistance and possible impairment of function from the first trimester of pregnancy demonstrated by an increased UAPI in the SGA group. However, the magnitude of such impairment is considerably less than in PE. These observed differences have been seen repeatedly in similar studies and the reason for this is likely to be multifactorial with three predominant explanations.

First, use of SGA as a proxy for FGR is not ideal; with studies reporting that up to 50% of growth-restricted neonates can be born with a birthweight above the 10th centile (Breeze & Lees 2007). In a recent study from the same population Akolekar *et al.,* showed that addition of UAPI to maternal factors improved the prediction of Early PE (<34wks) from 33% to 54.1% for FPR of 5% (Akolekar *et al.,* 2010), in contrast to the more modest increase from 23% to 33% for severe SGA (<37wks) for the same FPR. Similar effects have been seen in another study where the sensitivity of screening only by uterine artery Doppler (UAD) in the first trimester was highest for SGA with PE compared with SGA without PE (Melchiorre *et al.,* 2009). Interestingly in the same study, the investigators attempted to differentiate true FGR from constitutional SGA by evaluating third-trimester Dopplers and considering those pregnancies that required iatrogenic preterm delivery. They showed a graded increase in the sensitivity of UAD (24.5% for FGR and 37.5% for preterm FGR) supporting the notion that the decreased performance on UAD in the SGA group

might represent the “dilution effect” of the outcome group by a large number of normal-small pregnancies.

Second, the observed difference in UAD screening can be attributed to fundamental pathophysiological differences between FGR and PE (Goswami *et al.,* 2006). Evidence of structural differences between cases of isolated SGA and PE has been described in the literature, however, a robust mechanism to explain these differences does not exist. In a study examining placentas from pregnancies with PE, PE with SGA and isolated SGA it was shown that isolated SGA had significantly smaller placentas with a reduction in the total area occupied by the villi. In PE, with and without FGR, the total area occupied by villi or intervillous space was not altered (Daayana *et al.,* 2004). At the same time in vitro studies of pure cytotrophoblast show strong differences between the patterns of differentiation of cells cultured from isolate FGR, FGR with PE and PE alone (Newhouse *et al.,* 2007). These pathophysiological differences have also been seen in chorionic villi of cases obtained in the first trimester after Chorionic villous sampling (Huisman *et al.,* 2010). However in order to move these observations into a more robust theory around the causation of FGR and PE one would need to correlate these observed pathophysiological differences with the presence or absence of increased UA resistance through obtaining chorionic villi from the first trimester of pregnancy and prospectively investigating the outcomes of pregnancies that are seen to have high UA resistance, something which by no doubt is challenging from a methodological point of view. In a recent study a similar but more simplified technique for the investigation of early placental samples has been described, where samples from first-trimester elective terminations are separated by examining UA resistance (Fraser *et al.,* 2012). This technique although useful in the investigation of early pathophysiological changes in placentas with inadequate trophoblast invasion still would not be able to the differential between SGA and PE due to the obvious lack of knowledge of the final outcome.

Third, it is possible that the phenomenon of a differential maternal response to inadequate trophoblast invasion is due to the presence or absence of the metabolic syndrome (Ramsay *et al.,* 2004). Observations between the PE and isolated SGA populations between the SGA study and the PE screening study from the same population mentioned before show that the PE population has a higher mean maternal weight (70kg vs 61kg) supporting in part the metabolic syndrome theory (Akolekar *et al.,* 2010).

MAP was found to be significantly raised in the SGA group at the time of screening even if subsequently the subjects did not develop gestational hypertension or PE. Again the effect seems to be much less prominent when compared with PE. It is possible that the degree of vasculopathy has not exceeded the thresholds for the development of PE in later pregnancy but still some degree of hypertension is present in the first trimester. Another consideration for the association identified is that a proportion of these cases are undiagnosed chronic hypertension cases. Chronic hypertension is a well-recognized risk factor for SGA and our classification of chronic hypertension depends on maternal reporting. It is possible that some of the subjects did not volunteer this information or hypertension has not been picked up in those that had their booking appointment prior to the first-trimester scan. Another explanation can be that the technique that we follow as part of our research project is likely to be more thorough and consistent than blood pressure measurements performed in midwifery booking visits. Irrespective of the cause, measurement of MAP in the first trimester has a small but significant correlation with SGA and can be used as part of the screening algorithm.

We also report a negative correlation between NT and fetal growth. Similar results have been found in another study (aOR of 0.57(0.38—0.86) (Papastefanou *et al.,*

2011). The association is weak with minimal value in the overall prediction but non-the-less statistically significant. Published data on the association between fetal NT and birth weight are confined to only one study, which reported that an increased fetal NT in euploid pregnancies is associated with macrosomia (Kelecki *et al.,* 2005). The suggested underlying pathophysiology for this association is that enhanced capillary permeability, resulting in an increase in fetal NT, may be a result of maternal hyperglycemia, which by itself may increase birth weight (Bartha *et al*., 2003; Kelecki *et al.,* 2005). Whether the reverse of this hypothesis may provide a possible reason for our findings remains to be established. Given the availability of the NT measurement at the same time of the application of the screening algorithm, we advocate its use.

Overall it has been shown that the use of the above biophysical markers in combination with the a-priori derived risk for SGA in pregnancy can improve the detection algorithm especially for those pregnancies that suffer from severe SGA requiring delivery before 37 weeks. It also verifies results of previous studies that show a degree of inadequate placentation reflected by increased resistance to blood flow. Given that all the above measurements are recommended for screening for PE and some are already widely used for screening for aneuploidies it is logical that they are applied concurrently for the prediction of SGA. Further improvement in screening is likely to be achieved with the inclusion of serum metabolites measuring placental function.

CHAPTER 5. BIOCHEMICAL MARKERS AT 11-13 WEEKS IN THE PREDICTION OF FETAL GROWTH RESTRICTION

ABSTRACT

Objective: To develop a model for prediction of small-for-gestational age (SGA) neonates in the absence of preeclampsia (PE) based on maternal factors and biochemical markers at 11-13 weeks' gestation.

Method: Screening study in 1,536 SGA and 31,314 non-SGA pregnancies based on maternal characteristics, PAPP-A, β-hCG and a series of case-controlled studies on PLGF, PP13, sENG, FT3, FT4 and TSH. Regression analysis was used to develop a model for the prediction of SGA.

Results: In the SGA group, serum PAPP-A, β-hCG, PLGF and PP13 were significantly decreased while no difference was found for sENG, FT3, FT4 and TSH. The performance of screening for the combination of the above markers showed a DR of 30% for a FPR 5% for all-SGA and 50% respectively for SGA<37wks.

Conclusion: Screening performance for the delivery of an SGA neonate can be improved with the addition of biochemical markers of placental function.

5. BIOCHEMICAL MARKERS IN THE PREDICTION OF SGA

This chapter is based on:

Karagiannis G, Akolekar R, Sarquis R, Wright D, Nicolaides KH. Prediction of small-for-gestation neonates from biophysical and biochemical markers at 11-13 weeks. Fetal Diagn Ther. 2011;29(2):148-54

Karagiannis G, Ashoor G, Maiz N, Jawdat F, Nicolaides KH. Maternal thyroid function at eleven to thirteen weeks of gestation and subsequent delivery of small for gestational age neonates. Thyroid. 2011 Oct;21(10):1127-3

INTRODUCTION

There is growing evidence that birth weight is related to placental function in early pregnancy. The complex process of early placentation and subsequent placental function is thought to involve a multitude of processes regulated by a variety of signalling molecules, angiogenic factors, and other growth factors. We performed a thorough review of the current literature and identified markers that have shown promise in the detection of placental insufficiency (chapter 1). Some markers have been shown to have a value in the early prediction of PE but evidence for the association with normotensive SGA tends to be less available.

Several studies reported that in pregnancies delivering small-for-gestational-age (SGA) neonates, serum PAPP-A at 11 to 13 weeks' was decreased (Ong *et al*., 2000; Smith *et al*., 2002; Yaron *et al*., 2002; Tul *et al*., 2003; Dugoff *et al*., 2004; Smith *et al*., 2006; Canini *et al*., 2008; Spencer *et al*., 2008; Fox *et al*., 2009; Law *et al*.,2009; Montanari *et al*., 2009; Pihl *et al*., 2009). At the same time, other placental growth factors have shown promising evidence as markers of placental function in the first trimester. PP13 and ADAM12 are thought to be involved in growth regulation of the placenta and an association with SGA neonates has been reported in previous studies (Chafez *et al.,* 2007; Cowans *et al.,* 2008; Matwejew *et al.,* 2010, Poon *et al.,* 2008).

Angiogenic factors have featured a central role in pathophysiological theories of PE and FGR. The most widely investigated molecule is PLGF, low levels of which have been associated with placental insufficiency in both the presence and absence of PE (Thadhani *et al.,* 2004; Smith *et al.,* 2007; Erez *et al.,* 2008; Poon *et al.,* 2008). Soluble endoglin (sENG) which is an anti-angiogenic factor has also been reported to be

increased in PE and is thought to reflect an anti-angiogenic state with only limited results of value in normotensive SGA (Erez *et al.,* 2008).

Both In-vitro studies (Barber *et al.,* 2005; Oki *et al.,* 2004) and some clinical studies have reported an association with thyroid hormones (TSH, FT3, FT4), placental function and birthweight (Leung *et al.,* 1993; Levine *et al.,* 2009; Sahu *et al.,* 2010). There is also contradictory evidence that clinical and subclinical hypothyroidism is associated with increased risk for both PE and the birth of SGA neonates in the absence of PE (Blazer *et al.,* 2003; Casey *et al.,* 2007; Allan *et al.,* 2000). In a recent study it has been reported that in pregnancies that develop PE, maternal serum thyroid stimulating hormone (TSH) at 11-13 weeks' gestation was higher and free thyroxine (FT4) was lower than in normotensive controls (Ashoor *et al.,* 2010).

We choose to investigate the above markers based on promising preliminary results from our group or other studies and also based on availability of stored samples and analytic assays. We bring together results from previous case-control studies performed from our group for some of these markers (PAPP-A, PLGF, ADAM12, PP13) and also investigate the value of some additional biochemical markers (sENG, TSH, FT3, FT4) and examine their combined predictive value in the prediction of SGA in the first trimester.

5.2 PATIENTS AND METHODS

This was a prospective screening study for adverse obstetric outcomes in women attending for their routine first hospital visit in pregnancy at 11-13 weeks of gestation. (See Chapter 2 for description of the population, data collection and outcome measures and measurement methodology of the individual markers.)

5.2.1 Statistical analysis

The measured maternal serum PAPP-A and β-hCG were converted to multiples of the expected normal median (MoM) corrected for fetal CRL, maternal age, weight, smoking, parity, racial origin and method of conception as previously described (Kagan *et al.,* 2008a).

The measured concentration of PLGF was log transformed to make the distribution Gaussian. Multiple regression in a previous study demonstrated that for log PLGF significant independent contributions were provided by fetal CRL, maternal weight, smoking and racial origin. (Akolekar *et al.,* 2008). In each patient, we used the formula below to derive the expected log PLGF and then expressed the observed value as a MoM of the expected value.

log expected PlGF = 1.150 + *(*0.008 × CRL in mm*)*−*(*0.002 × weight in kg*)*+ *(*0.199 if smoker, 0 if not*)*+ *(*0.177 if African-American, 0.100 if Indian or Pakistani, 0 if other racial origin*)*; *r*2 = 0.237*, P* < 0.0001*.*

The measured concentration of PP13 was log transformed to make the distribution Gaussian. Multiple regression in a previous study demonstrated that for log PP13 significant independent contributions were provided by maternal weight and smoking (Akolekar *et al.,* 2009). In each patient, we used the formula below to derive the expected log PP13 and then expressed the observed value as a MoM of the expected value.

log expected PP13 = 2.089 − 0.004× maternal weight in kilograms + (−0.214 if smoker, 0 if non-smoker); *R*2 = 0.154, *p* < 0.0001.

The measured concentration of ADAM12 was log-transformed to make the distribution Gaussian. Multiple regression analysis in the control group demonstrated that for log ADAM12, significant independent contributions were provided by fetal CRL, maternal weight, and racial origin. In each patient, we used the formula below to derive the expected log ADAM12 and then expressed the observed value as a MoM of the expected value.

log expected ADAM12 = 2.789 − 0.005× maternal weight in kilograms + (0.006 x CRL in mm) + (0.040 if African-American, 0 if other racial origin); $R2 = 0.272$, $p < 0.001$.

The measured sENG concentration was made Gaussian using the following transformation: Y = log10 (sEng−10,000). The data could not be made Gaussian by more standard transformations, such as log10 or square root. Distributions were confirmed to be Gaussian using the Kolmogorov–Smirnov test. Multiple regression analysis in the control group demonstrated that for Y significant contribution was provided by fetal CRL and maternal weight. In each patient, we used the formula below to derive the expected logsENG and then expressed the observed value as a MoM of the expected value.

Log10 (sENG-10,000) = 4.9327234491 + (-0.0025306978 x CRL in mm) + (-0.0024068511 x maternal weight in kilograms) R2 = 0.106 p < 0.001.

The measured concentrations of FT3, FT4 and TSH were converted to multiples of the expected normal median (MoM) corrected for gestational age and maternal age, racial origin, and body mass index. We reported previously that serum TSH increases whereas FT3 and FT4 decrease with gestational age and all three are lower in women of African racial origin than Caucasians, serum FT3, and FT4 decrease but TSH does not change significantly with maternal age and serum TSH and FT3 increase whereas

FT4 decreases with body mass index (Ashoor *et al.,* 2010). The SGA and unaffected groups were compared for median TSH MoM, FT3 MoM and FT4 MoM using the Mann-Whitney test and for the proportion of cases with serum TSH above the 97.5th percentile and serum FT3 and FT4 below the 2,5th percentile by the Chi-square test.

All mean MoM values for the unaffected and SGA groups are summarized in table 5.1.

Subsequently the biophysical and biochemical markers that were found to be predictive of SGA were simulated for 500,000 pregnancies from the SGA populations and the non-SGA distributions as described in chapter 2. The a priori and a posteriori risks in the SGA and non-SGA groups were used to calculate the detection rates at fixed false positive rates of 5 and 10%.

5.3 RESULTS

PAPP-A and β-hCG were measured in the whole screened population (31,314 non-SGA and 1,536 non-SGA) and were found to be significantly reduced in all three SGA groups apart from the case of β-hCG in the SGA <37 which was decreased but did not reach significance.

Serum PLGF was measured in 1,869 Non-SGA and 274 SGA cases and was found to be significantly reduced in all three SGA groups. A graded response was observed with the lowest mean values in the SGA<37 group.

Serum PP13 was measured in 877 Non-SGA and 173 SGA cases and was found to be significantly reduced in all three SGA groups. Again a graded response was observed with the lowest mean values in the SGA<37 group.

Serum ADAM12 was measured in 830 Non-SGA cases and 168 SGA cases. It was also found to be significantly reduced in all three SGA groups with the lowest values in the SGA<37 group.

Serum sENG was measured in 200 non-SGA cases and 100 SGA cases. There was no significant difference between SGA and controls ($p = 0.087$). Also, no difference was detected between the SGA<37 group and controls.

Maternal FT3, FT4 and TSH were previously measured in 3,592 non-SGA cases and as part of this study in 212 SGA cases. There were no significant differences between any of the SGA groups and controls ($p=0.543$).

Table 5.1 Median and interquartile range (IQR) of PAPP-A, free β-hCG, PLGF, PP13, ADAM12 and sENG in the non-SGA group and in those delivering SGA neonates.

Bio physical markers	Non-SGA		All SGA		SGA < 37wks		SGA ≥ 37wks	
	n	MoM	n	MoM	n	MoM	n	MoM
PAPP-A	31,314	1.03 (0.71-1.45)	1,536	0.82 (0.55-1.12)*	163	0.71 (0.40-0.095)*	1,373	0.83 (0.58-1.40)*
Free β-hCG	31,314	0.97 (0.66-1.47)	1,536	0.89 (0.58-1.40)*	163	0.93 (0.54-1.47)	1,373	0.89 (0.58-1.40)*
PLGF	1,869	1.00 (0.78-1.28)	274	0.90 (0.63–1.24)*	37	0.79 (0.50–1.14)†	237	0.93 (0.64–1.26)†
PP13	877	1.00 (0.76-1.34)	173	0.82 (0.62–1.07)*	20	0.79 (0.70–1.00)‡	153	0.85 (0.60–1.08)*
ADAM12	830	0.99 (0.81-1.20)	168	0.86 (0.69–1.08)*	19	0.80 (0.56–0.92)†	149	0.87 (0.70–1.08)*
sENG	200	1.03 (0.85-1.17)	100	0.97 (0.82-1.11)	17	0.98 (0.82-1.13)	83	0.97 (0.82-1.11)
Free T3	3592	0.099 (0.935-1.059)	212	0.990 (0.940-1.063)	31	1.008 (0.979-1.070)	181	0.987 (0.938-1.061)
Free T4	3592	0.992 (0.908-1.086)	212	1.010 (0.919-1.090)	31	0.961 (0.887-1.144)	181	1.013 (0.923-1.081)
TSH	3592	1.007 (0.608-1.511)	212	1.061 (0.651-1.562)	31	0.758 (0.316-1.470)	181	1.096 (0.724-1.583)

Comparisons between the SGA and the non-SGA groups by Mann-Whitney U test.
Significance level * $p < 0.00001$, † $p < 0.01$, ‡ $p < 0.025$.

Figure 5.1 Box plot with median, interquartile range and 95% CI for placental plasma protein A (MoM) in the non-SGA group and in those delivering SGA neonates

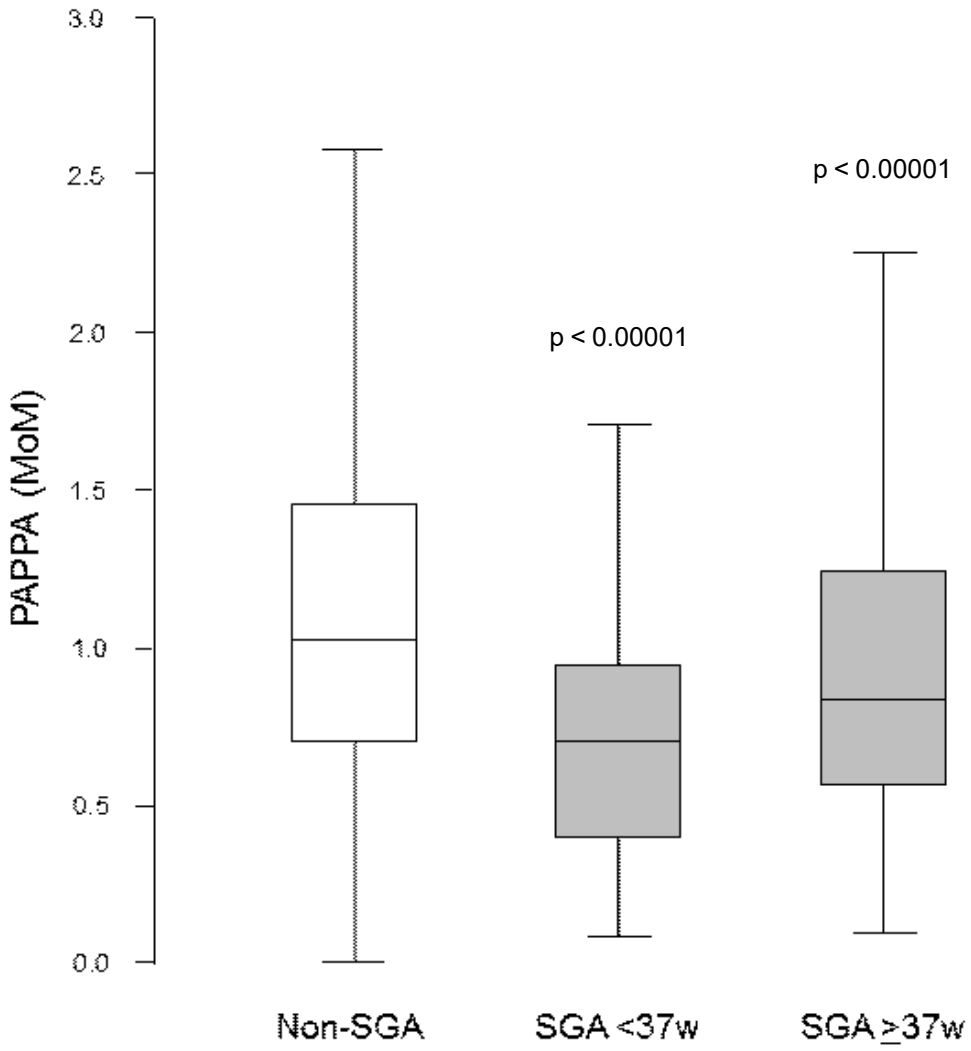


Figure 5.2 Box plot with median, interquartile range and 95% CI for beta chorionic gonadotrophin (MoM) in the non-SGA group and in those delivering SGA neonates

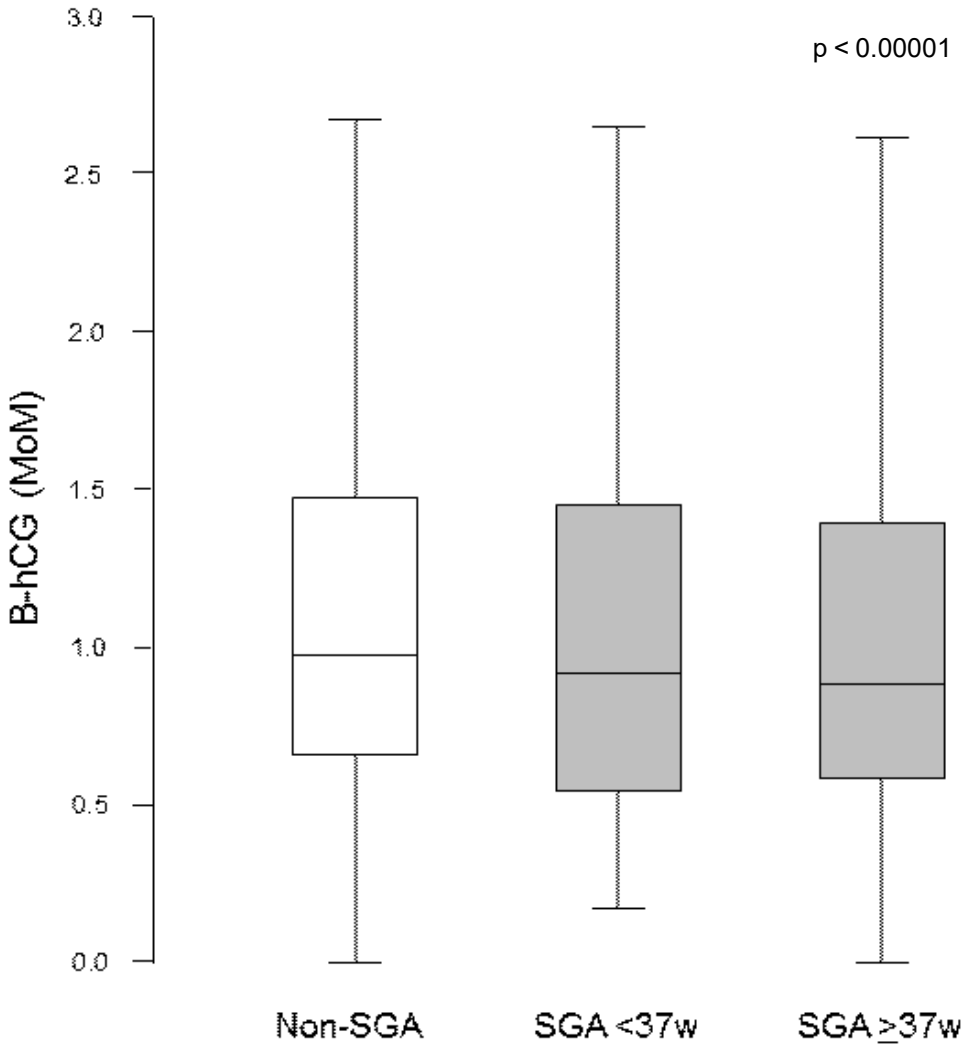


Figure 5.3 Box plot with median, interquartile range and 95% CI for placental growth factorl (MoM) in the non-SGA group and in those delivering SGA neonates

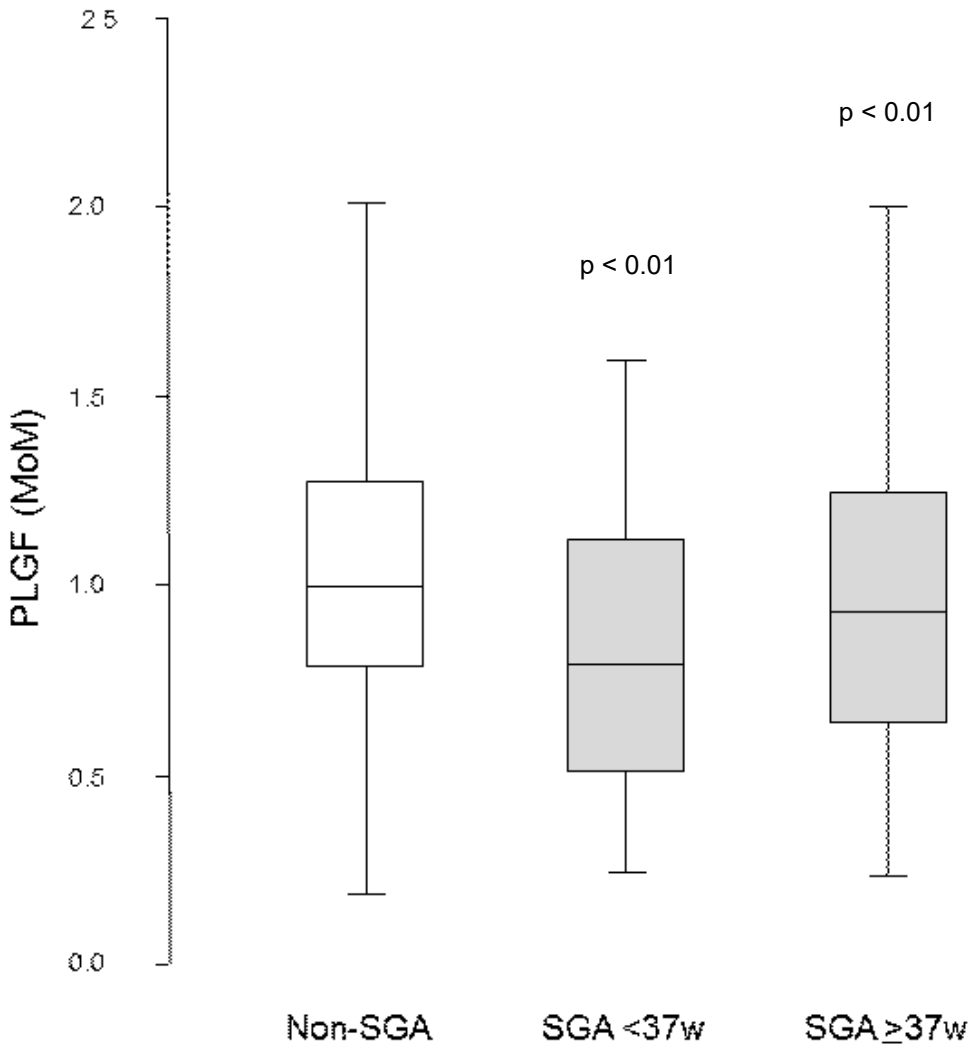


Figure 5.4 Box plot with median, interquartile range and 95% CI for placental protein 13 (MoM) in the non-SGA group and in those delivering SGA neonates

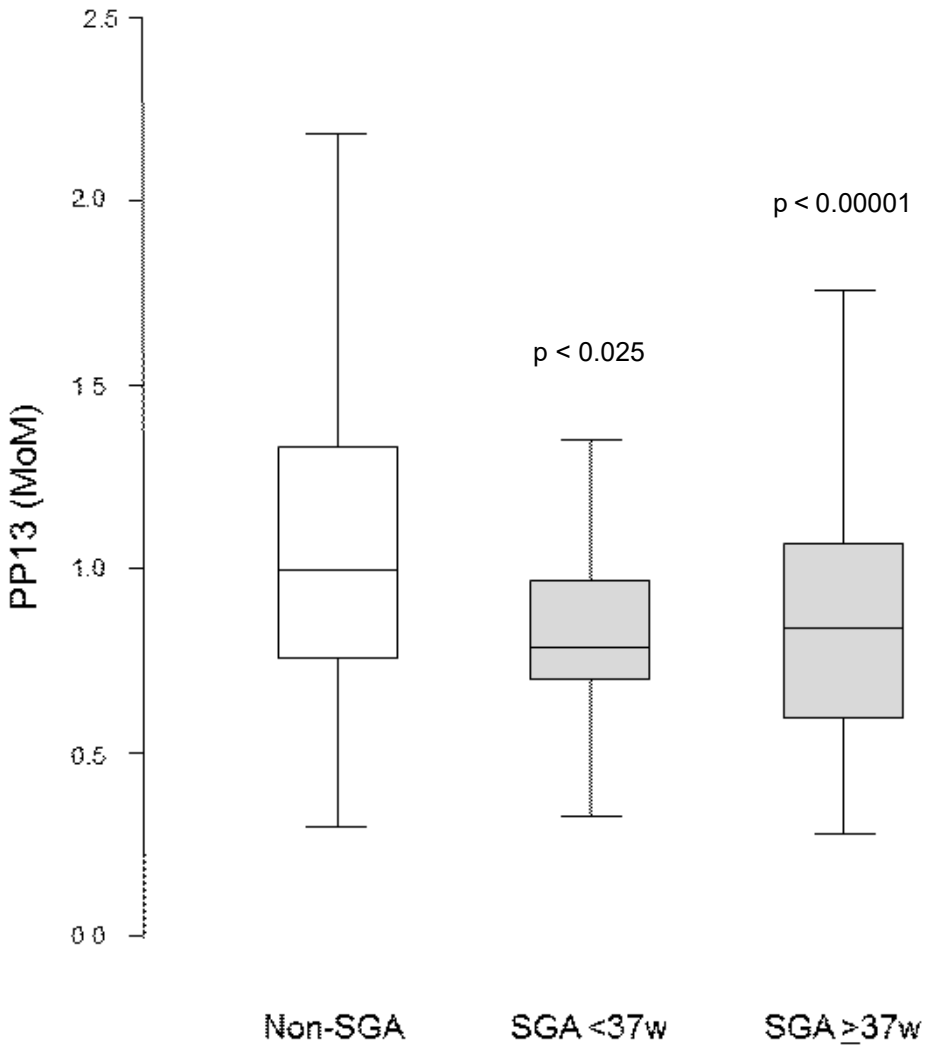
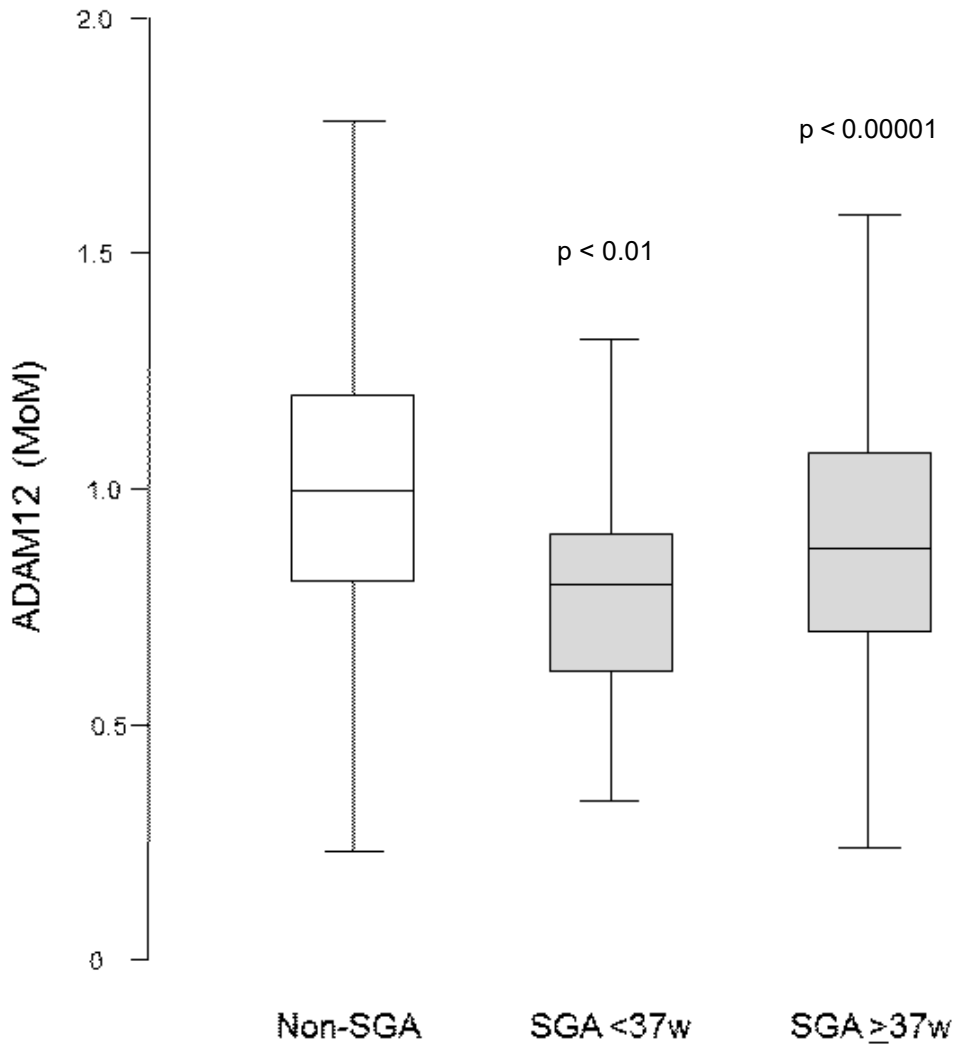


Figure 5.5 Box plot with median, interquartile range and 95% CI for ADAM 12 (MoM) in the non-SGA group and in those delivering SGA neonates



5.3.1 Performance of screening

Only the markers who showed a significant difference between SGA and controls were included in the prediction analysis

The DR for Maternal factors plus PAPP-A increased to 25.5% for FPR of 5% and 37.5% for FPR of 10%. Free β-hCG has a small effect over maternal factors only with a DR of 22.4% for FPR of 5% and 34.1% for FPR of 10%. PLGF, PP13, and ADAM12 show very similar performance with individual detection rates over maternal factors only of around 25% for FPR of 5% and 37% for FPR of 10%. Overall detection rate with the inclusion of all biochemical markers was 30.2% for FPR of 5% and 42.7% for FPR of 10%.

The results were also analysed by stratifying SGA to <37 weeks and >37weeks. The detection rate of maternal factors plus all biochemical factors for SGA< 37 weeks was 50.1% for FPR of 5% and 63.0% for FPR of 10%. The breakdown of screening performance is summarized in table 5.2

Table 5.2 Performance of screening for delivery of SGA neonates by maternal factors only, maternal serum pregnancy-associated plasma protein-A, free b-human chorionic gonadotrophin, placental growth factor, placental protein 13, A Disintegrin And Metalloprotease and their combinations.

Method of screening	ALL SGA		SGA<37 weeks		SGA >37 weeks	
	DR for FPR 5%	DR for FPR 10%	DR for FPR 5%	DR for FPR 10%	DR for FPR 5%	DR for FPR 10%
Maternal factors	21.0	34.0	23.3	35	20.8	33.9
Maternal factors plus						
PAPP-A	25.5	37.5	35.5	47.8	24.5	36.6
Free β-hCG	22.4	34.1	22.9	34.8	22.4	34.1
PLGF	25.1	37.0	34.3	46.2	23.9	35.7
PP13	24.7	37.0	25.9	38.6	24.8	37.1
ADAM12	25.1	37.3	29.5	42.6	24.7	37.1
All biochemical markers	30.4	42.7	50.1	63.0	29.0	41.7

DR = Detection rate; FPR = false positive rate

5.4 DISCUSSION

This study demonstrates that utilizing biochemical markers of placental function in the first trimester improves the prediction of SGA neonates and the effect is more pronounced in the SGA <37 group (from 23.3% to 50.1% for FPR of 5%). The data confirm the results of previous studies and indicate that, as in the case of PE, in pregnancies with SGA in the absence of PE there is evidence of impaired placental perfusion and function from the first trimester of pregnancy. Below we discuss in details the results of the individual markers examined.

5.4.1 PAPP-A and β-hCG

Previous studies have reported that maternal serum PAPP-A below the 5th percentile in early pregnancy could detect 10% to 18% of pregnancies delivering SGA neonates, and the reported odds ratios varied between 1.7 and 3.3 (Ong *et al*., 2000; Smith *et al*., 2002; Yaron *et al*., 2002; Tul *et al*., 2003; Dugoff *et al*., 2004; Smith *et al*., 2006; Canini *et al*., 2008; Spencer *et al*., 2008; Fox *et al*., 2009; Law *et al*., 2009; Montanari *et al*., 2009; Pihl *et al*., 2009). The findings of this study confirm the results of these reports that the levels of maternal serum PAPP-A during the first trimester are low in women who subsequently deliver small babies. Additionally, serum free *β*-hCG was low in the SGA group. The finding of such an association implies that birth weight is predetermined by placental development during the first trimester of pregnancy. It is uncertain to what extent genetic factors affect fetal growth through placentation, although there is some evidence that imprinted genes play a role in regulating the supply of nutrients to the fetus (Angiolini *et al*., 2006). The gene encoding for insulin-like growth factor (IGF) is imprinted and IGF is thought to have a key role in the control of placental growth and ability to transfer nutrients (Reik *et al*., 2003). PAPP-A has been shown to be a syncytiotrophoblast-derived protease for IGF binding proteins,

and the cleavage of these proteins by PAPP-A increases the bioavailability of IGF (Bonno *et al*., 1994; Irwin *et al*., 1999; Lawrence *et al*., 1999). It is therefore not surprising that low serum PAPP-A is associated with a higher incidence of SGA.

Measurement of maternal serum PAPP-A in early pregnancy is in itself not an effective method of screening for SGA neonates. Nevertheless, the observation of low levels of PAPP-A in euploid pregnancies in the first trimester has led to recommendations that such pregnancies should have follow-up scans for monitoring fetal growth (Ong *et al*., 2000; Smith *et al*., 2002; Yaron *et al*., 2002; Tul *et al*., 2003; Dugoff *et al*., 2004; Smith *et al*., 2006; Canini *et al*., 2008; Spencer *et al*., 2008; Fox *et al*., 2009; Law *et al*., 2009; Montanari *et al*.,2009; Pihl *et al*., 2009). Our results demonstrate that the PAPP-A-related patient-specific risk for SGA is substantially affected by various maternal factors and is modifiable by the addition of the measurement of fetal NT. These variables should be taken into account in calculating the patient-specific risk and therefore in defining the real need and frequency of subsequent growth scans.

5.4.2 PLGF and sENG

As shown in previous studies in normal pregnancies the maternal serum concentrations of PlGF increase with gestational age, decrease with maternal weight and are higher in Black than in White women. It has also been shown that cigarette smokers have increased levels of PlGF and hence the measured concentrations of PlGF were also adjusted for smoking before comparing results between the SGA and control groups (Akolekar *et al*., 2008; Poon *et al.,* 2008). The results are in accordance with previous data showing low levels of PLFG in SGA pregnancies. A previous study examining 40 SGA pregnancies and 80 AGA pregnancies showed an association between low PLGF levels and SGA but when corrected for covariates such as race,

smoking and BMI the significance was lost (Thadhani *et al.,* 2004). In two larger studies with appropriate adjustment the association between low PLGF and SGA has been shown to be significant with reported ORs of 0.990 (0.980–0.999) and 0.92(0.87–0.96) (Erez *et al.,* 2008; Smith *et al.,* 2007).

Soluble endoglin was not found to be correlated with the delivery of an SGA neonate in our data. Studies have shown changes in sENG in the second and third trimester mostly in PE but also in normotensive SGA (Rana *et al.,* 2007; Kim *et al.,* 2009; Jeyabalan *et al.,* 2008; Savvidou *et al.,* Stepan *et al.,* 2007). A study among normotensive women delivering an SGA neonate showed a small increase in sENG levels beginning at 17 through 20 weeks with a large increase at 37 through 42 weeks (Levine *et al.,* 2006). When examining sENG in the first trimester studies have demonstrated a change in sENG levels in PE cases, albeit much less pronounced (Foidart *et al.,* 2010, Lim *et al.,* 2009).

Data on sENG and normotensive SGA in the first trimester are limited with only two studies in the literature. Erez *et al.* did not demonstrate a difference in sENG in the first trimester between SGA neonate and AGA controls and only a possible value in the longitudinal assessment of this marker between the first and second trimesters (Erez *et al.,* 2008). Another study comparing 46 controls with 56 SGA pregnancies in the first trimester reports higher levels of sENG in the SGA group (Romero *et al.,* 2008). We examined a sample of 100 SGA pregnancies with a strict definition of SGA (< 5^{th} centile) with appropriate correction for covariates known to affect sENG levels in a normal population and failed to show a significant difference between the all-SGA groups and controls but also between the severe SGA (<37wks) and controls. Our study is similar in numbers with the study by Erez *et al.,* and shows similar results. In similarity with previously reported behaviour of angiogenic related biomarkers the effect appears to be more pronounced in cases of PE but in the case of sENG its

value does not appear to be significant in the prediction of normotensive SGA in the first trimester.

Based on the theory of angiogenic/anti-angiogenic imbalance researchers have investigated the value of various ratios between pro-angiogenic factors and anti-angiogenic factors mostly when studying longitudinal changes of these markers at different gestations with weakly positive results (Romero *et al.,* 2008; Erez *et al.,* 2008). However, with a focus on a screening algorithm applied in a one-stop first-trimester visit the value of longitudinal assessment becomes less relevant.

5.4.3 PP13 and ADAM12

PP13 and ADAM 12, both placental products, were found to be reduced in the SGA group with the highest effect in the SGA<37wks group. The results are in keeping with previous studies (Cowans *et al.,* 2008; Chafez *et al.,* 2007; Matwejew *et al.,* 2010, Poon *et al.,* 2008; Cowans *et al.,* 2007). ADAM12 demonstrated a better predictive value than PP13 however both markers showed a good correlation with serum PAPP-A ($r=0.513$, $p<0.0001$ and $r=0.381$, $p<0.0001$). Both markers appear to have a small additional benefit in the overall prediction over PAPP-A. The utilization will likely depend on the availability of these markers from concurrent use in the PE algorithm (Akolekar *et al.,* 2011).

5.4.4 Thyroid Hormones

In the investigation of thyroid hormones, results show that maternal thyroid function at 11-13 weeks' gestation is not significantly different from those delivering appropriately grown neonates and there is no evidence that in the SGA group the incidence of impaired thyroid function is increased. A previous screening study in

which maternal thyroid function was assessed at 15-18 weeks' gestation reported that there was no significant difference in mean birth weight between euthyroid pregnancies and those with subclinical hypothyroidism (Allan *et al.,* 2000). In contrast, a case-control study reported that the mean birth weight in pregnancies with subclinical hypothyroidism was significantly lower than in euthyroid controls (Blazer *et al.,* 2003). Another screening study before 20 weeks reported that the incidence of neonates with birth weight below 2.5 Kg was not significantly different between euthyroid pregnancies and those with subclinical hypothyroidism (Casey *et al.,* 2007). However, use of mean birth weight or a cut-off in birth weight without appropriate adjustments for gestation are not appropriate for the investigation of thyroid function on fetal growth.

The strengths of our study are first, examination of a large number of appropriately documented cases of SGA and normal controls, secondly, use of normal ranges of thyroid function corrected for maternal characteristics, including age, racial origin and body mass index (Ashoor *et al.,* 2010a), and thirdly, assessment of thyroid function in the first-trimester of pregnancy providing the option for therapeutic interventions in future studies to determine if the incidence of SGA can be reduced. The weakness of the study was its cross-sectional nature, which did not allow longitudinal assessment of thyroid function from early pregnancy to the development of SGA.

The findings that firstly, SGA is not associated with maternal thyroid hypofunction and secondly, that there is no correlation between gestation at delivery and TSH, FT3 or FT4 suggest that the results of *in vitro* studies concerning the role of thyroid hormones on trophoblast proliferation and invasion (Barber *et al.,* 2005; Oki *et al.,* 2004) may not be clinically relevant. Histological studies reported that impaired placentation is observed in association with PE with or without SGA and in about half of pregnancies with SGA in the absence of PE (Brosens *et al.,* 1977). However, Doppler studies of

the uterine arteries documented that the incidence of high impedance to flow in pregnancies with PE depends on the gestational age at the onset of the disease. The uterine artery PI was above the 95th percentile in 82% of those that developed PE requiring delivery before 34 weeks (early-PE) and in 40% of those delivering at or after 34 weeks (late-PE) (Yu *et al.,* 2008). Similarly, in SGA without PE high PI was observed in 44% of those that delivered before 34 weeks and in 10% of those delivering at or after 34 weeks. Although the basis of the possible association between hypothyroidism, PE and SGA was the suggested role of thyroid hormones in promoting placentation and trophoblastic invasion, we previously found that thyroid hypofunction is observed in pregnancies developing late- rather than early-PE (Ashoor *et al.,* 2010b). We therefore suggested that the association is unlikely to be mediated by impaired trophoblastic invasion but rather by a metabolic derangement with increased insulin resistance, which is thought to underlie late-PE (Egbor *et al.,* 2006; Moldenhauer *et al.,* 2003; Bosio *et al.,* 1999; D'Anna *et al.,* 2006).

To conclude, in pregnancies delivering SGA neonates maternal thyroid function at 11-13 weeks' gestation is not impaired. Consequently, irrespective of the possible effect of thyroid hormones on placentation, hypothyroidism does not have a significant contribution to the incidence of SGA neonates.

5.5 CONCLUSION

In conclusion, we report on a variety of markers (PAPP-A, PLGF, ADAM12 and PP13) that show promising performance in the prediction of SGA in the first trimester along with their performance in a combined predictive model. We demonstrate a significant increase in the detection rate of SGA from the predictive model utilizing only maternal factors (From 21% to 30.4% for all SGA for FPR of 5%). We also report on a variety of markers, with conflicting results in the existing literature that did not demonstrate a

value in early screening (sENG, TSH, FT3, and FT4). In the next chapter of this thesis, we examine the value of these biochemical markers in combination with maternal history and biophysical markers in the prediction of the delivery of an SGA neonate.

CHAPTER 6. PREDICTION OF SGA NEONATES FROM BIOPHYSICAL AND BIOCHEMICAL MARKERS AT 11-13 WEEKS' GESTATION

ABSTRACT

Objective To develop a model for prediction of small-for-gestational age (SGA) neonates in the absence of preeclampsia (PE) based on maternal factors and biochemical markers at 11-13 weeks' gestation.

Method Screening study in 1,536 SGA and 31,314 non-SGA pregnancies based on maternal characteristics, fetal nuchal translucency (NT) thickness, serum pregnancy-associated plasma protein-A (PAPP-A) and free b-human chorionic gonadotrophin (β-hCG), mean arterial pressure (MAP), uterine pulsatility index (PI) in combination with case controlled studies of maternal serum concentration of placental growth factor (PLGF), placental protein 13 (PP13) and A Disintegrin And Metalloprotease (ADAM12). Regression analysis was used to develop a model for the prediction of SGA.

Results At a false positive rate of 10%, the estimated detection rate by a combination of maternal factors and biophysical and biochemical markers at 11-13 weeks was 73% for SGA requiring delivery before 37 weeks and 46% for those delivering at term.

Conclusion Half of pregnancies with SGA neonates in the absence f PE could potentially be identified at 11-13 weeks.

This chapter is based on:

Karagiannis G, Akolekar R, Sarquis R, Wright D, Nicolaides KH. Prediction of small-for-gestation neonates from biophysical and biochemical markers at 11-13 weeks. Fetal Diagn Ther. 2011;29(2):148-54

6.1 INTRODUCTION

Screening and management of placental insufficiency with or without PE has proven to be one of the great challenges of modern obstetric care. With increasing evidence placing FGR as one of the main causative factors of stillbirth as well as accumulating evidence for metabolic imprinting of FGR fetuses having effects late into adult life, an effective algorithm for the early prediction of the pregnancies at risk is essential. In the previous chapters, we risk-stratified the pregnant population based on factors associated with the delivery of SGA neonates and examined different modalities thought to reflect placenta function for their predictive value in detecting SGA in the first trimester. In large, results were in accordance with previous studies showing individual markers having modest predictive value if used in isolation. For a 5% FPR maternal risk factors appeared to predict 21% of SGA fetuses and when combined with the use of biophysical markers show a sensitivity of 25.5% while in combination with biochemical markers from maternal serum can predict 30.4%.

In similarity with screening protocols for aneuploidies in the first trimester the natural conclusion and one that has been proposed by many authors is that a combined screening algorithm utilizing all available modalities is likely to be the most successful screening method (Breeze & Lees 2007, Zhong *et al.,* 2010, Imdad *et al.,* 2011). If we take into consideration that the currently accepted surveillance protocol for the screen-positive group is serial growth scans at 28, 32 and 36 weeks of gestation, the aim of an effective combined protocol would be not only to achieve the highest possible DR but also allow for a FPR which would be sustainable by antenatal care providers. A recent update of the RCOG guidance on screening for SGA in pregnancy proposes an algorithm that stratifies risk factors based on the Odds ratios of having an SGA neonate in combination with selective screening with UAPI at 24 weeks and serial ultrasound scans in the high-risk group (figure 1.4, chapter 1). However, the

effectiveness of the RCOG algorithm has not been validated with no data in the literature on its performance. In a model that treats each risk factor as an independent screening test, the false positive rate in the presence of more than one risk factor are cumulative resulting is higher screen positive rates. Authors of the guideline mention: "There is insufficient evidence to determine how risk factors relate to each other in the individual woman and consequently how these risk factors should be managed. This includes abnormal maternal Down syndrome serum markers" (RCOG Green-top guideline No. 31). So the need for individualised assessment of mothers at risk of having an SGA baby is evident.

Data from screening studies in the first trimester utilizing combined algorithms are limited. In a recent study combined the use of PAPP-A, bhCG and NT report a DR of 55% for FPR of 20% (Papastefanou *et al.,* 2012). A second study combining maternal characteristics PLGF and PAPP-A reports a DR of 36.8% for FPR 0f 10% (Poon *et al.,* 2008). We perform the largest screening study to date, for the delivery of an SGA neonate in the first trimester of pregnancy by using a combination of maternal characteristics, biophysical and biochemical markers.

6.2 PATIENTS AND METHODS

In this chapter, we analyse data derived from the previous studies with a view to producing cumulative values for screening performance by using the combination of available markers and examine the intercorrelation between the markers examined.

The data for this study were derived from prospective screening for adverse obstetric outcomes in women attending for their routine first-trimester screening hospital visit

in pregnancy between March 2006 and September 2009. We record maternal characteristics and medical history and perform combined screening for aneuploidies by measurement of the fetal crown-rump length (CRL) and NT thickness and maternal serum PAPP-A and free β-hCG (Robinson & Fleming 1975; Snijders *et al.,* 1998; Kagan *et al.,* 2008). In the second part of the study period, we also measured the maternal MAP by automated devices (Poon *et al.,* 2010) and used transabdominal colour Doppler ultrasound to visualise the left and right uterine artery, measure the PI in each vessel and calculate the mean PI (Plasencia *et al.,* 2007). We performed case-control studies for PLGF, PP13, and ADAM12 at 11–13 weeks' gestation in pregnancies complicated by the birth of SGA and non-SGA neonates. Detailed methodology is described in the previous chapters.

6.2.1 Statistical analysis

All the biophysical and biochemical parameters were adjusted for maternal characteristics as previously described. Markers that were not found to be significantly different between non-SGA and SGA cases (sENG, TSH, FT3, FT4) were not included in the analysis.

The following steps were used to develop a model for predicting the birth of SGA neonates. First, in the total screened population there were 1,536 SGA and 31,314 non-SGA pregnancies. In each patient, the risk for SGA was calculated using multivariate logistic regression analysis of maternal factors as previously described [6]. Secondly, gaussian distributions of markers in SGA and non-SGA pregnancies were fitted. These fitted distributions define the likelihood ratios for the screening tests that can be combined with the prior risk to produce a posterior risk. Third, the maternal factors related a priori risks and log 10 MoM values of the biophysical and biochemical markers were simulated for 500,000 pregnancies from the SGA populations and the

non-SGA distributions. For the markers this involved sampling from the fitted multivariate gaussian distributions. For the prior risks, this involved drawing samples, with replacement, 500,000 records from the screening samples of SGA and non-SGA pregnancies. These records were then used to define maternal factors related a priori risks for SGA that were multiplied by the likelihood ratios of the biophysical and biochemical markers to derive the a-posterior risks in simulated samples of 500,000 SGA and 500,000 non-SGA pregnancies. Fifth, the a priori and a posteriori risks in the SGA and non-SGA groups were used to calculate the detection rates at fixed false positive rates of 5 and 10%. The process of sampling with replacement from the SGA and non-SGA screening data means that the modelled screening performance reflects the screening population. The samples of 500,000 were chosen to make the error resulting from the simulation negligible. The statistical software package SPSS 16.0 (SPSS Inc., Chicago,Ill., USA) was used for data analyses. Monte-Carlo simulations were programmed in R (The R Foundation for Statistical Computing, R version 2.11.0, ISBN 3–900051–070–0, http://www.rproject.org).

Regression analysis was used to determine the significance of the interrelations between all measured biochemical markers.

6.3 RESULTS

The total study population included 32,850 cases, were 1,536 (4.7%) were SGA and 31,314 (95.3%) non-SGA pregnancies. In the SGA group, the maternal weight and height were significantly lower, and there was a higher prevalence of women of African and Asian racial origin, cigarette smokers and those who had assisted conception, a history of chronic hypertension and previous pregnancies with SGA neonates (Poon *et al.,* 2010). In the SGA group, compared to the non-SGA group, the median MoM uterine artery PI and MAP were increased and serum PAPP-A, free β-

hCG, PLGF, PP13 and ADAM12 as well as fetal NT were decreased (table 6.1). The differences between the SGA and non-SGA groups were greater for the subgroup of SGA delivering before 37 weeks (n = 163) than those delivering at or after 37 weeks (n = 1,373) for most markers apart from MAP, fetal NT, and serum free β-hCG. The inter-correlations between biophysical and biochemical markers in the SGA and Non-SGA pregnancies are shown in table 6.3.

The estimated detection rates at fixed false positive rates of 5% and 10% in screening by maternal factors only and by combinations of maternal factors with biophysical and biochemical markers are given in table 6.2.

Table 6.1 Median and interquartile range (IQR) of uterine artery PI, MAP, Δ-NT thickness, PAPP-A, free β-hCG, PLGF, PP13 and ADAM12 in the non-SGA group and in those delivering SGA neonates

Biophysical markers	Non-SGA		All SGA		SGA < 37wks		SGA ≥ 37wks	
	n	MoM	n	MoM	n	MoM	n	MoM
Uterine artery PI	19,957	1.02 (0.84–1.22)	1,133	1.14 (0.90–1.34)*	126	1.27 (0.96–1.53)*	1,007	1.12 (0.89–1.32)*
MAP	12,854	1.00 (0.95–1.06)	661	1.01 (0.96–1.07)*	68	1.00 (0.96–1.07)	593	1.02 (0.96–1.07)*
Δ NT	31,314	0.12 (–0.08–0.34)	1,536	0.10 (–0.12–0.30)*	163	0.11 (–0.09–0.31)	1,373	0.10 (–0.12–0.30)*
PAPP-A	31,314	1.03 (0.71-1.45)	1,536	0.82 (0.55-1.12)*	163	0.71 (0.40-0.095)	1,373	0.083 (0.58-1.40)*
Free β-hCG	31,314	0.97 (0.66-1.47)	1,536	0.89 (0.58-1.40)*	163	0.93 (0.54-1.47)	1,373	0.89 (0.58-1.40)*
PLGF	1,869	1.00 (0.78-1.28)	274	0.90 (0.63–1.24)*	37	0.79 (0.50–1.14)†	237	0.93 (0.64–1.26)†
PP13	877	1.00 (0.76-1.34)	173	0.82 (0.62–1.07)*	20	0.79 (0.70–1.00)‡	153	0.85 (0.60–1.08)*
ADAM12	830	0.99 (0.81-1.20)	168	0.86 (0.69–1.08)*	19	0.80 (0.56–0.92)†	149	0.87 (0.70–1.08)*

Comparisons between the SGA and the non-SGA groups by Mann-Whitney U test.
Significance level * $p < 0.00001$, † $p < 0.01$, ‡ $p < 0.025$.

Table 6.2 Performance of screening for delivery of SGA neonates by maternal factors only and maternal factors with UAPI, MAP, Δ NT, PAPP-A, free β-hCG, PLGF, PP13, ADAM12 and their combinations

Method of screening	ALL SGA		SGA<37 weeks		SGA >37 weeks	
	DR for FPR 5%	DR for FPR 10%	DR for FPR 5%	DR for FPR 10%	DR for FPR 5%	DR for FPR 10%
M. Factors	21.0	34.0	23.3	35	20.8	33.9
M. Factors plus						
UA PI	23.9	35.9	32	44.5	23.1	34.9
MAP	22.5	34.5	22.6	34.6	22.6	34.6
NT	21.8	33.9	22.5	34.5	22.0	33.6
PAPP-A	25.5	37.5	35.5	47.8	24.5	36.6
Free b-HCG	22.4	34.1	22.9	34.8	22.4	34.1
PLGF	25.1	37.0	34.3	46.2	23.9	35.7
PP13	24.7	37.0	25.9	38.6	24.8	37.1
ADAM12	25.1	37.3	29.5	42.6	24.7	37.1
Biophysical markers	25.5	37.7	33.7	46.8	24.3	36.8
Biochemical markers	30.4	42.7	50.1	63.0	29.0	41.7
All markers	**34.3**	**47.3**	**60.7**	**73.2**	**32.5**	**45.8**

DR = Detection rate; FPR = False positive rate

Figure 6.1 Receiver operating characteristics curves in the prediction of SGA neonates in the absence of PE by maternal factors only (– – –) and by a combination of maternal factors, uterine artery PI, MAP, fetal NT, maternal serum PAPP-A, free β-hCG, PLGF, PP13 and ADAM (———).

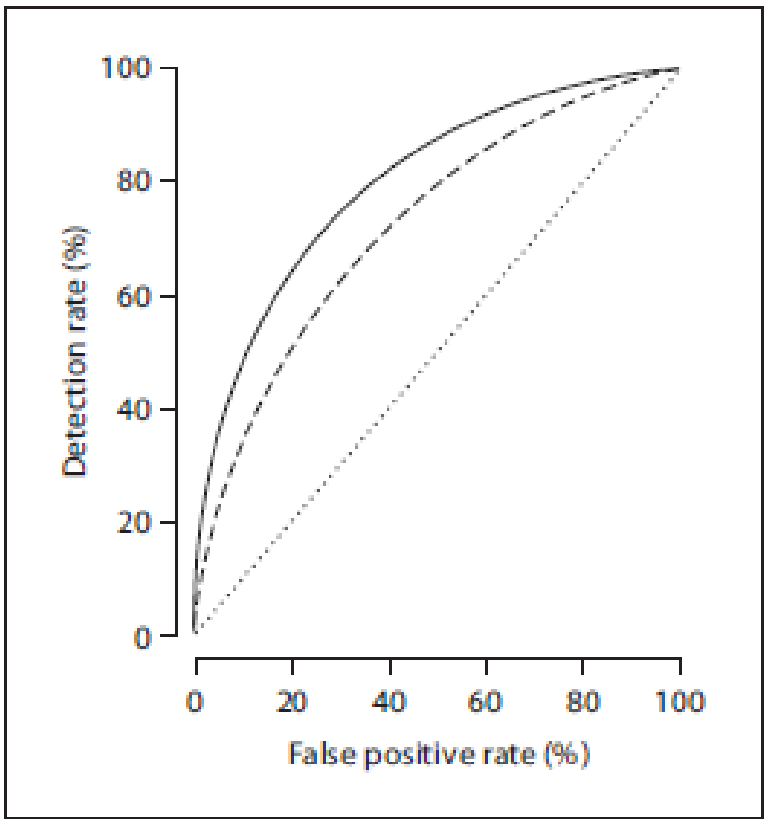


Table 6.3 Inter-correlation between log10 MoM values of uterine artery PI, MAP, PAPP-A, free β-hCG, PLGF and PP13 and square root ADAM12 MoM and Δ- NT thickness, in pregnancies delivering non-SGA and SGA neonates

Variable	Uterine artery PI		MAP		NT	
	r	p	r	P	r	p
Non-SGA pregnancies						
Uterine artery PI	1.000	-	-0.044	<0.0001	-0.015	0.038
MAP	-0.044	<0.0001	1.000	-	0.011	0.233
NT	-0.015	0.038	0.011	0.233	1.000	-
PAPP-A	-0.146	<0.0001	-0.014	0.121	0.010	0.082
Free β-hCG	-0.014	0.055	-0.006	0.464	-0.024	<0.0001
PLGF	-0.116	<0.0001	0.019	0.408	0.043	0.064
PP13	-0.089	0.009	0.035	0.312	0.090	0.008
ADAM12	-0.005	0.881	0.077	0.029	0.054	0.121
SGA pregnancies						
Uterine artery PI	1.000	-	-0.152	<0.0001	-0.040	0.182
MAP	-0.152	<0.0001	1.000	-	-0.079	0.041
NT	-0.040	0.182	-0.079	0.041	1.000	-
PAPP-A	-0.184	<0.0001	0.001	0.979	0.016	0.536
Free β-hCG	-0.015	0.060	0.055	0.157	-0.023	0.370
PLGF	-0.261	<0.0001	-0.073	0.288	0.082	0.178
PP13	-0.254	0.001	0.148	0.053	0.026	0.733
ADAM12	-0.030	0.696	0.043	0.577	-0.028	0.715

r = Pearson correlation coefficient, p = significant value

Table 6.3 (continued) Inter-coofelation between log10 MoM values of uterine artery PI, MAP, PAPP-A, free β-hCG, PLGF and PP13 and square root ADAM12 MoM and Δ- NT thickness, in pregnancies delivering non-SGA and SGA neonates

PAPP-A		Free β-Hcg		PLGF		PP13		ADAM12	
r	p	r	P	r	p	r	P	r	p
-0.146	<0.0001	-0.014	0.055	-0.116	<0.0001	-0.089	0.009	-0.005	
-0.014	0.121	-0.006	0.464	0.019	0.408	0.035	0.312	0.077	
0.010	0.082	-0.024	<0.0001	0.043	0.064	0.090	0.008	0.054	
1.000	-	0.215	<0.0001	0.317	<0.0001	0.300	<0.0001	0.414	
0.215	<0.0001	1.000	-	0.138	<0.0001	0.378	<0.0001	0.238	
0.317	<0.0001	0.138	<0.0001	1	-	0.037	0.290	0.259	
0.300	<0.0001	0.378	<0.0001	0.037	0.290	1	-	0.413	
0.414	<0.0001	0.238	<0.0001	0.259	<0.0001	0.413	<0.0001	1.000	
-0.184	<0.0001	-0.015	0.606	-0.261	<0.0001	-0.254	0.001	-0.030	0.696
0.001	0.979	0.055	0.157	-0.073	0.288	0.148	0.053	0.043	0.577
0.016	0.536	-0.023	0.370	0.082	0.178	0.026	0.733	-0.028	0.715
1.000	-	0.174	<0.0001	0.438	<0.0001	0.381	<0.0001	0.513	<0.0001
0.174	<0.0001	1.000	-	0.049	0.421	0.444	<0.0001	0.207	<0.0001
0.438	<0.0001	0.049	0.421	1.000	-	0.054	0.490	0.303	<0.0001
0.381	<0.0001	0.444	<0.0001	0.054	0.0490	1.000	-	0.404	<0.0001
0.513	<0.0001	0.207	0.007	0.303	<0.0001	0.404	<0.0001	1.000	-

r = Pearson correlation coefficient, p = significant value

6.4 DISCUSSION

This study has demonstrated that an algorithm combining maternal characteristics and biophysical and biochemical tests at 11–13 weeks could potentially identify half of pregnancies that deliver SGA neonates in the absence of PE, at a 10% false positive rate. We used logistic regression analysis to derive the a-priori risk for SGA from maternal characteristics in a prospective screening study involving more than 30,000 pregnancies. The patient-specific a posteriori odds for SGA were then calculated by multiplying the a priori odds with the likelihood ratio of a series of biophysical and biochemical markers after appropriate adjustments for the inter-correlations of all markers.

The data confirm the results of previous studies and indicate that, as in the case of PE, in pregnancies with SGA in the absence of PE there is evidence of impaired placental perfusion and function from the first trimester of pregnancy. However, the magnitude of such impairment is considerably less than in PE (Akolekar *et al.,* 2010). This is not surprising because, unlike PE which is a pathological disorder, SGA is a heterogeneous condition which includes constitutionally small fetuses, at no or minimally increased risk of perinatal death and handicap, and growth restricted fetuses (FGR) due to impaired placentation, genetic disease or environmental damage. In our study, we excluded fetal abnormalities but did not distinguish between constitutional SGA and FGR by such measures as performing Doppler studies in the third trimester of pregnancy (Wright *et al.,* 2008) because the diagnosis of SGA was made retrospectively. Nevertheless, we found that the differences in uterine artery PI and serum PAPP-A, PLGF, PP13 and ADAM12 between the SGA and non-SGA groups were greater for the subgroup of SGA delivering before 37 weeks than those delivering at or after 37 weeks. Since the proportion of FGR to constitutional SGA is likely to be higher in the preterm rather than term SGA, our findings imply that the

early biophysical and biochemical markers could be identifying the FGR subgroup amongst the SGA.

Assessment of the patient-specific risk for SGA at 11–13 weeks is a by-product of early screening for aneuploidies. In this hospital visit a series of maternal characteristics are recorded because these are essential for the correct interpretation of the measured free β-hCG and PAPP-A (Kagan *et al.,* 2008). Additionally, an ultrasound scan is performed for examination of the fetal anatomy and assessment of markers of aneuploidy. We have shown that the use of an algorithm derived from multiple regression analysis of a series of basic maternal characteristics and the results of the combined test for aneuploidies can identify 37% of pregnancies that will deliver SGA neonates, at a false positive rate of 10% (Poon *et al.,* 2010). We have previously advocated that the same ultrasound examination at 11–13 weeks should include measurement of uterine artery PI because this in combination with maternal characteristics, MAP and biomarkers could identify about 90% of pregnancies that will develop PE (Akolekar *et al.,* 2010, Poon *et al.,* 2009). This study has shown that inclusion of such additional biochemical and biophysical markers could improve the early detection of SGA in the absence of PE to 73% for those requiring delivery before 37 weeks and 46% for those delivering at term. Early estimation of patient-specific risks for SGA could potentially improve pregnancy outcome by shifting antenatal care from a series of routine visits to a more individualized approach both in terms of the schedule and content of such visits.

Considering the existing antenatal care pathway, use of a first-trimester screening algorithm necessitates a surveillance protocol for the high-risk group that currently in the United Kingdom is serial growth scans at 28, 32, 36 weeks. This surveillance protocol has been shown to perform optimally when applied to a high-risk population (Chang *et al.,* 1992; Chauhan *et al.,* 2006). In our attempt to define a high-risk

population we have shown that the multifactorial predictive model that we apply in the first trimester can risk-stratify the pregnancy population better that any modalities applied in isolation. Once a pregnancy has been labelled as high risk and enters the close surveillance pathway a combination of biometric measurements, liquor volume, and Doppler studies can be used to ascertain whether the fetus is suffering from placental insufficiency.

Care must be taken by providers applying such early screening algorithms because unlike screening for aneuploidies where diagnostic tests are available immediately; the diagnosis of FGR can only be made late in gestation or even after birth. The psychological cost for a mother being labelled as “high risk” can be high, could potentially increase anxiety throughout pregnancy, might increase hospital attendances, result in increased number of CTGs and consequently increased the rate of intervention. It is essential that appropriate attention is taken into counselling women about the implications of these tests and being able to offer on-going support throughout the pregnancy. If our screening algorithm was applied with a 5% FPR then the screen-positive group would be 6.4% of the whole population and out of those women 74% would deliver AGA babies. Similarly, if the 10% FPR was applied then the screen-positive group would be 11.7% and out of those, 81% would be normal. For this reason robust evidence of FGR must exist in order to justify medical intervention.

6.5 CONCLUSION

In conclusion, we see that a large proportion of pregnancies that are destined to develop an SGA neonate can be predicted in the first trimester by a combined screening algorithm. Validation of the findings prospectively and in different

populations is required. This algorithm can be made part of the routine screening regime of every pregnancy and help shape a more targeted antenatal care plan for the subsequent gestation.

CHAPTER 7. FUTURE STUDIES

At the time of publication, our study provided a comprehensive evaluation of the most promising markers of fetal growth restriction. Factors that increase the validity of our results include the fact that we examined a large low-risk population from which we derived our own birthweight centiles. In addition, we utilised validated and quality controlled techniques for obtaining the measurements of the ultrasound and biochemical markers which make the effects demonstrated more reliable.

Our findings agree with previous studies in that no individual marker appears to perform well in detecting SGA in pregnancy. The combination of markers was able to detect around 50% of cases of SGA for a FPR of 10% but showed improved detection rates for the preterm group.

Placental disease and its different manifestations represent a complex and varied pathophysiological process which is not yet fully understood. Looking into the future there are certain areas that progress is likely to be observed and will be briefly discussed below.

7.1 IMPROVED DEFINITION OF FGR

As discussed previously, one of the reasons of the variable and sometimes poor performance of screening algorithms for FGR lies within the heterogeneity of the definition of the outcome measure. Commonly an SGA fetus will be used as a proxy for FGR but not all SGA fetuses will suffer from true FGR and some fetuses which do not fall below the centile threshold for the definition of SGA might be actually suffering

from growth restriction. A definition of the outcome measure incorporating evidence of placental disease in combination with a reduction in growth is likely to represent better the affected population and hence an improved performance of the screening algorithm might be able to be demonstrated. A recent attempt at standardisation of the definition of FGR was performed via a Delphi procedure. The Delphi procedure aims for refinement of opinions by participating experts, while minimizing confounding factors present in other group response methods (Sinha *et al.,* 2011). The definition of FGR was separated into early (<32 weeks) and late (>32 weeks) and criteria were based on prenatal ultrasound biometry plus Doppler indices (Gordijn *et al.,* 2016). The exact definitions are presented in table (7.1).

In a screening study performed the same year the performance of an algorithm utilising maternal characteristics, biophysical parameters and biochemical markers in the first trimester of pregnancy was compared when the outcome measure was defined as the birth of an SGA fetus <10^{th} centile versus an antenatal definition including EFW <10^{th} plus Doppler evidence of placental insufficiency. Results showed a significantly improved definition of the model utilising the improved FGR definition (AUC 0.78 vs 0.68, $p<0.001$) thus demonstrating that there is scope for improvement of first-trimester prediction if FGR is optimally defined.

Table 7.1 Definitions of FGR from Delphi procedure (Gordijn *et al.*, 2016).

Definition of early FGR (<32 weeks of gestation): either

1. Abdominal circumference below the 3rd centile OR estimated fetal weight below the 3rd centile OR absent end-diastolic flow in the umbilical artery

   OR

2. Both of the following
   - Estimated fetal weight or abdominal circumference below the 10th centile

     AND

   - Pulsatility index of the uterine artery above the 95th centile OR pulsatility index in the umbilical artery above the 95th centile.

Definition of late FGR (≥32 weeks of gestation):

1. Abdominal circumference below the 3rd centile, or estimated fetal weight below the 3rd centile

   OR

2. At least two out of three of the following:
   - Abdominal circumference below the 10th centile OR estimated fetal weight below the 10th centile
   - Abdominal circumference OR estimated fetal weight crossing centiles >2 quartiles
   - Cerebro-placental ratio below the 5th centile OR pulsatility index in the umbilical artery above the 95th centile.

7.2 DISCOVERY OF NEW BIOMARKERS OF PLACENTAL DISEASE

There is no doubt that a marker that can demonstrate an increased risk of placental disease before the development of symptoms would be of great interest with regards to patient selection. Traditionally techniques such as enzyme-linked immunosorbent assay (ELISA), western blot, immunostaining and polymerase chain reaction (PCR) have been used to identify and compare proteins derived by pathophysiological pathways that are thought to have relevance to the disease in question (Law *et al.,* 2015). Due to the highly complex nature of the disease process, it is likely that multiple markers will perform better that a single one and hence improved techniques for identification of proteins are being increasingly employed. Proteomics research, the analysis of the entire set of proteins of a biological system is such an emerging technique. It utilises Mass spectrometry in combination with a variety of other separation tools to analyse simultaneously hundreds of proteins (Sydor & Nock 2003, Law *et al.,* 2015, Hernandez-Nunez & Valdes-Yong, 2015). In one study high-throughput analysis of proteins in maternal plasma in women with PET and/or FGR was performed that identified 166 different proteins between groups including previously undetected proteins. Furthermore, it demonstrated differences in protein levels between isolated PET and PET associated with FGR (Auer *et al.,* 2009). Few other studies applying proteomic techniques in a variety of tissues such as placentas, amniotic fluid or umbilical cord blood obtained from fetuses affected by FGR have demonstrated differences between protein profiles when compared to controls (Shehab *et al.,* 2009, karamessinis *et al.,* 2008, Cecconi *et al.,* 2011, Wolter *et al.,* 2012). These potential biomarkers need to be validated in clinical studies with the hope that they could be integrated into a screening algorithm.

7.3 ASPIRIN FOR THE PREVENTION OF PREECLAMPSIA AND FETAL GROWTH RESTRICTION

Preeclampsia and fetal growth restriction being mostly unexplained with regards to their pathophysiological mechanisms have suffered from an outright absence of any effective preventive or therapeutic interventions that could potentially alter the course of the disease, short of delivery of the placenta. The drug with the best evidence to date is Aspirin. One meta-analysis on the use of Aspirin has reported a reduction in rates of both PET (RR 0.47) and FGR (RR 0.44) (Bujold *et al.,* 2010). The strongest effect has been demonstrated when Aspirin was administered before 16 weeks of gestation and the highest reduction was in women who suffered from preterm PE (Roberge *et al.,* 2012). It is most likely that an effect will be demonstrated if Aspirin is given to a high-risk population and hence applying an algorithm in the first trimester of pregnancy will facilitate the selection of a high-risk group for subsequent randomisation. A large multicentre randomised controlled trial is registered with the aim of testing the use of Aspirin in women found to be high risk for PET by using a first-trimester screening algorithm (O'Gorman *et al.,* 2016). The screening algorithm involves the same markers as the SGA algorithm in this thesis and if results are promising it is possible that further investigation will extend to women from the risk of SGA without PET.

7.4 CONCLUSION

In conclusion, the future looks promising with regards to improvement in the management of placental diseases in pregnancy and a hope for an effective therapeutic intervention. Our study at the time set a benchmark on the prediction of SGA fetuses in the first trimester by utilising the most extensive complement of predictive markers. Our algorithm while not accurate enough to be applied to clinical practice has demonstrated that a standardised and quality controlled process of multifactorial assessment of pregnant women in the first trimester of pregnancy is a promising format upon which future studies can expand and improve.

REFERENCES

Abu Shehab, M., Inoue, S., Han, V. K. M., & Gupta, M. B. (2009). Site specific phosphorylation of insulin-like growth factor binding protein-1 (IGFBP-1) for evaluating clinical relevancy in fetal growth restriction. *Journal of Proteome Research*, *8*(11), 5325–5335. article. https://doi.org/10.1021/pr900633x

Abumaree, M. H., Stone, P. R., & Chamley, L. W. (2006). The effects of apoptotic, deported human placental trophoblast on macrophages: possible consequences for pregnancy. *Journal of Reproductive Immunology*, *72*(1–2), 33–45. https://doi.org/10.1016/j.jri.2006.03.001

Aggarwal, P. K., Jain, V., Sakhuja, V., Karumanchi, S. A., & Jha, V. (2006). Low urinary placental growth factor is a marker of pre-eclampsia. *Kidney International*, *69*(3), 621–4. https://doi.org/10.1038/sj.ki.5000075

Akolekar, R., de Cruz, J., Foidart, J.-M., Munaut, C., & Nicolaides, K. H. (2010). Maternal plasma soluble fms-like tyrosine kinase-1 and free vascular endothelial growth factor at 11 to 13 weeks of gestation in preeclampsia. *Prenatal Diagnosis*, *30*(3), 191–7. https://doi.org/10.1002/pd.2433

Akolekar, R., Syngelaki, A., Beta, J., Kocylowski, R., & Nicolaides, K. H. (2009). Maternal serum placental protein 13 at 11-13 weeks of gestation in preeclampsia. *Prenatal Diagnosis*, *29*(12), 1103–1108. article. https://doi.org/10.1002/pd.2375

Akolekar, R., Syngelaki, A., Sarquis, R., Zvanca, M., & Nicolaides, K. H. (2011). Prediction of early, intermediate and late pre-eclampsia from maternal factors, biophysical and biochemical markers at 11-13 weeks. *Prenatal Diagnosis*, *31*(1), 66–74. article. https://doi.org/10.1002/pd.2660

Alexander, G. R., Wingate, M. S., Mor, J., & Boulet, S. (2007). Birth outcomes of Asian-Indian-Americans. *International Journal of Gynaecology and Obstetrics: The Official Organ of the International Federation of Gynaecology and Obstetrics*, *97*(3), 215–220. article. https://doi.org/10.1016/j.ijgo.2007.02.017

Alfirevic, Z., & Neilson, J. P. (1995). Doppler ultrasonography in high-risk pregnancies: systematic review with meta-analysis. *American Journal of Obstetrics and Gynecology*, *172*(5), 1379–1387. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7755042

Allan, W. C., Haddow, J. E., Palomaki, G. E., Williams, J. R., Mitchell, M. L., Hermos, R. J., Klein, R. Z. (2000). Maternal thyroid deficiency and pregnancy complications: implications for population screening. *Journal of Medical Screening*, *7*(3), 127–30. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11126160

Allen, V. M., Joseph, K. S., Murphy, K. E., Magee, L. A., & Ohlsson, A. (2004). The effect of hypertensive disorders in pregnancy on small for gestational age and stillbirth: a population based study. *BMC Pregnancy and Childbirth*, *4*(1), 17. article. https://doi.org/10.1186/1471-2393-4-17

Altman DG, Hytten F. Assessment of fetal size and fetal growth. In: Chalmers I, Enkin M, Keirse MJNC editor(s). (1989) Effective care in pregnancy and childbirth. Oxford: Oxford University Press 411–8.

Ananth, C. V, Peltier, M. R., Chavez, M. R., Kirby, R. S., Getahun, D., & Vintzileos, A. M. (2007). Recurrence of ischemic placental disease. *Obstetrics and Gynecology*, *110*(1), 128–133. article. https://doi.org/10.1097/01.AOG.0000266983.77458.71

Angiolini, E., Fowden, A., Coan, P., Sandovici, I., Smith, P., Dean, W., Constância, M. (2006). Regulation of placental efficiency for nutrient transport by imprinted genes. *Placenta*, *27 Suppl A*, S98-102. https://doi.org/10.1016/j.placenta.2005.12.008

Anin, S. A., Vince, G., & Quenby, S. (2004). Trophoblast invasion. *Human Fertility (Cambridge, England)*, *7*(3), 169–174. article. https://doi.org/10.1080/14647270400006911

Aplin, J. D., Charlton, A. K., & Ayad, S. (1988). An immunohistochemical study of human endometrial extracellular matrix during the menstrual cycle and first trimester of pregnancy. *Cell and Tissue Research*, *253*(1), 231–240. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/3416340

Ashoor, G., Kametas, N. A., Akolekar, R., Guisado, J., & Nicolaides, K. H. (2010). Maternal thyroid function at 11-13 weeks of gestation. *Fetal Diagnosis and Therapy*, *27*(3), 156–163. article. https://doi.org/10.1159/000313301

Ashoor, G., Maiz, N., Rotas, M., Kametas, N. A., & Nicolaides, K. H. (2010). Maternal thyroid function at 11 to 13 weeks of gestation and subsequent development of preeclampsia. *Prenatal Diagnosis*, *30*(11), 1032–1038. article. https://doi.org/10.1002/pd.2566

Askie, L. M., Duley, L., Henderson-Smart, D. J., Stewart, L. A., & PARIS Collaborative Group. (2007). Antiplatelet agents for prevention of pre-eclampsia: a meta-analysis of individual patient data. *Lancet (London, England)*, *369*(9575), 1791–1798. article. https://doi.org/10.1016/S0140-6736(07)60712-0

Athapathu, H., Jayawardana, M. A. J., & Senanayaka, L. (2003). A study of the incidence of apoptosis in the human placental cells in the last weeks of pregnancy. *Journal of Obstetrics and Gynaecology : The Journal of the Institute of Obstetrics and Gynaecology*, *23*(5), 515–517. article. https://doi.org/10.1080/0144361031000153756

Auer, J., Camoin, L., Guillonneau, F., Rigourd, V., Chelbi, S. T., Leduc, M., Vaiman, D. (2010). Serum profile in preeclampsia and intra-uterine growth restriction revealed by iTRAQ technology. *Journal of Proteomics*, *73*(5), 1004–17. https://doi.org/10.1016/j.jprot.2009.12.014

Aviram, R., T, B. S., & Kidron, D. (2010). Placental aetiologies of foetal growth restriction: clinical and pathological differences. *Early Human Development*, *86*(1), 59–63. https://doi.org/10.1016/j.earlhumdev.2010.01.020

Bailey, S. M., Sarmandal, P., & Grant, J. M. (1989). A comparison of three methods of assessing inter-observer variation applied to measurement of the symphysis-fundal height. *British Journal of Obstetrics and Gynaecology*, *96*(11), 1266–1271. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2611167

Bais, J. M. J., Eskes, M., Pel, M., Bonsel, G. J., & Bleker, O. P. (2004). Effectiveness of detection of intrauterine growth retardation by abdominal palpation as screening test in a low risk population: an observational study. *European Journal of Obstetrics, Gynecology, and Reproductive Biology*, *116*(2), 164–169. article. https://doi.org/10.1016/j.ejogrb.2004.01.037

Bakewell, J. M., Stockbauer, J. W., & Schramm, W. F. (1997). Factors associated with repetition of low birthweight: Missouri longitudinal study. *Paediatric and Perinatal Epidemiology*, *11 Suppl 1*, 119–29. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9018721

Ball, E., Bulmer, J. N., Ayis, S., Lyall, F., & Robson, S. C. (2006). Late sporadic miscarriage is associated with abnormalities in spiral artery transformation and trophoblast invasion. *The Journal of Pathology*, *208*(4), 535–542. article. https://doi.org/10.1002/path.1927

Barber, K. J., Franklyn, J. A., McCabe, C. J., Khanim, F. L., Bulmer, J. N., Whitley, G. S. J., & Kilby, M. D. (2005). The in vitro effects of triiodothyronine on epidermal growth factor-induced trophoblast function. *The Journal of Clinical Endocrinology and Metabolism*, *90*(3), 1655–1661. article. https://doi.org/10.1210/jc.2004-0785

Barker, D. J. P., Larsen, G., Osmond, C., Thornburg, K. L., Kajantie, E., & Eriksson, J. G. (2012). The placental origins of sudden cardiac death. *International Journal of Epidemiology*, *41*(5), 1394–9. https://doi.org/10.1093/ije/dys116

Bartha, J. L., Wood, J., Kyle, P. M., & Soothill, P. W. (2003). The effect of metabolic control on fetal nuchal translucency in women with insulin-dependent diabetes: a preliminary study. *Ultrasound in Obstetrics & Gynecology : The Official Journal of the International Society of Ultrasound in Obstetrics and Gynecology*, *21*(5), 451–4. https://doi.org/10.1002/uog.118

Baschat, A. A. (2005). Arterial and venous Doppler in the diagnosis and management of early onset fetal growth restriction. *Early Human Development*, *81*(11), 877–887. article. https://doi.org/10.1016/j.earlhumdev.2005.09.008

Baschat, A. A. (2011). Venous Doppler evaluation of the growth-restricted fetus. *Clinics in Perinatology*, *38*(1), 103--12, vi. article. https://doi.org/10.1016/j.clp.2010.12.001

Basso, O., Olsen, J., & Christensen, K. (1998). Risk of preterm delivery, low birthweight and growth retardation following spontaneous abortion: a registry-based study in Denmark. *International Journal of Epidemiology*, *27*(4), 642–646. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9758119

Batech, M., Tonstad, S., Job, J. S., Chinnock, R., Oshiro, B., Allen Merritt, T., Singh, P. N. (2013). Estimating the impact of smoking cessation during pregnancy: the San Bernardino County experience. *Journal of Community Health*, *38*(5), 838–846. article. https://doi.org/10.1007/s10900-013-9687-8

Bateson, P., Barker, D., Clutton-Brock, T., Deb, D., D'Udine, B., Foley, R. A., Sultan, S. E. (2004). Developmental plasticity and human health. *Nature*, *430*(6998), 419–21. https://doi.org/10.1038/nature02725

Bateson, P., Barker, D., Clutton-Brock, T., Deb, D., D'Udine, B., Foley, R. A., Sultan, S. E. (2004). Developmental plasticity and human health. *Nature*, *430*(6998), 419–21. https://doi.org/10.1038/nature02725

Battaglia, F. C., & Regnault, T. R. (n.d.). Placental transport and metabolism of amino acids. *Placenta*, *22*(2–3), 145–161. article. https://doi.org/10.1053/plac.2000.0612

Berends, A. L., Steegers, E. A., Isaacs, A., Aulchenko, Y. S., Liu, F., de Groot, C. J., van Duijn, C. M. (2008). Familial aggregation of preeclampsia and intrauterine growth restriction in a genetically isolated population in The Netherlands. *European Journal of Human Genetics : EJHG*, *16*(12), 1437–42. https://doi.org/10.1038/ejhg.2008.118

Bernstein, P. S., & Divon, M. Y. (1997). Etiologies of fetal growth restriction. *Clinical Obstetrics and Gynecology*, *40*(4), 723–729. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9429786

Blazer, S., Moreh-waterman, Y., Miller-lotan, R., Tamir, A., & Hochberg, Z. (2003). Maternal Hypothyroidism May Affect Fetal Growth and Neonatal Thyroid Function, *102*(2), 232–241. https://doi.org/10.1016/S0029-7844(03)00513-1

Bonno, M., Oxvig, C., Kephart, G. M., Wagner, J. M., Kristensen, T., Sottrup-Jensen, L., & Gleich, G. J. (1994). Localization of pregnancy-associated plasma protein-A and colocalization of pregnancy-associated plasma protein-A messenger ribonucleic acid and eosinophil granule major basic protein messenger ribonucleic acid in placenta. *Laboratory Investigation; a Journal of Technical Methods and Pathology*, *71*(4), 560–6. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7526035

Bosio, P. M., McKenna, P. J., Conroy, R., & O'Herlihy, C. (1999). Maternal central hemodynamics in hypertensive disorders of pregnancy. *Obstetrics and Gynecology*, *94*(6), 978–84. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10576186

Bradley, R. J., Nicolaides, K. H., & Brudenell, J. M. (1988). Are all infants of diabetic mothers "macrosomic"? *BMJ (Clinical Research Ed.)*, *297*(6663), 1583–1584. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/3147084

Breeze, A. C. G., & Lees, C. C. (2007). Prediction and perinatal outcomes of fetal growth restriction. *Seminars in Fetal {&} Neonatal Medicine*, *12*(5), 383–397. article. https://doi.org/10.1016/j.siny.2007.07.002

Bricker, L., Neilson, J. P., & Dowswell, T. (2008). Routine ultrasound in late pregnancy (after 24 weeks' gestation). *The Cochrane Database of Systematic Reviews*, (4), CD001451. article. https://doi.org/10.1002/14651858.CD001451.pub3

Brosens IA. The utero-placental vessels at term: the distribution and extent of physiological changes. (1988) Trophoblast Res 3: 61-7.

Brosens, I. A., Robertson, W. B., & Dixon, H. G. (1972). The role of the spiral arteries in the pathogenesis of preeclampsia. *Obstetrics and Gynecology Annual*, *1*, 177–191. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/4669123

Brosens, I., Dixon, H. G., & Robertson, W. B. (1977). Fetal growth retardation and the arteries of the placental bed. *British Journal of Obstetrics and Gynaecology*, *84*(9), 656–663. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/911717

Brosens, I., & Renaer, M. (1972). On the pathogenesis of placental infarcts in pre-eclampsia. *The Journal of Obstetrics and Gynaecology of the British Commonwealth*, *79*(9), 794–799. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/4651288

Brosens, I., Pijnenborg, R., Vercruysse, L., & Romero, R. (2011). The “Great Obstetrical Syndromes” are associated with disorders of deep placentation. *American Journal of Obstetrics and Gynecology*, *204*(3), 193–201. article. https://doi.org/10.1016/j.ajog.2010.08.009

Brosens, J. J., Pijnenborg, R., & Brosens, I. A. (2002). The myometrial junctional zone spiral arteries in normal and abnormal pregnancies: a review of the literature. *American Journal of Obstetrics and Gynecology*, *187*(5), 1416–1423. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12439541

Buhimschi, C. S., Norwitz, E. R., Funai, E., Richman, S., Guller, S., Lockwood, C. J., & Buhimschi, I. A. (2005). Urinary angiogenic factors cluster hypertensive disorders and identify women with severe preeclampsia. *American Journal of Obstetrics and Gynecology*, *192*(3), 734–741. article. https://doi.org/10.1016/j.ajog.2004.12.052

Bujold, E., Roberge, S., Lacasse, Y., Bureau, M., Audibert, F., Marcoux, S., Giguère, Y. (2010). Prevention of preeclampsia and intrauterine growth restriction with aspirin started in early pregnancy: a meta-analysis. *Obstetrics and Gynecology*, *116*(2 Pt 1), 402–414. article. https://doi.org/10.1097/AOG.0b013e3181e9322a

Bukowski, R., Smith, G. C. S., Malone, F. D., Ball, R. H., Nyberg, D. A., Comstock, C. H., FASTER Research Consortium. (2007). Fetal growth in early pregnancy and risk of delivering low birth weight infant: prospective cohort study. *BMJ (Clinical Research Ed.)*, *334*(7598), 836. article. https://doi.org/10.1136/bmj.39129.637917.AE

Burger, O., Pick, E., Zwickel, J., Klayman, M., Meiri, H., Slotky, R., Gonen, R. (2004). Placental protein 13 (PP-13): effects on cultured trophoblasts, and its detection in human body fluids in normal and pathological pregnancies. *Placenta*, *25*(7), 608–622. article. https://doi.org/10.1016/j.placenta.2003.12.009

Burke, S. D., & Karumanchi, S. A. (2013). Spiral artery remodeling in preeclampsia revisited. *Hypertension (Dallas, Tex. : 1979)*, *62*(6), 1013–1014. article. https://doi.org/10.1161/HYPERTENSIONAHA.113.02049

Burrows, T. D., King, A., & Loke, Y. W. (n.d.). Trophoblast migration during human placental implantation. *Human Reproduction Update*, *2*(4), 307–321. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9080228

Burton, G. J., Reshetnikova, O. S., Milovanov, A. P., & Teleshova, O. V. (1996). Stereological evaluation of vascular adaptations in human placental villi to differing forms of hypoxic stress. *Placenta*, *17*(1), 49–55. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8710813

Caniggia, I., Winter, J., Lye, S. J., & Post, M. (n.d.). Oxygen and placental development during the first trimester: implications for the pathophysiology of pre-eclampsia. *Placenta*, *21 Suppl A*, S25-30. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10831118

Canini, S., Prefumo, F., Pastorino, D., Crocetti, L., Afflitto, C. G., Venturini, P. L., & De Biasio, P. (2008). Association between birth weight and first-trimester free beta-human chorionic gonadotropin and pregnancy-associated plasma protein A. *Fertility and Sterility*, *89*(1), 174–8. https://doi.org/10.1016/j.fertnstert.2007.02.024

Canini, S., Prefumo, F., Pastorino, D., Crocetti, L., Afflitto, C. G., Venturini, P. L., & De Biasio, P. (2008). Association between birth weight and first-trimester free beta-human chorionic gonadotropin and pregnancy-associated plasma protein A. *Fertility and Sterility*, *89*(1), 174–8. https://doi.org/10.1016/j.fertnstert.2007.02.024

Casey, B. M., Dashe, J. S., Spong, C. Y., McIntire, D. D., Leveno, K. J., & Cunningham, G. F. (2007). Perinatal significance of isolated maternal

hypothyroxinemia identified in the first half of pregnancy. *Obstetrics and Gynecology*, *109*(5), 1129–35. https://doi.org/10.1097/01.AOG.0000262054.03531.24

Cecconi, D., Lonardoni, F., Favretto, D., Cosmi, E., Tucci, M., Visentin, S., Ferrara, S. D. (2011). Changes in amniotic fluid and umbilical cord serum proteomic profiles of foetuses with intrauterine growth retardation. *Electrophoresis*, *32*(24), 3630–3637. article. https://doi.org/10.1002/elps.201100256

Cerdeira, A. S., & Karumanchi, S. A. (2012). Angiogenic factors in preeclampsia and related disorders. *Cold Spring Harbor Perspectives in Medicine*, *2*(11). https://doi.org/10.1101/cshperspect.a006585

Cerdeira, A. S., & Karumanchi, S. A. (2012). Angiogenic factors in preeclampsia and related disorders. *Cold Spring Harbor Perspectives in Medicine*, *2*(11). https://doi.org/10.1101/cshperspect.a006585

Cervera, R., Font, J., Carmona, F., & Balasch, J. (2002). Pregnancy outcome in systemic lupus erythematosus: good news for the new millennium. *Autoimmunity Reviews*, *1*(6), 354–359. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12848991

Cetin, I., & Alvino, G. (2009). Intrauterine growth restriction: implications for placental metabolism and transport. A review. *Placenta*, *30 Suppl A*, S77-82. https://doi.org/10.1016/j.placenta.2008.12.006

Chafetz, I., Kuhnreich, I., Sammar, M., Tal, Y., Gibor, Y., Meiri, H., Wolf, M. (2007). First-trimester placental protein 13 screening for preeclampsia and intrauterine growth restriction. *American Journal of Obstetrics and Gynecology*, *197*(1), 35.e1-7. https://doi.org/10.1016/j.ajog.2007.02.025

Chafetz, I., Kuhnreich, I., Sammar, M., Tal, Y., Gibor, Y., Meiri, H., Wolf, M. (2007). First-trimester placental protein 13 screening for preeclampsia and intrauterine growth restriction. *American Journal of Obstetrics and Gynecology*, *197*(1), 35.e1-7. https://doi.org/10.1016/j.ajog.2007.02.025

Chaiworapongsa, T., Chaemsaithong, P., Yeo, L., & Romero, R. (2014). Pre-eclampsia part 1: current understanding of its pathophysiology. *Nature*

Reviews. Nephrology, *10*(8), 466–480. article. https://doi.org/10.1038/nrneph.2014.102

Chang, T. C., Robson, S. C., Boys, R. J., & Spencer, J. A. (1992). Prediction of the small for gestational age infant: which ultrasonic measurement is best? *Obstetrics and Gynecology*, *80*(6), 1030–1038. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1448248

Chang, T. C., Robson, S. C., Spencer, J. A., & Gallivan, S. (1993). Identification of fetal growth retardation: comparison of Doppler waveform indices and serial ultrasound measurements of abdominal circumference and fetal weight. *Obstetrics and Gynecology*, *82*(2), 230–236. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8336870

Chappell, L. C., Enye, S., Seed, P., Briley, A. L., Poston, L., & Shennan, A. H. (2008). Adverse perinatal outcomes and risk factors for preeclampsia in women with chronic hypertension: a prospective study. *Hypertension (Dallas, Tex. : 1979)*, *51*(4), 1002–1009. article. https://doi.org/10.1161/HYPERTENSIONAHA.107.107565

Chauhan, S. P., & Magann, E. F. (2006). Screening for fetal growth restriction. *Clinical Obstetrics and Gynecology*, *49*(2), 284–294. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/16721107

Cheifetz, S., Bellón, T., Calés, C., Vera, S., Bernabeu, C., Massagué, J., & Letarte, M. (1992). Endoglin is a component of the transforming growth factor-beta receptor system in human endothelial cells. *The Journal of Biological Chemistry*, *267*(27), 19027–19030. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1326540

Clark, D. E., Smith, S. K., Licence, D., Evans, A. L., & Charnock-Jones, D. S. (1998). Comparison of expression patterns for placenta growth factor, vascular endothelial growth factor (VEGF), VEGF-B and VEGF-C in the human placenta throughout gestation. *The Journal of Endocrinology*, *159*(3), 459–467. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9834463

Clausson, B., Cnattingius, S., & Axelsson, O. (1998). Preterm and term births of small for gestational age infants: a population-based study of risk

factors among nulliparous women. *British Journal of Obstetrics and Gynaecology*, *105*(9), 1011–1017. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9763054

Clowse, M. E. B., Magder, L. S., Witter, F., & Petri, M. (2005). The impact of increased lupus activity on obstetric outcomes. *Arthritis and Rheumatism*, *52*(2), 514–521. article. https://doi.org/10.1002/art.20864

Cnossen, J. S., Morris, R. K., ter Riet, G., Mol, B. W. J., van der Post, J. A. M., Coomarasamy, A., Khan, K. S. (2008). Use of uterine artery Doppler ultrasonography to predict pre-eclampsia and intrauterine growth restriction: a systematic review and bivariable meta-analysis. *CMAJ : Canadian Medical Association Journal = Journal de l'Association Medicale Canadienne*, *178*(6), 701–711. article. https://doi.org/10.1503/cmaj.070430

Cnossen, J. S., Vollebregt, K. C., de Vrieze, N., ter Riet, G., Mol, B. W. J., Franx, A., van der Post, J. A. M. (2008). Accuracy of mean arterial pressure and blood pressure measurements in predicting pre-eclampsia: systematic review and meta-analysis. *BMJ (Clinical Research Ed.)*, *336*(7653), 1117–1120. article. https://doi.org/10.1136/bmj.39540.522049.BE

Cockell, A. P., Learmont, J. G., Smárason, A. K., Redman, C. W., Sargent, I. L., & Poston, L. (1997). Human placental syncytiotrophoblast microvillous membranes impair maternal vascular endothelial function. *British Journal of Obstetrics and Gynaecology*, *104*(2), 235–240. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9070146

Cohen, M., & Bischof, P. (2007). Factors regulating trophoblast invasion. *Gynecologic and Obstetric Investigation*, *64*(3), 126–130. article. https://doi.org/10.1159/000101734

Conde-Agudelo, A., Rosas-Bermúdez, A., & Kafury-Goeta, A. C. (2006). Birth spacing and risk of adverse perinatal outcomes: a meta-analysis. *JAMA*, *295*(15), 1809–1823. article. https://doi.org/10.1001/jama.295.15.1809

Confidential Enquiry into stillbirths with fetal growth restriction 2007. Perinatal Institute, 2007. Available from URL:www.pi.nhs.uk/rpnm/CE_SB_Final.pdf/.

Conover, C. A., Bale, L. K., Overgaard, M. T., Johnstone, E. W., Laursen, U. H., Füchtbauer, E.-M., van Deursen, J. (2004). Metalloproteinase pregnancy-associated plasma protein A is a critical growth regulatory factor during fetal development. *Development (Cambridge, England)*, *131*(5), 1187–1194. article. https://doi.org/10.1242/dev.00997

Cooper, C., Fall, C., Egger, P., Hobbs, R., Eastell, R., & Barker, D. (1997). Growth in infancy and bone mass in later life. *Annals of the Rheumatic Diseases*, *56*(1), 17–21. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9059135

Cooper, C., Fall, C., Egger, P., Hobbs, R., Eastell, R., & Barker, D. (1997). Growth in infancy and bone mass in later life. *Annals of the Rheumatic Diseases*, *56*(1), 17–21. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9059135

Cowans, N. J., Stamatopoulou, A., Matwejew, E., Kaisenberg, C. S. Von, & Spencer, K. (2010). First-trimester placental growth factor as a marker for hypertensive disorders and SGA †, (May), 565–570. https://doi.org/10.1002/pd

Cowans, N. J., & Spencer, K. (2007). First-trimester ADAM12 and PAPP-A as markers for intrauterine fetal growth restriction through their roles in the insulin-like growth factor system. *Prenatal Diagnosis*, *27*(3), 264–271. https://doi.org/10.1002/pd

Craven, C. M., Morgan, T., & Ward, K. (1998). Decidual spiral artery remodelling begins before cellular interaction with cytotrophoblasts. *Placenta*, *19*(4), 241–52. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9639319

Craven, C. M., Morgan, T., & Ward, K. (1998). Decidual spiral artery remodelling begins before cellular interaction with cytotrophoblasts. *Placenta*, *19*(4), 241–52. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9639319

Crovetto, F., Triunfo, S., Crispi, F., Rodriguez-Sureda, V., Dominguez, C., Figueras, F., & Gratacos, E. (2016). Differential performance of first trimester screening in predicting small for gestational age neonates or fetal growth restriction. *Ultrasound in Obstetrics {&} Gynecology : The Official Journal of the International Society of Ultrasound in Obstetrics and Gynecology*. article. https://doi.org/10.1002/uog.15919

Daayana, S., Baker, P., & Crocker, I. (2004). An image analysis technique for the investigation of variations in placental morphology in pregnancies complicated by preeclampsia with and without intrauterine growth restriction. *Journal of the Society for Gynecologic Investigation*, *11*(8), 545–552. article. https://doi.org/10.1016/j.jsgi.2004.06.009

Dane, B., Dane, C., Kiray, M., Cetin, A., Koldas, M., & Erginbas, M. (2010). Correlation between first-trimester maternal serum markers, second-trimester uterine artery Doppler indices and pregnancy outcome. *Gynecologic and Obstetric Investigation*, *70*(2), 126–131. article. https://doi.org/10.1159/000303260

D'Anna, R., Baviera, G., Corrado, F., Giordano, D., De Vivo, A., Nicocia, G., & Di Benedetto, A. (2006). Adiponectin and insulin resistance in early- and late-onset pre-eclampsia. *BJOG : An International Journal of Obstetrics and Gynaecology*, *113*(11), 1264–9. https://doi.org/10.1111/j.1471-0528.2006.01078.x

Davey Smith, G., Hart, C., Ferrell, C., Upton, M., Hole, D., Hawthorne, V., & Watt, G. (1997). Birth weight of offspring and mortality in the Renfrew and Paisley study: prospective observational study. *BMJ (Clinical Research Ed.)*, *315*(7117), 1189–1193. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9393220

Davey, D. A., & MacGillivray, I. (1988). The classification and definition of the hypertensive disorders of pregnancy. *American Journal of Obstetrics and Gynecology*, *158*(4), 892–898. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/3364501

De Falco, S. (2012). The discovery of placenta growth factor and its biological activity. *Experimental {&} Molecular Medicine*, *44*(1), 1–9. article. https://doi.org/10.3858/emm.2012.44.1.025

De Jong, C. L., Francis, A., Van Geijn, H. P., & Gardosi, J. (2000). Customized fetal weight limits for antenatal detection of fetal growth restriction. *Ultrasound in Obstetrics {&} Gynecology : The Official Journal of the International Society of Ultrasound in Obstetrics and Gynecology*, *15*(1), 36–40. article. https://doi.org/10.1046/j.1469-0705.2000.00001.x

De Onis, M., Blössner, M., & Villar, J. (1998). Levels and patterns of intrauterine growth retardation in developing countries. *European Journal of Clinical Nutrition*, *52 Suppl 1*, S5--15. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9511014

De Wolf, F., Brosens, I., & Renaer, M. (1980). Fetal growth retardation and the maternal arterial supply of the human placenta in the absence of sustained hypertension. *British Journal of Obstetrics and Gynaecology*, *87*(8), 678–685. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7426529

Demir, R. (2009). Expression of VEGF receptors VEFGR-1 and VEGFR-2, angiopoietin receptors Tie-1 and Tie-2 in chorionic villi tree during early pregnancy. *Folia Histochemica et Cytobiologica / Polish Academy of Sciences, Polish Histochemical and Cytochemical Society*, *47*(3), 435–445. article. https://doi.org/10.2478/v10042-009-0100-5

Divers, M. J., Bulmer, J. N., Miller, D., & Lilford, R. J. (1995). Beta 1 integrins in third trimester human placentae: no differential expression in pathological pregnancy. *Placenta*, *16*(3), 245–260. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7543673

Dugoff, L., Hobbins, J. C., Malone, F. D., Porter, T. F., Luthy, D., Comstock, C. H., D'Alton, M. E. (2004). First-trimester maternal serum PAPP-A and free-beta subunit human chorionic gonadotropin concentrations and nuchal translucency are associated with obstetric complications: a population-based screening study (the FASTER Trial). *American Journal of Obstetrics and Gynecology*, *191*(4), 1446–51. https://doi.org/10.1016/j.ajog.2004.06.052

Dugoff, L., Lynch, A. M., Cioffi-Ragan, D., Hobbins, J. C., Schultz, L. K., Malone, F. D., & D'Alton, M. E. (2005). First trimester uterine artery Doppler abnormalities predict subsequent intrauterine growth restriction.

American Journal of Obstetrics and Gynecology, *193*(3 Pt 2), 1208–12. https://doi.org/10.1016/j.ajog.2005.06.054

Dunk, C., Shams, M., Nijjar, S., Rhaman, M., Qiu, Y., Bussolati, B., & Ahmed, A. (2000). Angiopoietin-1 and angiopoietin-2 activate trophoblast Tie-2 to promote growth and migration during placental development. *The American Journal of Pathology*, *156*(6), 2185–2199. article. https://doi.org/10.1016/S0002-9440(10)65089-4

Egbor, M., Ansari, T., Morris, N., Green, C. J., & Sibbons, P. D. (2006). Morphometric placental villous and vascular abnormalities in early- and late-onset pre-eclampsia with and without fetal growth restriction. *BJOG : An International Journal of Obstetrics and Gynaecology*, *113*(5), 580–589. article. https://doi.org/10.1111/j.1471-0528.2006.00882.x

Egbor, M., Ansari, T., Morris, N., Green, C. J., & Sibbons, P. D. (2006). Pre-eclampsia and fetal growth restriction: how morphometrically different is the placenta? *Placenta*, *27*(6–7), 727–34. https://doi.org/10.1016/j.placenta.2005.06.002

Eide, I. P., Rolfseng, T., Isaksen, C. V, Mecsei, R., Roald, B., Lydersen, S., Austgulen, R. (2006). Serious foetal growth restriction is associated with reduced proportions of natural killer cells in decidua basalis. *Virchows Archiv : An International Journal of Pathology*, *448*(3), 269–276. article. https://doi.org/10.1007/s00428-005-0107-z

Empson, M., Lassere, M., Craig, J., & Scott, J. (2005). Prevention of recurrent miscarriage for women with antiphospholipid antibody or lupus anticoagulant. *The Cochrane Database of Systematic Reviews*, (2), CD002859. article. https://doi.org/10.1002/14651858.CD002859.pub2

Endo, H., Okamoto, A., Yamada, K., Nikaido, T., & Tanaka, T. (2005). Frequent apoptosis in placental villi from pregnancies complicated with intrauterine growth restriction and without maternal symptoms. *International Journal of Molecular Medicine*, *16*(1), 79–84. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/15942681

Erez, O., Romero, R., Espinoza, J., Fu, W., Todem, D., Kusanovic, J. P., Hassan, S. S. (2008). The change in concentrations of angiogenic and

anti-angiogenic factors in maternal plasma between the first and second trimesters in risk assessment for the subsequent development of preeclampsia and small-for-gestational age. *The Journal of Maternal-Fetal & Neonatal Medicine : The Official Journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstetricians*, *21*(5), 279–87. https://doi.org/10.1080/14767050802034545

Erlebacher, A. (2013). Immunology of the maternal-fetal interface. *Annual Review of Immunology*, *31*, 387–411. article. https://doi.org/10.1146/annurev-immunol-032712-100003

Fall, C. H., Osmond, C., Barker, D. J., Clark, P. M., Hales, C. N., Stirling, Y., & Meade, T. W. (1995). Fetal and infant growth and cardiovascular risk factors in women. *BMJ (Clinical Research Ed.)*, *310*(6977), 428–32. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7873947

Fall, C. H., Osmond, C., Barker, D. J., Clark, P. M., Hales, C. N., Stirling, Y., & Meade, T. W. (1995). Fetal and infant growth and cardiovascular risk factors in women. *BMJ (Clinical Research Ed.)*, *310*(6977), 428–32. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7873947

FMF 11-13 weeks scan online (2010) https://fetalmedicine.org/education/the-11-13-weeks-scan

Figueras, F., Figueras, J., Meler, E., Eixarch, E., Coll, O., Gratacos, E., Carbonell, X. (2007). Customised birthweight standards accurately predict perinatal morbidity. *Archives of Disease in Childhood. Fetal and Neonatal Edition*, *92*(4), F277-80. https://doi.org/10.1136/adc.2006.108621

Figueras, F., & Gardosi, J. (2011). Intrauterine growth restriction: new concepts in antenatal surveillance, diagnosis, and management. *American Journal of Obstetrics and Gynecology*, *204*(4), 288–300. article. https://doi.org/10.1016/j.ajog.2010.08.055

Figueras, F., & Gratacós, E. (2014). Update on the diagnosis and classification of fetal growth restriction and proposal of a stage-based management

protocol. *Fetal Diagnosis and Therapy*, *36*(2), 86–98. article. https://doi.org/10.1159/000357592

Filippi, E., Staughton, J., Peregrine, E., Jones, P., Huttly, W., Peebles, D. M., David, A. L. (2011). Uterine artery Doppler and adverse pregnancy outcome in women with extreme levels of fetoplacental proteins used for Down syndrome screening. *Ultrasound in Obstetrics & Gynecology : The Official Journal of the International Society of Ultrasound in Obstetrics and Gynecology*, *37*(5), 520–7. https://doi.org/10.1002/uog.8901

Filippi, E., Staughton, J., Peregrine, E., Jones, P., Huttly, W., Peebles, D. M., David, A. L. (2011). Uterine artery Doppler and adverse pregnancy outcome in women with extreme levels of fetoplacental proteins used for Down syndrome screening. *Ultrasound in Obstetrics & Gynecology : The Official Journal of the International Society of Ultrasound in Obstetrics and Gynecology*, *37*(5), 520–7. https://doi.org/10.1002/uog.8901

Flenady, V., Middleton, P., Smith, G. C., Duke, W., Erwich, J. J., Khong, T. Y., Lancet's Stillbirths Series steering committee. (2011). Stillbirths: the way forward in high-income countries. *Lancet (London, England)*, *377*(9778), 1703–1717. article. https://doi.org/10.1016/S0140-6736(11)60064-0

Foidart, J.-M., Munaut, C., Chantraine, F., Akolekar, R., & Nicolaides, K. H. (2010). Maternal plasma soluble endoglin at 11-13 weeks' gestation in pre-eclampsia. *Ultrasound in Obstetrics {&} Gynecology : The Official Journal of the International Society of Ultrasound in Obstetrics and Gynecology*, *35*(6), 680–687. article. https://doi.org/10.1002/uog.7621

Fox, H. (1970). Effect of hypoxia on trophoblast in organ culture. A morphologic and autoradiographic study. *American Journal of Obstetrics and Gynecology*, *107*(7), 1058–1064. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/5429972

Fox, N. S., & Chasen, S. T. (2009). First trimester pregnancy associated plasma protein-A as a marker for poor pregnancy outcome in patients with early-onset fetal growth restriction. *Prenatal Diagnosis*, *29*(13), 1244–1248. article. https://doi.org/10.1002/pd.2397

Fraser, R., Whitley, G. S., Johnstone, A. P., Host, A. J., Sebire, N. J., Thilaganathan, B., & Cartwright, J. E. (2012). Impaired decidual natural killer cell regulation of vascular remodelling in early human pregnancies with high uterine artery resistance. *The Journal of Pathology*, *228*(3), 322–32. https://doi.org/10.1002/path.4057

Fraser, R., Whitley, G. S., Johnstone, A. P., Host, A. J., Sebire, N. J., Thilaganathan, B., & Cartwright, J. E. (2012). Impaired decidual natural killer cell regulation of vascular remodelling in early human pregnancies with high uterine artery resistance. *The Journal of Pathology*, *228*(3), 322–32. https://doi.org/10.1002/path.4057

Gardosi, J. (2006). New definition of small for gestational age based on fetal growth potential. *Hormone Research*, *65 Suppl 3*, 15–18. article. https://doi.org/10.1159/000091501

Gardosi, J. O., Mongelli, J. M., & Mul, T. (1995). Intrauterine growth retardation. *Baillière's Clinical Obstetrics and Gynaecology*, *9*(3), 445–463. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8846549

Gardosi, J., Chang, A., Kalyan, B., Sahota, D., & Symonds, E. M. (1992). Customised antenatal growth charts. *Lancet (London, England)*, *339*(8788), 283–287. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1346292

Gardosi, J., Mongelli, M., Wilcox, M., & Chang, a. (1995). An adjustable fetal weight standard. *Ultrasound in Obstetrics & Gynecology : The Official Journal of the International Society of Ultrasound in Obstetrics and Gynecology*, *6*(3), 168–74. https://doi.org/10.1046/j.1469-0705.1995.06030168.x

Gardosi, J., & Francis, A. (2009). Adverse pregnancy outcome and association with small for gestational age birthweight by customized and population-based percentiles. *American Journal of Obstetrics and Gynecology*, *201*(1), 28.e1--8. article. https://doi.org/10.1016/j.ajog.2009.04.034

Gardosi, J., Kady, S. M., McGeown, P., Francis, A., & Tonks, A. (2005). Classification of stillbirth by relevant condition at death (ReCoDe):

population based cohort study. *BMJ (Clinical Research Ed.)*, *331*(7525), 1113–1117. article. https://doi.org/10.1136/bmj.38629.587639.7C

Gardosi, J., Madurasinghe, V., Williams, M., Malik, A., & Francis, A. (2013). Maternal and fetal risk factors for stillbirth: population based study. *BMJ (Clinical Research Ed.)*, *346*, f108. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/23349424

Gerretsen, G., Huisjes, H. J., & Elema, J. D. (1981). Morphological changes of the spiral arteries in the placental bed in relation to pre-eclampsia and fetal growth retardation. *British Journal of Obstetrics and Gynaecology*, *88*(9), 876–881. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7272259

Ghezzi, F., Tibiletti, M. G., Raio, L., Di Naro, E., Lischetti, B., Taborelli, M., & Franchi, M. (2003). Idiopathic fetal intrauterine growth restriction: a possible inheritance pattern. *Prenatal Diagnosis*, *23*(3), 259–64. https://doi.org/10.1002/pd.571

Gilbert, W. M., Young, A. L., & Danielsen, B. (2007). Pregnancy outcomes in women with chronic hypertension: a population-based study. *The Journal of Reproductive Medicine*, *52*(11), 1046–1051. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/18161404

Gluckman, P. D., Hanson, M. a, Cooper, C., & Thornburg, K. L. (2008). Effect of in utero and early-life conditions on adult health and disease. *The New England Journal of Medicine*, *359*(1), 61–73. https://doi.org/10.1056/NEJMra0708473

Gómez, O., Figueras, F., Fernández, S., Bennasar, M., Martínez, J. M., Puerto, B., & Gratacós, E. (2008). Reference ranges for uterine artery mean pulsatility index at 11-41 weeks of gestation. *Ultrasound in Obstetrics & Gynecology : The Official Journal of the International Society of Ultrasound in Obstetrics and Gynecology*, *32*(2), 128–32. https://doi.org/10.1002/uog.5315

Gordijn, S. J., Beune, I. M., Thilaganathan, B., Papageorghiou, A., Baschat, A. A., Baker, P. N., Ganzevoort, W. (2016). Consensus definition of fetal growth restriction: a Delphi procedure. *Ultrasound in Obstetrics {&}*

Gynecology : The Official Journal of the International Society of Ultrasound in Obstetrics and Gynecology, *48*(3), 333–339. article. https://doi.org/10.1002/uog.15884

Goswami, D., Tannetta, D. S., Magee, L. A., Fuchisawa, A., Redman, C. W. G., Sargent, I. L., & von Dadelszen, P. (2006). Excess syncytiotrophoblast microparticle shedding is a feature of early-onset pre-eclampsia, but not normotensive intrauterine growth restriction. *Placenta*, *27*(1), 56–61. article. https://doi.org/10.1016/j.placenta.2004.11.007

Groom, K. M., Poppe, K. K., North, R. A., & McCowan, L. M. E. (2007). Small-for-gestational-age infants classified by customized or population birthweight centiles: impact of gestational age at delivery. *American Journal of Obstetrics and Gynecology*, *197*(3), 239.e1-5. https://doi.org/10.1016/j.ajog.2007.06.038

Guller S, Ma Y, Di Santo S, Krikun G, Malek A, Schneider H. Microparticles shed from human placenta contain anti-fibrinolytic and anti-angiogenic compounds: implications in pre-eclampsia. (2007) Reprod Sci;14 (Suppl 1):219A.

Gülmezoglu, A. M., & Hofmeyr, G. J. (2000). Maternal nutrient supplementation for suspected impaired fetal growth. *The Cochrane Database of Systematic Reviews*, (2), CD000148. article. https://doi.org/10.1002/14651858.CD000148

Gülmezoglu, A. M. (2003). Promoting standards for quality of maternal health care. *British Medical Bulletin*, *67*, 73–83. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/14711755

Haapsamo, M., Martikainen, H., & Räsänen, J. (2008). Low-dose aspirin reduces uteroplacental vascular impedance in early and mid gestation in IVF and ICSI patients: a randomized, placebo-controlled double-blind study. *Ultrasound in Obstetrics {&} Gynecology : The Official Journal of the International Society of Ultrasound in Obstetrics and Gynecology*, *32*(5), 687–693. article. https://doi.org/10.1002/uog.6215

Hackman, E., Emanuel, I., van Belle, G., & Daling, J. (1983). Maternal birth weight and subsequent pregnancy outcome. *JAMA*, *250*(15), 2016–9. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/6620503

Hackman, E., Emanuel, I., van Belle, G., & Daling, J. (1983). Maternal birth weight and subsequent pregnancy outcome. *JAMA*, *250*(15), 2016–9. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/6620503

Haider, B. A., & Bhutta, Z. A. (2006). Multiple-micronutrient supplementation for women during pregnancy. *The Cochrane Database of Systematic Reviews*, (4), CD004905. article. https://doi.org/10.1002/14651858.CD004905.pub2

Hanna, J., Goldman-Wohl, D., Hamani, Y., Avraham, I., Greenfield, C., Natanson-Yaron, S., Mandelboim, O. (2006). Decidual NK cells regulate key developmental processes at the human fetal-maternal interface. *Nature Medicine*, *12*(9), 1065–1074. article. https://doi.org/10.1038/nm1452

Hare, J. W., & White, P. (1977). Pregnancy in diabetes complicated by vascular disease. *Diabetes*, *26*(10), 953–955. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/908464

Heazell, A. E. P., & Crocker, I. P. (2008). Live and let die - regulation of villous trophoblast apoptosis in normal and abnormal pregnancies. *Placenta*, *29*(9), 772–783. article. https://doi.org/10.1016/j.placenta.2008.07.003

Heazell, A. E. P., Lacey, H. A., Jones, C. J. P., Huppertz, B., Baker, P. N., & Crocker, I. P. (2008). Effects of oxygen on cell turnover and expression of regulators of apoptosis in human placental trophoblast. *Placenta*, *29*(2), 175–186. article. https://doi.org/10.1016/j.placenta.2007.11.002

Helske, S., Vuorela, P., Carpén, O., Hornig, C., Weich, H., & Halmesmäki, E. (2001). Expression of vascular endothelial growth factor receptors 1, 2 and 3 in placentas from normal and complicated pregnancies. *Molecular Human Reproduction*, *7*(2), 205–210. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11160848

Hernández-Núñez, J., & Valdés-Yong, M. (2015). Utility of proteomics in obstetric disorders: a review. *International Journal of Women's Health*, *7*, 385–391. article. https://doi.org/10.2147/IJWH.S79577

Herr, F., Liang, O. D., Herrero, J., Lang, U., Preissner, K. T., Han, V. K. M., & Zygmunt, M. (2003). Possible angiogenic roles of insulin-like growth factor II and its receptors in uterine vascular adaptation to pregnancy. *The Journal of Clinical Endocrinology and Metabolism*, *88*(10), 4811–4817. article. https://doi.org/10.1210/jc.2003-030243

Hiby, S. E., Apps, R., Sharkey, A. M., Farrell, L. E., Gardner, L., Mulder, A. Moffett, A. (2010). Maternal activating KIRs protect against human reproductive failure mediated by fetal HLA-C2. *The Journal of Clinical Investigation*, *120*(11), 4102–4110. article. https://doi.org/10.1172/JCI43998

Hiby, S. E., Walker, J. J., O'shaughnessy, K. M., Redman, C. W. G., Carrington, M., Trowsdale, J., & Moffett, A. (2004). Combinations of maternal KIR and fetal HLA-C genes influence the risk of preeclampsia and reproductive success. *The Journal of Experimental Medicine*, *200*(8), 957–965. article. https://doi.org/10.1084/jem.20041214

Higgins, J. R., Walshe, J. J., Halligan, A., O'Brien, E., Conroy, R., & Darling, M. R. (1997). Can 24-hour ambulatory blood pressure measurement predict the development of hypertension in primigravidae? *British Journal of Obstetrics and Gynaecology*, *104*(3), 356–362. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9091016

Howarth, C., Gazis, A., & James, D. (2007). Associations of Type 1 diabetes mellitus, maternal vascular disease and complications of pregnancy. *Diabetic Medicine : A Journal of the British Diabetic Association*, *24*(11), 1229–1234. article. https://doi.org/10.1111/j.1464-5491.2007.02254.x

Huang, S. J., Chen, C.-P., Schatz, F., Rahman, M., Abrahams, V. M., & Lockwood, C. J. (2008). Pre-eclampsia is associated with dendritic cell recruitment into the uterine decidua. *The Journal of Pathology*, *214*(3), 328–336. article. https://doi.org/10.1002/path.2257

Huckle, W. R., & Roche, R. I. (2004). Post-transcriptional control of expression of sFlt-1, an endogenous inhibitor of vascular endothelial growth factor. *Journal of Cellular Biochemistry*, *93*(1), 120–32. https://doi.org/10.1002/jcb.20142

Huisman, M. A., Timmer, B., Stegehuis, J., Swart, B., Aarnoudse, J. G., & Erwich, J. H. M. (2010). Vascularization in first-trimester chorionic villi in complicated and uncomplicated pregnancies. *American Journal of Obstetrics and Gynecology*, *202*(1), 88.e1-7. https://doi.org/10.1016/j.ajog.2009.08.036

Hung, T.-H., & Burton, G. J. (2006). Hypoxia and reoxygenation: a possible mechanism for placental oxidative stress in preeclampsia. *Taiwanese Journal of Obstetrics {&} Gynecology*, *45*(3), 189–200. article. https://doi.org/10.1016/S1028-4559(09)60224-2

Hunt, J. S. (n.d.). Current topic: the role of macrophages in the uterine response to pregnancy. *Placenta*, *11*(6), 467–475. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2290798

Hustin, J., Foidart, J. M., & Lambotte, R. (1983). Maternal vascular lesions in pre-eclampsia and intrauterine growth retardation: light microscopy and immunofluorescence. *Placenta*, *4 Spec No*, 489–498. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/6369298

Hutcheon, J. a, Zhang, X., Cnattingius, S., Kramer, M. S., & Platt, R. W. (2008). Customised birthweight percentiles: does adjusting for maternal characteristics matter? *BJOG : An International Journal of Obstetrics and Gynaecology*, *115*(11), 1397–404. https://doi.org/10.1111/j.1471-0528.2008.01870.x

Idris, I., Srinivasan, R., Simm, A., & Page, R. C. (2005). Maternal hypothyroidism in early and late gestation: effects on neonatal and obstetric outcome. *Clinical Endocrinology*, *63*(5), 560–5. https://doi.org/10.1111/j.1365-2265.2005.02382.x

Imdad, A., Yakoob, M. Y., Siddiqui, S., & Bhutta, Z. A. (2011). Screening and triage of intrauterine growth restriction (IUGR) in general population and high risk pregnancies: a systematic review with a focus on reduction of

IUGR related stillbirths. *BMC Public Health*, *11 Suppl 3*, S1. https://doi.org/10.1186/1471-2458-11-S3-S1

Irgens, H. U., Reisaeter, L., Irgens, L. M., & Lie, R. T. (2001). Long term mortality of mothers and fathers after pre-eclampsia: population based cohort study. *BMJ (Clinical Research Ed.)*, *323*(7323), 1213–1217. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11719411

Irwin, J. C., Suen, L. F., Martina, N. A., Mark, S. P., & Giudice, L. C. (1999). Role of the IGF system in trophoblast invasion and pre-eclampsia. *Human Reproduction (Oxford, England)*, *14 Suppl 2*, 90–96. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10690804

Jackson, R. A., Gibson, K. A., Wu, Y. W., & Croughan, M. S. (2004). Perinatal outcomes in singletons following in vitro fertilization: a meta-analysis. *Obstetrics and Gynecology*, *103*(3), 551–563. article. https://doi.org/10.1097/01.AOG.0000114989.84822.51

Jaddoe, V. W. V, Bakker, R., Hofman, A., Mackenbach, J. P., Moll, H. A., Steegers, E. A. P., & Witteman, J. C. M. (2007). Moderate alcohol consumption during pregnancy and the risk of low birth weight and preterm birth. The generation R study. *Annals of Epidemiology*, *17*(10), 834–840. article. https://doi.org/10.1016/j.annepidem.2007.04.001

Jaquet, D., Swaminathan, S., Alexander, G. R., Czernichow, P., Collin, D., Salihu, H. M., Lévy-Marchal, C. (2005). Significant paternal contribution to the risk of small for gestational age. *BJOG : An International Journal of Obstetrics and Gynaecology*, *112*(2), 153–159. article. https://doi.org/10.1111/j.1471-0528.2004.00313.x

Jauniaux, E., Watson, A. L., Hempstock, J., Bao, Y. P., Skepper, J. N., & Burton, G. J. (2000). Onset of maternal arterial blood flow and placental oxidative stress. A possible factor in human early pregnancy failure. *The American Journal of Pathology*, *157*(6), 2111–2122. article. https://doi.org/10.1016/S0002-9440(10)64849-3

Jeyabalan, a, McGonigal, S., Gilmour, C., Hubel, C. a, & Rajakumar, a. (2008). Circulating and placental endoglin concentrations in pregnancies

complicated by intrauterine growth restriction and preeclampsia. *Placenta*, *29*(6), 555–63. https://doi.org/10.1016/j.placenta.2008.03.006

Jolly, M. C., Sebire, N., Harris, J., Robinson, S., & Regan, L. (2000). Obstetric risks of pregnancy in women less than 18 years old. *Obstetrics and Gynecology*, *96*(6), 962–966. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11084186

Jolly, M., Sebire, N., Harris, J., Robinson, S., & Regan, L. (2000). The risks associated with pregnancy in women aged 35 years or older. *Human Reproduction (Oxford, England)*, *15*(11), 2433–2437. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11056148

Jurkovic, D., Jauniaux, E., Kurjak, A., Hustin, J., Campbell, S., & Nicolaides, K. H. (1991). Transvaginal color Doppler assessment of the uteroplacental circulation in early pregnancy. *Obstetrics and Gynecology*, *77*(3), 365–369. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1992400

Kagan, K. O., Wright, D., Baker, A., Sahota, D., & Nicolaides, K. H. (2008). Screening for trisomy 21 by maternal age, fetal nuchal translucency thickness, free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A. *Ultrasound in Obstetrics {&} Gynecology : The Official Journal of the International Society of Ultrasound in Obstetrics and Gynecology*, *31*(6), 618–624. article. https://doi.org/10.1002/uog.5331

Kagan, K. O., Wright, D., Spencer, K., Molina, F. S., & Nicolaides, K. H. (2008). First-trimester screening for trisomy 21 by free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A: impact of maternal and pregnancy characteristics. *Ultrasound in Obstetrics {&} Gynecology : The Official Journal of the International Society of Ultrasound in Obstetrics and Gynecology*, *31*(5), 493–502. article. https://doi.org/10.1002/uog.5332

Kam, E. P., Gardner, L., Loke, Y. W., & King, A. (1999). The role of trophoblast in the physiological change in decidual spiral arteries. *Human Reproduction (Oxford, England)*, *14*(8), 2131–2138. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10438439

Kaminopetros, P., Higueras, M. T., & Nicolaides, K. H. (1991). Doppler study of uterine artery blood flow: comparison of findings in the first and second trimesters of pregnancy. *Fetal Diagnosis and Therapy*, *6*(1–2), 58–64. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1768347

Karagiannis, G., Akolekar, R., Sarquis, R., Wright, D., & Nicolaides, K. H. (2011). Prediction of small-for-gestation neonates from biophysical and biochemical markers at 11-13 weeks. *Fetal Diagnosis and Therapy*, *29*(2), 148–154. article. https://doi.org/10.1159/000321694

Karamessinis, P. M., Malamitsi-Puchner, A., Boutsikou, T., Makridakis, M., Vougas, K., Fountoulakis, M., Chrousos, G. (2008). Marked defects in the expression and glycosylation of alpha2-HS glycoprotein/fetuin-A in plasma from neonates with intrauterine growth restriction: proteomics screening and potential clinical implications. *Molecular {&} Cellular Proteomics : MCP*, *7*(3), 591–599. article. https://doi.org/10.1074/mcp.M700422-MCP200

Karamysheva, A. F. (2008). Mechanisms of angiogenesis. *Biochemistry. Biokhimiia*, *73*(7), 751–762. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/18707583

Kaufmann P, Castellucci M. Extravillous trophoblast in the human placenta. (1997) Trophoblast Res; 10:21–65. "

Kaufmann, P., Black, S., & Huppertz, B. (2003). Endovascular trophoblast invasion: implications for the pathogenesis of intrauterine growth retardation and preeclampsia. *Biol. Reprod*, *69*, 1–7doi101095/biolreprod102014977.

Kayisli, U. A., Cayli, S., Seval, Y., Tertemiz, F., Huppertz, B., & Demir, R. (n.d.). Spatial and temporal distribution of Tie-1 and Tie-2 during very early development of the human placenta. *Placenta*, *27*(6–7), 648–659. article. https://doi.org/10.1016/j.placenta.2005.05.013

Kelekci, S., Yilmaz, B., Savan, K., & Sonmez, S. (2005). Can increased nuchal translucency in the first trimester of pregnancy predict gestational diabetes mellitus. *Journal of Obstetrics and Gynaecology : The Journal of*

the Institute of Obstetrics and Gynaecology, *25*(6), 579–582. article. https://doi.org/10.1080/01443610500231518

Khong, T. Y., De Wolf, F., Robertson, W. B., & Brosens, I. (1986). Inadequate maternal vascular response to placentation in pregnancies complicated by pre-eclampsia and by small-for-gestational age infants. *British Journal of Obstetrics and Gynaecology*, *93*(10), 1049–59. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/3790464

Khong, T. Y., Liddell, H. S., & Robertson, W. B. (1987). Defective haemochorial placentation as a cause of miscarriage: a preliminary study. *British Journal of Obstetrics and Gynaecology*, *94*(7), 649–655. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/3620413

Kim, J.-S., Romero, R., Cushenberry, E., Kim, Y. M., Erez, O., Nien, J. K., Kim, C. J. (n.d.). Distribution of CD14+ and CD68+ macrophages in the placental bed and basal plate of women with preeclampsia and preterm labor. *Placenta*, *28*(5–6), 571–576. article. https://doi.org/10.1016/j.placenta.2006.07.007

Kim, Y. N., Lee, D. S., Jeong, D. H., Sung, M. S., & Kim, K. T. (2009). The relationship of the level of circulating antiangiogenic factors to the clinical manifestations of preeclampsia. *Prenatal Diagnosis*, *29*(5), 464–470. article. https://doi.org/10.1002/pd.2203

Klebanoff, M. A., Mednick, B. R., Schulsinger, C., Secher, N. J., & Shiono, P. H. (1998). Father's effect on infant birth weight. *American Journal of Obstetrics and Gynecology*, *178*(5), 1022–1026. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9609578

Kleijer, M. E., Dekker, G. A., & Heard, A. R. (2005). Risk factors for intrauterine growth restriction in a socio-economically disadvantaged region. *The Journal of Maternal-Fetal {&} Neonatal Medicine : The Official Journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstetricians*, *18*(1), 23–30. article. https://doi.org/10.1080/14767050500127674

Koopman, L. A., Kopcow, H. D., Rybalov, B., Boyson, J. E., Orange, J. S., Schatz, F., Strominger, J. L. (2003). Human decidual natural killer cells are a unique NK cell subset with immunomodulatory potential. *The Journal of Experimental Medicine*, *198*(8), 1201–1212. article. https://doi.org/10.1084/jem.20030305

Korteweg, F. J., Gordijn, S. J., Timmer, A., Holm, J. P., Ravisé, J. M., & Erwich, J. J. H. M. (2008). A placental cause of intra-uterine fetal death depends on the perinatal mortality classification system used. *Placenta*, *29*(1), 71–80. article. https://doi.org/10.1016/j.placenta.2007.07.003

Koudstaal, J., Braat, D. D., Bruinse, H. W., Naaktgeboren, N., Vermeiden, J. P., & Visser, G. H. (2000). Obstetric outcome of singleton pregnancies after IVF: a matched control study in four Dutch university hospitals. *Human Reproduction (Oxford, England)*, *15*(8), 1819–1825. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10920110

Kramer, M. S. (1987). Determinants of low birth weight: methodological assessment and meta-analysis. *Bulletin of the World Health Organization*, *65*(5), 663–737. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/3322602

Kramer, M. S., Platt, R., Yang, H., McNamara, H., & Usher, R. H. (1999). Are all growth-restricted newborns created equal(ly)? *Pediatrics*, *103*(3), 599–602. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10049963

Krantz, D., Goetzl, L., Simpson, J. L., Thom, E., Zachary, J., Hallahan, T. W., First Trimester Maternal Serum Biochemistry and Fetal Nuchal Translucency Screening (BUN) Study Group. (2004). Association of extreme first-trimester free human chorionic gonadotropin-beta, pregnancy-associated plasma protein A, and nuchal translucency with intrauterine growth restriction and other adverse pregnancy outcomes. *American Journal of Obstetrics and Gynecology*, *191*(4), 1452–8. https://doi.org/10.1016/j.ajog.2004.05.068

Krantz, D., Goetzl, L., Simpson, J. L., Thom, E., Zachary, J., Hallahan, T. W., First Trimester Maternal Serum Biochemistry and Fetal Nuchal Translucency Screening (BUN) Study Group. (2004). Association of

extreme first-trimester free human chorionic gonadotropin-beta, pregnancy-associated plasma protein A, and nuchal translucency with intrauterine growth restriction and other adverse pregnancy outcomes. *American Journal of Obstetrics and Gynecology*, *191*(4), 1452–8. https://doi.org/10.1016/j.ajog.2004.05.068

Krulewitch, C. J., Herman, A. A., Yu, K. F., & Johnson, Y. R. (1997). Does changing paternity contribute to the risk of intrauterine growth retardation? *Paediatric and Perinatal Epidemiology*, *11 Suppl 1*, 41–47. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9018714

Kwik, M., & Morris, J. (2003). Association between first trimester maternal serum pregnancy associated plasma protein-A and adverse pregnancy outcome. *The Australian {&} New Zealand Journal of Obstetrics {&} Gynaecology*, *43*(6), 438–442. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/14712947

Lackman, F., Capewell, V., Gagnon, R., & Richardson, B. (2001). Fetal umbilical cord oxygen values and birth to placental weight ratio in relation to size at birth. *American Journal of Obstetrics and Gynecology*, *185*(3), 674–682. article. https://doi.org/10.1067/mob.2001.116686

Langer, O., Levy, J., Brustman, L., Anyaegbunam, A., Merkatz, R., & Divon, M. (1989). Glycemic control in gestational diabetes mellitus--how tight is tight enough: small for gestational age versus large for gestational age? *American Journal of Obstetrics and Gynecology*, *161*(3), 646–653. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2782347

Larsen, T., Petersen, S., Greisen, G., & Larsen, J. F. (1990). Normal fetal growth evaluated by longitudinal ultrasound examinations. *Early Human Development*, *24*(1), 37–45. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2265597

Law, K. P., Han, T.-L., Tong, C., & Baker, P. N. (2015). Mass spectrometry-based proteomics for pre-eclampsia and preterm birth. *International Journal of Molecular Sciences*, *16*(5), 10952–10985. article. https://doi.org/10.3390/ijms160510952

Law, L. W., Leung, T. Y., Sahota, D. S., Chan, L. W., Fung, T. Y., & Lau, T. K. (2009). Which ultrasound or biochemical markers are independent predictors of small-for-gestational age? *Ultrasound in Obstetrics & Gynecology : The Official Journal of the International Society of Ultrasound in Obstetrics and Gynecology*, *34*(3), 283–7. https://doi.org/10.1002/uog.6455

Lawrence, J. B., Oxvig, C., Overgaard, M. T., Sottrup-Jensen, L., Gleich, G. J., Hays, L. G., Conover, C. a. (1999). The insulin-like growth factor (IGF)-dependent IGF binding protein-4 protease secreted by human fibroblasts is pregnancy-associated plasma protein-A. *Proceedings of the National Academy of Sciences of the United States of America*, *96*(6), 3149–53. Retrieved from http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=15910&tool=pmcentrez&rendertype=abstract

Lei, Z. M., Reshef, E., & Rao, V. (1992). The expression of human chorionic gonadotropin/luteinizing hormone receptors in human endometrial and myometrial blood vessels. *The Journal of Clinical Endocrinology and Metabolism*, *75*(2), 651–659. article. https://doi.org/10.1210/jcem.75.2.1379262

Leung, a S., Millar, L. K., Koonings, P. P., Montoro, M., & Mestman, J. H. (1993). Perinatal outcome in hypothyroid pregnancies. *Obstetrics and Gynecology*, *81*(3), 349–53. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8437784

Leung, D. N., Smith, S. C., To, K. F., Sahota, D. S., & Baker, P. N. (2001). Increased placental apoptosis in pregnancies complicated by preeclampsia. *American Journal of Obstetrics and Gynecology*, *184*(6), 1249–1250. article. https://doi.org/10.1067/mob.2001.112906

Levine, R. J., Lam, C., Qian, C., Yu, K. F., Maynard, S. E., Sachs, B. P., Karumanchi, S. A. (2006). Soluble endoglin and other circulating antiangiogenic factors in preeclampsia. *The New England Journal of Medicine*, *355*(10), 992–1005. https://doi.org/10.1056/NEJMoa055352

Levine, R. J., Thadhani, R., Qian, C., Lam, C., Lim, K.-H., Yu, K. F., Karumanchi, S. A. (2005). Urinary placental growth factor and risk of

preeclampsia. *JAMA*, *293*(1), 77–85. article. https://doi.org/10.1001/jama.293.1.77

Levine, R. J., Vatten, L. J., Horowitz, G. L., Qian, C., Romundstad, P. R., Yu, K. F., Karumanchi, S. A. (2009). Pre-eclampsia, soluble fms-like tyrosine kinase 1, and the risk of reduced thyroid function: nested case-control and population based study. *BMJ (Clinical Research Ed.)*, *339*, b4336. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/19920004

Levy, R., & Nelson, D. M. (2000). To be, or not to be, that is the question. Apoptosis in human trophoblast. *Placenta*, *21*(1), 1–13. article. https://doi.org/10.1053/plac.1999.0450

Levy, R., Smith, S. D., Chandler, K., Sadovsky, Y., & Nelson, D. M. (2000). Apoptosis in human cultured trophoblasts is enhanced by hypoxia and diminished by epidermal growth factor. *American Journal of Physiology. Cell Physiology*, *278*(5), C982--8. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10794672

Lieppman, R. E., Williams, M. A., Cheng, E. Y., Resta, R., Zingheim, R., Hickok, D. E., & Luthy, D. A. (1993). An association between elevated levels of human chorionic gonadotropin in the midtrimester and adverse pregnancy outcome. *American Journal of Obstetrics and Gynecology*, *168*(6 Pt 1), 1852–1857. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8317532

Lim, J. H., Kim, S. Y., Park, S. Y., Lee, M. H., Yang, J. H., Kim, M. Y., Ryu, H. M. (2009). Soluble endoglin and transforming growth factor-beta1 in women who subsequently developed preeclampsia. *Prenatal Diagnosis*, *29*(5), 471–476. article. https://doi.org/10.1002/pd.2217

Lindley, A. A., Becker, S., Gray, R. H., & Herman, A. A. (2000). Effect of continuing or stopping smoking during pregnancy on infant birth weight, crown-heel length, head circumference, ponderal index, and brain:body weight ratio. *American Journal of Epidemiology*, *152*(3), 219–225. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10933268

Lindqvist, P. G., & Molin, J. (2005). Does antenatal identification of small-for-gestational age fetuses significantly improve their outcome? *Ultrasound*

in Obstetrics {&} Gynecology : The Official Journal of the International Society of Ultrasound in Obstetrics and Gynecology, *25*(3), 258–264. article. https://doi.org/10.1002/uog.1806

Lockwood, C. J., Romero, R., Feinberg, R. F., Clyne, L. P., Coster, B., & Hobbins, J. C. (1989). The prevalence and biologic significance of lupus anticoagulant and anticardiolipin antibodies in a general obstetric population. *American Journal of Obstetrics and Gynecology*, *161*(2), 369–373. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2504043

Lockwood, C. J., Matta, P., Krikun, G., Koopman, L. A., Masch, R., Toti, P., Schatz, F. (2006). Regulation of monocyte chemoattractant protein-1 expression by tumor necrosis factor-alpha and interleukin-1beta in first trimester human decidual cells: implications for preeclampsia. *The American Journal of Pathology*, *168*(2), 445–452. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/16436659

Lumley, J., Chamberlain, C., Dowswell, T., Oliver, S., Oakley, L., & Watson, L. (2009). Interventions for promoting smoking cessation during pregnancy. *The Cochrane Database of Systematic Reviews*, (3), CD001055. article. https://doi.org/10.1002/14651858.CD001055.pub3

Luttun, A., Tjwa, M., & Carmeliet, P. (2002). Placental growth factor (PlGF) and its receptor Flt-1 (VEGFR-1): novel therapeutic targets for angiogenic disorders. *Annals of the New York Academy of Sciences*, *979*, 80–93. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12543719

Lyall, F., Bulmer, J. N., Duffie, E., Cousins, F., Theriault, A., & Robson, S. C. (2001). Human trophoblast invasion and spiral artery transformation: the role of PECAM-1 in normal pregnancy, preeclampsia, and fetal growth restriction. *The American Journal of Pathology*, *158*(5), 1713–1721. article. https://doi.org/10.1016/S0002-9440(10)64127-2

Lynch, A., Marlar, R., Murphy, J., Davila, G., Santos, M., Rutledge, J., & Emlen, W. (1994). Antiphospholipid antibodies in predicting adverse pregnancy outcome. A prospective study. *Annals of Internal Medicine*, *120*(6), 470–475. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8093135

M Kady, S., & Gardosi, J. (2004). Perinatal mortality and fetal growth restriction. *Best Practice {&} Research. Clinical Obstetrics {&} Gynaecology*, *18*(3), 397–410. article. https://doi.org/10.1016/j.bpobgyn.2004.02.009

Magnus, P., Bakketeig, L. S., & Skjaerven, R. (n.d.). Correlations of birth weight and gestational age across generations. *Annals of Human Biology*, *20*(3), 231–8. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8489198

Magnus, P., Bakketeig, L. S., & Skjaerven, R. (n.d.). Correlations of birth weight and gestational age across generations. *Annals of Human Biology*, *20*(3), 231–8. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8489198

Magnus, P., Gjessing, H. K., Skrondal, A., & Skjaerven, R. (2001). Paternal contribution to birth weight. *Journal of Epidemiology and Community Health*, *55*(12), 873–877. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11707480

Manaster, I., & Mandelboim, O. (2010). The unique properties of uterine NK cells. *American Journal of Reproductive Immunology (New York, N.Y.: 1989)*, *63*(6), 434–444. article. https://doi.org/10.1111/j.1600-0897.2009.00794.x

Mandruzzato, G., Antsaklis, A., Botet, F., Chervenak, F. A., Figueras, F., Grunebaum, A., WAPM. (2008). Intrauterine restriction (IUGR). *Journal of Perinatal Medicine*, *36*(4), 277–281. article. https://doi.org/10.1515/JPM.2008.050

Martin, a M., Bindra, R., Curcio, P., Cicero, S., & Nicolaides, K. H. (2001). Screening for pre-eclampsia and fetal growth restriction by uterine artery Doppler at 11-14 weeks of gestation. *Ultrasound in Obstetrics & Gynecology : The Official Journal of the International Society of Ultrasound in Obstetrics and Gynecology*, *18*(6), 583–6. https://doi.org/10.1046/j.0960-7692.2001.00594.x

Maruo, T., Matsuo, H., & Mochizuki, M. (1991). Thyroid hormone as a biological amplifier of differentiated trophoblast function in early

pregnancy. *Acta Endocrinologica*, *125*(1), 58–66. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1872126

Masuyama, H., Nakatsukasa, H., Takamoto, N., & Hiramatsu, Y. (2007). Correlation between soluble endoglin, vascular endothelial growth factor receptor-1, and adipocytokines in preeclampsia. *The Journal of Clinical Endocrinology and Metabolism*, *92*(7), 2672–2679. article. https://doi.org/10.1210/jc.2006-2349

Matijevic, R., Meekins, J. W., Walkinshaw, S. A., Neilson, J. P., & McFadyen, I. R. (1995). Spiral artery blood flow in the central and peripheral areas of the placental bed in the second trimester. *Obstetrics and Gynecology*, *86*(2), 289–292. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7617363

Matwejew, E., Cowans, N. J., Stamatopoulou, a, Spencer, K., & von Kaisenberg, C. S. (2010). Maternal serum ADAM-12 as a potential marker for different adverse pregnancy outcomes. *Fetal Diagnosis and Therapy*, *27*(1), 32–9. https://doi.org/10.1159/000275669

Mayhew, T. M., Wijesekara, J., Baker, P. N., & Ong, S. S. (2004). Morphometric evidence that villous development and fetoplacental angiogenesis are compromised by intrauterine growth restriction but not by pre-eclampsia. *Placenta*, *25*(10), 829–833. article. https://doi.org/10.1016/j.placenta.2004.04.011

Maynard, S. E., Moore Simas, T. A., Solitro, M. J., Rajan, A., Crawford, S., Soderland, P., & Meyer, B. A. (2008). Circulating angiogenic factors in singleton vs multiple-gestation pregnancies. *American Journal of Obstetrics and Gynecology*, *198*(2), 200.e1--7. article. https://doi.org/10.1016/j.ajog.2007.08.042

McCowan, L. M., Buist, R. G., North, R. A., & Gamble, G. (1996). Perinatal morbidity in chronic hypertension. *British Journal of Obstetrics and Gynaecology*, *103*(2), 123–129. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8616127

McCowan, L. M. E., Dekker, G. A., Chan, E., Stewart, A., Chappell, L. C., Hunter, M., consortium, S. (2009). Spontaneous preterm birth and small

for gestational age infants in women who stop smoking early in pregnancy: prospective cohort study. *BMJ (Clinical Research Ed.)*, *338*, b1081. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/19325177

McDonald, S. D., Han, Z., Mulla, S., Murphy, K. E., Beyene, J., Ohlsson, A., & Knowledge Synthesis Group. (2009). Preterm birth and low birth weight among in vitro fertilization singletons: a systematic review and meta-analyses. *European Journal of Obstetrics, Gynecology, and Reproductive Biology*, *146*(2), 138–148. article. https://doi.org/10.1016/j.ejogrb.2009.05.035

McIntire, D. D., Bloom, S. L., Casey, B. M., & Leveno, K. J. (1999). Birth weight in relation to morbidity and mortality among newborn infants. *The New England Journal of Medicine*, *340*(16), 1234–8. https://doi.org/10.1056/NEJM199904223401603

McKenna, D., Tharmaratnam, S., Mahsud, S., Bailie, C., Harper, A., & Dornan, J. (2003). A randomized trial using ultrasound to identify the high-risk fetus in a low-risk population. *Obstetrics and Gynecology*, *101*(4), 626–632. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12681862

Melchiorre, K., Leslie, K., Prefumo, F., Bhide, A., & Thilaganathan, B. (2009). First-trimester uterine artery Doppler indices in the prediction of small-for-gestational age pregnancy and intrauterine growth restriction. *Ultrasound in Obstetrics & Gynecology : The Official Journal of the International Society of Ultrasound in Obstetrics and Gynecology*, *33*(5), 524–9. https://doi.org/10.1002/uog.6368

Merviel, P., Challier, J. C., Carbillon, L., Foidart, J. M., & Uzan, S. (n.d.). The role of integrins in human embryo implantation. *Fetal Diagnosis and Therapy*, *16*(6), 364–371. article. https://doi.org/53942

Meyer, M. B., & Comstock, G. W. (1972). Maternal cigarette smoking and perinatal mortality. *American Journal of Epidemiology*, *96*(1), 1–10. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/5039725

Mikolajczyk, R. T., Zhang, J., Betran, A. P., Souza, J. P., Mori, R., Gülmezoglu, A. M., & Merialdi, M. (2011). A global reference for fetal-weight and

birthweight percentiles. *Lancet (London, England)*, *377*(9780), 1855–1861. article. https://doi.org/10.1016/S0140-6736(11)60364-4

Miller, J., Turan, S., & Baschat, A. A. (2008). Fetal growth restriction. *Seminars in Perinatology*, *32*(4), 274–280. article. https://doi.org/10.1053/j.semperi.2008.04.010

Mills, J. L., Graubard, B. I., Harley, E. E., Rhoads, G. G., & Berendes, H. W. (1984). Maternal alcohol consumption and birth weight. How much drinking during pregnancy is safe? *JAMA*, *252*(14), 1875–1879. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/6471316

Mitchell, M. L. (2001). Fetal brain to liver weight ratio as a measure of intrauterine growth retardation: analysis of 182 stillborn autopsies. *Modern Pathology : An Official Journal of the United States and Canadian Academy of Pathology, Inc*, *14*(1), 14–9. https://doi.org/10.1038/modpathol.3880251

Moldenhauer, J. S., Stanek, J., Warshak, C., Khoury, J., & Sibai, B. (2003). The frequency and severity of placental findings in women with preeclampsia are gestational age dependent. *American Journal of Obstetrics and Gynecology*, *189*(4), 1173–1177. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/14586374

Mongelli, M., & Gardosi, J. (1996). Reduction of false-positive diagnosis of fetal growth restriction by application of customized fetal growth standards. *Obstetrics and Gynecology*, *88*(5), 844–848. article. https://doi.org/10.1016/0029-7844(96)00285-2

Montanari, L., Alfei, A., Albonico, G., Moratti, R., Arossa, A., Beneventi, F., & Spinillo, A. (2009). The impact of first-trimester serum free beta-human chorionic gonadotropin and pregnancy-associated plasma protein A on the diagnosis of fetal growth restriction and small for gestational age infant. *Fetal Diagnosis and Therapy*, *25*(1), 130–5. https://doi.org/10.1159/000207554

Montanari, L., Alfei, A., Albonico, G., Moratti, R., Arossa, A., Beneventi, F., & Spinillo, A. (2009). The impact of first-trimester serum free beta-human chorionic gonadotropin and pregnancy-associated plasma protein A on

the diagnosis of fetal growth restriction and small for gestational age infant. *Fetal Diagnosis and Therapy*, *25*(1), 130–5. https://doi.org/10.1159/000207554

Mook-Kanamori, D. O., Steegers, E. a P., Eilers, P. H., Raat, H., Hofman, A., & Jaddoe, V. W. V. (2010). Risk factors and outcomes associated with first-trimester fetal growth restriction. *JAMA : The Journal of the American Medical Association*, *303*(6), 527–34. https://doi.org/10.1001/jama.2010.78

Morris, R. K., Cnossen, J. S., Langejans, M., Robson, S. C., Kleijnen, J., Ter Riet, G., Khan, K. S. (2008). Serum screening with Down's syndrome markers to predict pre-eclampsia and small for gestational age: systematic review and meta-analysis. *BMC Pregnancy and Childbirth*, *8*, 33. https://doi.org/10.1186/1471-2393-8-33

Morris, R. K., Oliver, E. A., Malin, G., Khan, K. S., & Meads, C. (2013). Effectiveness of interventions for the prevention of small-for-gestational age fetuses and perinatal mortality: a review of systematic reviews. *Acta Obstetricia et Gynecologica Scandinavica*, *92*(2), 143–151. article. https://doi.org/10.1111/aogs.12029

Moutquin, J. M., Rainville, C., Giroux, L., Raynauld, P., Amyot, G., Bilodeau, R., & Pelland, N. (1985). A prospective study of blood pressure in pregnancy: prediction of preeclampsia. *American Journal of Obstetrics and Gynecology*, *151*(2), 191–196. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/3970084

National Heart Foundation of Australia. 2004. Hypertension Management Guide for Doctors. http://www.heartfoundation.org.au.

Napolitano, R., Rajakulasingam, R., Memmo, A., Bhide, A., & Thilaganathan, B. (2011). Uterine artery Doppler screening for pre-eclampsia: comparison of the lower, mean and higher first-trimester pulsatility indices. *Ultrasound in Obstetrics {&} Gynecology : The Official Journal of the International Society of Ultrasound in Obstetrics and Gynecology*, *37*(5), 534–537. article. https://doi.org/10.1002/uog.8848

Neilson, J. P. (2000). Symphysis-fundal height measurement in pregnancy. *The Cochrane Database of Systematic Reviews*, (2), CD000944. article. https://doi.org/10.1002/14651858.CD000944

Ness, R. B., & Sibai, B. M. (2006). Shared and disparate components of the pathophysiologies of fetal growth restriction and preeclampsia. *American Journal of Obstetrics and Gynecology*, *195*(1), 40–9. https://doi.org/10.1016/j.ajog.2005.07.049

Ness, R. B., & Sibai, B. M. (2006). Shared and disparate components of the pathophysiologies of fetal growth restriction and preeclampsia. *American Journal of Obstetrics and Gynecology*, *195*(1), 40–9. https://doi.org/10.1016/j.ajog.2005.07.049

Nevo, O., Soleymanlou, N., Wu, Y., Xu, J., Kingdom, J., Many, A., Caniggia, I. (2006). Increased expression of sFlt-1 in in vivo and in vitro models of human placental hypoxia is mediated by HIF-1. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, *291*(4), R1085--93. article. https://doi.org/10.1152/ajpregu.00794.2005

Newhouse, S. M., Davidge, S. T., Winkler-Lowen, B., Demianczuk, N., & Guilbert, L. J. (2007). In vitro differentiation of villous trophoblasts from pregnancies complicated by intrauterine growth restriction with and without pre-eclampsia. *Placenta*, *28*(10), 999–1003. article. https://doi.org/10.1016/j.placenta.2007.04.008

NHS FASP Condensed Education Module for Trisomy 21 resource, and the NHS FASP NT resource, http://cpd.screening.nhs.uk/fasp-elearning#fileid12012

Nicolaides, K. H., Bindra, R., Turan, O. M., Chefetz, I., Sammar, M., Meiri, H., Cuckle, H. S. (2006). A novel approach to first-trimester screening for early pre-eclampsia combining serum PP-13 and Doppler ultrasound. *Ultrasound in Obstetrics {&} Gynecology : The Official Journal of the International Society of Ultrasound in Obstetrics and Gynecology*, *27*(1), 13–17. article. https://doi.org/10.1002/uog.2686

Nicolaides, K. H. (2011). Turning the pyramid of prenatal care. *Fetal Diagnosis and Therapy*, *29*(3), 183–96. https://doi.org/10.1159/000324320

Niswander, K., & Jackson, E. C. (1974). Physical characteristics of the gravida and their association with birth weight and perinatal death. *American Journal of Obstetrics and Gynecology*, *119*(3), 306–313. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/4827373

Odibo, A. O., Nelson, D., Stamilio, D. M., Sehdev, H. M., & Macones, G. A. (2006). Advanced maternal age is an independent risk factor for intrauterine growth restriction. *American Journal of Perinatology*, *23*(5), 325–328. article. https://doi.org/10.1055/s-2006-947164

Office for National Statistics (2011). Death registration summary tables, England & Wales, 2010. 2012. www.ons.gov.uk/ons/publications/re-reference-tables.html?edition=tcm% 3A77-230730."

O'Gorman, N., Wright, D., Rolnik, D. L., Nicolaides, K. H., & Poon, L. C. (2016). Study protocol for the randomised controlled trial: combined multimarker screening and randomised patient treatment with ASpirin for evidence-based PREeclampsia prevention (ASPRE). *BMJ Open*, *6*(6), e011801. article. https://doi.org/10.1136/bmjopen-2016-011801

Oki, N., Matsuo, H., Nakago, S., Murakoshi, H., Laoag-Fernandez, J. B., & Maruo, T. (2004). Effects of 3,5,3'-triiodothyronine on the invasive potential and the expression of integrins and matrix metalloproteinases in cultured early placental extravillous trophoblasts. *The Journal of Clinical Endocrinology and Metabolism*, *89*(10), 5213–5221. article. https://doi.org/10.1210/jc.2004-0352

Ong, C. Y., Liao, a W., Cacho, a M., Spencer, K., & Nicolaides, K. H. (2001). First-trimester maternal serum levels of placenta growth factor as predictor of preeclampsia and fetal growth restriction. *Obstetrics and Gynecology*, *98*(4), 608–11. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11576576

Ong, C. Y., Liao, A. W., Spencer, K., Munim, S., & Nicolaides, K. H. (2000). First trimester maternal serum free beta human chorionic gonadotrophin and pregnancy associated plasma protein A as predictors of pregnancy complications. *BJOG : An International Journal of Obstetrics and Gynaecology*, *107*(10), 1265–1270. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11028579

Owen, P., Donnet, M. L., Ogston, S. A., Christie, A. D., Howie, P. W., & Patel, N. B. (1996). Standards for ultrasound fetal growth velocity. *British Journal of Obstetrics and Gynaecology*, *103*(1), 60–69. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8608100

Pallotto, E. K., & Kilbride, H. W. (2006). Perinatal outcome and later implications of intrauterine growth restriction. *Clinical Obstetrics and Gynecology*, *49*(2), 257–69. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/16721105

Papastefanou, I., Souka, A. P., Pilalis, A., Eleftheriades, M., Michalitsi, V., & Kassanos, D. (2012). First trimester prediction of small- and large-for-gestation neonates by an integrated model incorporating ultrasound parameters, biochemical indices and maternal characteristics. *Acta Obstetricia et Gynecologica Scandinavica*, *91*(1), 104–111. article. https://doi.org/10.1111/j.1600-0412.2011.01271.x

Pearce, J. M., & Campbell, S. (1987). A comparison of symphysis-fundal height and ultrasound as screening tests for light-for-gestational age infants. *British Journal of Obstetrics and Gynaecology*, *94*(2), 100–104. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/3548802

Pennington, K. A., Schlitt, J. M., Jackson, D. L., Schulz, L. C., & Schust, D. J. (2012). Preeclampsia: multiple approaches for a multifactorial disease. *Disease Models & Mechanisms*, *5*(1), 9–18. https://doi.org/10.1242/dmm.008516

Pennington, K. A., Schlitt, J. M., Jackson, D. L., Schulz, L. C., & Schust, D. J. (2012). Preeclampsia: multiple approaches for a multifactorial disease. *Disease Models & Mechanisms*, *5*(1), 9–18. https://doi.org/10.1242/dmm.008516

Persson, B., Stangenberg, M., Lunell, N. O., Brodin, U., Holmberg, N. G., & Vaclavinkova, V. (1986). Prediction of size of infants at birth by measurement of symphysis fundus height. *British Journal of Obstetrics and Gynaecology*, *93*(3), 206–211. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/3964594

Pickering TG, Hall JE, Appel LJ, Falkner BE, Graves J, Hill MN, Jones DW, Kurtz T, Sheps SG, Roccella EJ; Subcommittee of Professional and Public Education of the American Heart Association Council on High Blood Pressure Research. 2005. Recommendations for blood pressure measurement in humans and experimental animals: Part 1: blood pressure measurement in humans: a statement for professionals from the Subcommittee of Professional and Public Education of the American Heart Association Council on High Blood Pressure Research. Hypertension 45:142-61.

Phillips, D. I., Barker, D. J., Hales, C. N., Hirst, S., & Osmond, C. (1994). Thinness at birth and insulin resistance in adult life. *Diabetologia*, *37*(2), 150–4. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/8163048

Pihl, K., Larsen, T., Krebs, L., & Christiansen, M. (2008). First trimester maternal serum PAPP-A, beta-hCG and ADAM12 in prediction of small-for-gestational-age fetuses. *Prenatal Diagnosis*, *28*(12), 1131–1135. article. https://doi.org/10.1002/pd.2141

Pijnenborg, R., Anthony, J., Davey, D. A., Rees, A., Tiltman, A., Vercruysse, L., & van Assche, A. (1991). Placental bed spiral arteries in the hypertensive disorders of pregnancy. *British Journal of Obstetrics and Gynaecology*, *98*(7), 648–655. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1883787

Pijnenborg, R., Bland, J. M., Robertson, W. B., & Brosens, I. (n.d.). Uteroplacental arterial changes related to interstitial trophoblast migration in early human pregnancy. *Placenta*, *4*(4), 397–413. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/6634666

Pijnenborg, R., Dixon, G., Robertson, W. B., & Brosens, I. (n.d.). Trophoblastic invasion of human decidua from 8 to 18 weeks of pregnancy. *Placenta*, *1*(1), 3–19. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7443635

Pijnenborg, R., Vercruysse, L., & Hanssens, M. (2006). The uterine spiral arteries in human pregnancy: facts and controversies. *Placenta*, *27*(9–10), 939–58. https://doi.org/10.1016/j.placenta.2005.12.006

Pilalis, a, Souka, a P., Antsaklis, P., Daskalakis, G., Papantoniou, N., Mesogitis, S., & Antsaklis, a. (2007). Screening for pre-eclampsia and fetal growth restriction by uterine artery Doppler and PAPP-A at 11-14 weeks' gestation. *Ultrasound in Obstetrics & Gynecology : The Official Journal of the International Society of Ultrasound in Obstetrics and Gynecology*, *29*(2), 135–40. https://doi.org/10.1002/uog.3881

Plasencia, W., Maiz, N., Bonino, S., Kaihura, C., & Nicolaides, K. H. (2007). Uterine artery Doppler at 11 + 0 to 13 + 6 weeks in the prediction of pre-eclampsia. *Ultrasound in Obstetrics & Gynecology : The Official Journal of the International Society of Ultrasound in Obstetrics and Gynecology*, *30*(5), 742–9. https://doi.org/10.1002/uog.5157

Plasencia, W., Maiz, N., Bonino, S., Kaihura, C., & Nicolaides, K. H. (2007). Uterine artery Doppler at 11 + 0 to 13 + 6 weeks in the prediction of pre-eclampsia. *Ultrasound in Obstetrics & Gynecology : The Official Journal of the International Society of Ultrasound in Obstetrics and Gynecology*, *30*(5), 742–9. https://doi.org/10.1002/uog.5157

Plasencia, W., Maiz, N., Poon, L., Yu, C., & Nicolaides, K. H. (2008). Uterine artery Doppler at 11 + 0 to 13 + 6 weeks and 21 + 0 to 24 + 6 weeks in the prediction of pre-eclampsia. *Ultrasound in Obstetrics {&} Gynecology : The Official Journal of the International Society of Ultrasound in Obstetrics and Gynecology*, *32*(2), 138–146. article. https://doi.org/10.1002/uog.5402

Poon, L. C. Y., Staboulidou, I., Maiz, N., Plasencia, W., & Nicolaides, K. H. (2009). Hypertensive disorders in pregnancy: screening by uterine artery Doppler at 11-13 weeks. *Ultrasound in Obstetrics & Gynecology : The Official Journal of the International Society of Ultrasound in Obstetrics and Gynecology*, *34*(2), 142–8. https://doi.org/10.1002/uog.6452

Poon, L., Zaragoza, E., Akolekar, R., & E. (2008). Maternal serum placental growth factor (PlGF) in small for gestational age pregnancy at 11+ 0 to 13+ 6 weeks of gestation. *Prenatal Diagnosis*, *28*(November), 1110–1115. https://doi.org/10.1002/pd

Poon, L. C. Y., Chelemen, T., Granvillano, O., Pandeva, I., & Nicolaides, K. H. (2008). First-trimester maternal serum a disintegrin and metalloprotease

12 (ADAM12) and adverse pregnancy outcome. *Obstetrics and Gynecology*, *112*(5), 1082–90. https://doi.org/10.1097/AOG.0b013e318188d6f9

Poon, L. C. Y., Kametas, N. A., Maiz, N., Akolekar, R., & Nicolaides, K. H. (2009). First-trimester prediction of hypertensive disorders in pregnancy. *Hypertension (Dallas, Tex.: 1979)*, *53*(5), 812–818. article. https://doi.org/10.1161/HYPERTENSIONAHA.108.127977

Poon, L. C. Y., Kametas, N. A., Valencia, C., Chelemen, T., & Nicolaides, K. H. (2011). Hypertensive disorders in pregnancy: screening by systolic diastolic and mean arterial pressure at 11-13 weeks. *Hypertension in Pregnancy*, *30*(1), 93–107. article. https://doi.org/10.3109/10641955.2010.484086

Poon, L. C. Y., Karagiannis, G., Staboulidou, I., Shafiei, A., & Nicolaides, K. H. (2011). Reference range of birth weight with gestation and first-trimester prediction of small-for-gestation neonates. *Prenatal Diagnosis*, *31*(1), 58–65. article. https://doi.org/10.1002/pd.2520

Poon, L. C. Y., Volpe, N., Muto, B., Syngelaki, A., & Nicolaides, K. H. (2012). Birthweight with gestation and maternal characteristics in live births and stillbirths. *Fetal Diagnosis and Therapy*, *32*(3), 156–165. article. https://doi.org/10.1159/000338655

Potdar, N., Singh, R., Mistry, V., Evans, M. D., Farmer, P. B., Konje, J. C., & Cooke, M. S. (2009). First-trimester increase in oxidative stress and risk of small-for-gestational-age fetus. *BJOG : An International Journal of Obstetrics and Gynaecology*, *116*(5), 637–42. https://doi.org/10.1111/j.1471-0528.2008.02096.x

Prefumo, F., Güven, M., Ganapathy, R., & Thilaganathan, B. (2004). The longitudinal variation in uterine artery blood flow pattern in relation to birth weight. *Obstetrics and Gynecology*, *103*(4), 764–8. https://doi.org/10.1097/01.AOG.0000118310.51730.2d

Ramsay, J. E., Ferrell, W. R., Crawford, L., Wallace, A. M., Greer, I. A., & Sattar, N. (2004). Divergent metabolic and vascular phenotypes in pre-eclampsia and intrauterine growth restriction: relevance of adiposity.

Journal of Hypertension, *22*(11), 2177–2183. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/15480103

Rana, S., Karumanchi, S. A., Levine, R. J., Venkatesha, S., Rauh-Hain, J. A., Tamez, H., & Thadhani, R. (2007). Sequential changes in antiangiogenic factors in early pregnancy and risk of developing preeclampsia. *Hypertension (Dallas, Tex.: 1979)*, *50*(1), 137–142. article. https://doi.org/10.1161/HYPERTENSIONAHA.107.087700

Rasmussen, S., Irgens, L. M., Skjaerven, R., & Melve, K. K. (2009). Prior adverse pregnancy outcome and the risk of stillbirth. *Obstetrics and Gynecology*, *114*(6), 1259–1270. article. https://doi.org/10.1097/AOG.0b013e3181c22422

Redline, R. W. (2001). Macrophages in the basal plate of pre-eclamptic placentae. *Placenta*, *22*(10), 890–893. article. https://doi.org/10.1053/plac.2001.0726

Redline, R. W. (2008). Placental pathology: a systematic approach with clinical correlations. *Placenta*, *29 Suppl A*, S86--91. article. https://doi.org/10.1016/j.placenta.2007.09.003

Reece, E. A., Smikle, C., O'Connor, T. Z., Holford, T., Nelson-Robinson, L., Degennaro, N., & Hobbins, J. C. (1990). A longitudinal study comparing growth in diabetic pregnancies with growth in normal gestations: I. The fetal weight. *Obstetrical {&} Gynecological Survey*, *45*(3), 161–164. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2190130

Reeske, A., Kutschmann, M., Razum, O., & Spallek, J. (2011). Stillbirth differences according to regions of origin: an analysis of the German perinatal database, 2004-2007. *BMC Pregnancy and Childbirth*, *11*, 63. article. https://doi.org/10.1186/1471-2393-11-63

Regev, R. H., Lusky, A., Dolfin, T., Litmanovitz, I., Arnon, S., Reichman, B., & Israel Neonatal Network. (2003). Excess mortality and morbidity among small-for-gestational-age premature infants: a population-based study. *The Journal of Pediatrics*, *143*(2), 186–91. https://doi.org/10.1067/S0022-3476(03)00181-1

Reik, W., Constância, M., Fowden, A., Anderson, N., Dean, W., Ferguson-Smith, A., Sibley, C. (2003). Regulation of supply and demand for maternal nutrients in mammals by imprinted genes. *The Journal of Physiology*, *547*(Pt 1), 35–44. https://doi.org/10.1113/jphysiol.2002.033274

Reister, F., Frank, H. G., Heyl, W., Kosanke, G., Huppertz, B., Schröder, W., Rath, W. (n.d.). The distribution of macrophages in spiral arteries of the placental bed in pre-eclampsia differs from that in healthy patients. *Placenta*, *20*(2–3), 229–233. article. https://doi.org/10.1053/plac.1998.0373

Rieger, L., Segerer, S., Bernar, T., Kapp, M., Majic, M., Morr, A.-K., Kämmerer, U. (2009). Specific subsets of immune cells in human decidua differ between normal pregnancy and preeclampsia--a prospective observational study. *Reproductive Biology and Endocrinology : RB&E*, *7*, 132. https://doi.org/10.1186/1477-7827-7-132

Rieger, L., Segerer, S., Bernar, T., Kapp, M., Majic, M., Morr, A.-K., Kämmerer, U. (2009). Specific subsets of immune cells in human decidua differ between normal pregnancy and preeclampsia--a prospective observational study. *Reproductive Biology and Endocrinology : RB&E*, *7*, 132. https://doi.org/10.1186/1477-7827-7-132

Roberge, S., Nicolaides, K. H., Demers, S., Villa, P., & Bujold, E. (2013). Prevention of perinatal death and adverse perinatal outcome using low-dose aspirin: a meta-analysis. *Ultrasound in Obstetrics {&} Gynecology : The Official Journal of the International Society of Ultrasound in Obstetrics and Gynecology*, *41*(5), 491–499. article. https://doi.org/10.1002/uog.12421

Roberge, S., Giguère, Y., Villa, P., Nicolaides, K., Vainio, M., Forest, J.-C., Bujold, E. (2012). Early administration of low-dose aspirin for the prevention of severe and mild preeclampsia: a systematic review and meta-analysis. *American Journal of Perinatology*, *29*(7), 551–556. article. https://doi.org/10.1055/s-0032-1310527

Roberge, S., Villa, P., Nicolaides, K., Giguère, Y., Vainio, M., Bakthi, A., Bujold, E. (2012). Early administration of low-dose aspirin for the

prevention of preterm and term preeclampsia: a systematic review and meta-analysis. *Fetal Diagnosis and Therapy*, *31*(3), 141–146. article. https://doi.org/10.1159/000336662

Roberts, D. J., & Post, M. D. (2008). The placenta in pre-eclampsia and intrauterine growth restriction. *Journal of Clinical Pathology*, *61*(12), 1254–60. https://doi.org/10.1136/jcp.2008.055236

Robertson, W. B., Brosens, I., & Dixon, H. G. (1967). The pathological response of the vessels of the placental bed to hypertensive pregnancy. *The Journal of Pathology and Bacteriology*, *93*(2), 581–592. article. https://doi.org/10.1002/path.1700930219

Romero R, Nien JK, Espinoza J, Todem D, Fu W, Chung H, Kusanovic JP, Gotsch F, Erez O, Mazaki-Tovi S, Gomez R, Edwin S, Chaiworapongsa T, Levine RJ, Karumanchi SA. A longitudinal study of angiogenic (placental growth factor) and anti-angiogenic (soluble endoglin and soluble vascular endothelial growth factor receptor-1) factors in normal pregnancy and patients destined to develop preeclampsia and deliver a small for gestational age neonate. J Matern Fetal Neonatal Med. 2008 Jan;21(1):9-23. doi: 10.1080/14767050701830480.

Robinson, H. P., & Fleming, J. E. (1975). A critical evaluation of sonar “crown-rump length” measurements. *British Journal of Obstetrics and Gynaecology*, *82*(9), 702–710. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/1182090

Royston, P. (1995). Calculation of unconditional and conditional reference intervals for foetal size and growth from longitudinal measurements. *Statistics in Medicine*, *14*(13), 1417–1436. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7481181

Sahu, M. T., Das, V., Mittal, S., Agarwal, A., & Sahu, M. (2010). Overt and subclinical thyroid dysfunction among Indian pregnant women and its effect on maternal and fetal outcome. *Archives of Gynecology and Obstetrics*, *281*(2), 215–20. https://doi.org/10.1007/s00404-009-1105-1

Sahu, M. T., Das, V., Mittal, S., Agarwal, A., & Sahu, M. (2010). Overt and subclinical thyroid dysfunction among Indian pregnant women and its

effect on maternal and fetal outcome. *Archives of Gynecology and Obstetrics*, *281*(2), 215–20. https://doi.org/10.1007/s00404-009-1105-1

Sankaran, S., & Kyle, P. M. (2009). Aetiology and pathogenesis of IUGR. *Best Practice & Research. Clinical Obstetrics & Gynaecology*, *23*(6), 765–77. https://doi.org/10.1016/j.bpobgyn.2009.05.003

Sargent, I. L., Germain, S. J., Sacks, G. P., Kumar, S., & Redman, C. W. G. (2003). Trophoblast deportation and the maternal inflammatory response in pre-eclampsia. *Journal of Reproductive Immunology*, *59*(2), 153–160. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12896819

Sattar, N., Greer, I. A., Galloway, P. J., Packard, C. J., Shepherd, J., Kelly, T., & Mathers, A. (1999). Lipid and lipoprotein concentrations in pregnancies complicated by intrauterine growth restriction. *The Journal of Clinical Endocrinology and Metabolism*, *84*(1), 128–30. https://doi.org/10.1210/jcem.84.1.5419

Sattar, N., Greer, I. A., Galloway, P. J., Packard, C. J., Shepherd, J., Kelly, T., & Mathers, A. (1999). Lipid and lipoprotein concentrations in pregnancies complicated by intrauterine growth restriction. *The Journal of Clinical Endocrinology and Metabolism*, *84*(1), 128–30. https://doi.org/10.1210/jcem.84.1.5419

Savvidou, M. D., Noori, M., Anderson, J. M., Hingorani, a D., & Nicolaides, K. H. (2008). Maternal endothelial function and serum concentrations of placental growth factor and soluble endoglin in women with abnormal placentation. *Ultrasound in Obstetrics & Gynecology : The Official Journal of the International Society of Ultrasound in Obstetrics and Gynecology*, *32*(7), 871–6. https://doi.org/10.1002/uog.6126

Say, L., Gülmezoglu, A. M., & Hofmeyr, G. J. (2003). Maternal nutrient supplementation for suspected impaired fetal growth. *The Cochrane Database of Systematic Reviews*, (1), CD000148. article. https://doi.org/10.1002/14651858.CD000148

Schonkeren, D., van der Hoorn, M.-L., Khedoe, P., Swings, G., van Beelen, E., Claas, F., Scherjon, S. (2011). Differential distribution and phenotype of decidual macrophages in preeclamptic versus control pregnancies. *The*

American Journal of Pathology, *178*(2), 709–717. article. https://doi.org/10.1016/j.ajpath.2010.10.011

Seval, Y., Sati, L., Celik-ozenci, C., Taskin, O., & Demir, R. (2008). The Distribution of Angiopoietin-1, Angiopoietin-2 and Their Receptors Tie-1 and Tie-2 in the Very Early Human Placenta. *Placenta*, *29*, 809–815. https://doi.org/10.1016/j.placenta.2008.06.009

Shah, P. S., & Knowledge Synthesis Group on Determinants of LBW/PT births. (2010). Parity and low birth weight and preterm birth: a systematic review and meta-analyses. *Acta Obstetricia et Gynecologica Scandinavica*, *89*(7), 862–875. article. https://doi.org/10.3109/00016349.2010.486827

Shah, P. S., & Knowledge Synthesis Group on determinants of preterm/low birthweight births. (2010). Paternal factors and low birthweight, preterm, and small for gestational age births: a systematic review. *American Journal of Obstetrics and Gynecology*, *202*(2), 103–123. article. https://doi.org/10.1016/j.ajog.2009.08.026

Sheppard, B. L., & Bonnar, J. (1981). An ultrastructural study of utero-placental spiral arteries in hypertensive and normotensive pregnancy and fetal growth retardation. *British Journal of Obstetrics and Gynaecology*, *88*(7), 695–705. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7248226

Shi, Z., Xu, W., Loechel, F., Wewer, U. M., & Murphy, L. J. (2000). ADAM 12, a disintegrin metalloprotease, interacts with insulin-like growth factor-binding protein-3. *The Journal of Biological Chemistry*, *275*(24), 18574–18580. article. https://doi.org/10.1074/jbc.M002172200

Shibata, E., Rajakumar, A., Powers, R. W., Larkin, R. W., Gilmour, C., Bodnar, L. M., Hubel, C. a. (2005). Soluble fms-like tyrosine kinase 1 is increased in preeclampsia but not in normotensive pregnancies with small-for-gestational-age neonates: relationship to circulating placental growth factor. *The Journal of Clinical Endocrinology and Metabolism*, *90*(8), 4895–903. https://doi.org/10.1210/jc.2004-1955

Sinha, I. P., Smyth, R. L., & Williamson, P. R. (2011). Using the Delphi technique to determine which outcomes to measure in clinical trials:

recommendations for the future based on a systematic review of existing studies. *PLoS Medicine*, *8*(1), e1000393. article. https://doi.org/10.1371/journal.pmed.1000393

Smith, G. C., Pell, J. P., & Walsh, D. (2001). Pregnancy complications and maternal risk of ischaemic heart disease: a retrospective cohort study of 129,290 births. *Lancet (London, England)*, *357*(9273), 2002–2006. article. https://doi.org/10.1016/S0140-6736(00)05112-6

Smith, G. D., Harding, S., & Rosato, M. (2000). Relation between infants' birth weight and mothers' mortality: prospective observational study. *BMJ (Clinical Research Ed.)*, *320*(7238), 839–840. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10731177

Smith, G. C. S., Crossley, J. A., Aitken, D. A., Jenkins, N., Lyall, F., Cameron, A. D., Dobbie, R. (2007). Circulating angiogenic factors in early pregnancy and the risk of preeclampsia, intrauterine growth restriction, spontaneous preterm birth, and stillbirth. *Obstetrics and Gynecology*, *109*(6), 1316–1324. article. https://doi.org/10.1097/01.AOG.0000265804.09161.0d

Smith, G. C. S., & Fretts, R. C. (2007). Stillbirth. *Lancet (London, England)*, *370*(9600), 1715–1725. article. https://doi.org/10.1016/S0140-6736(07)61723-1

Smith, G. C. S., Shah, I., White, I. R., Pell, J. P., & Dobbie, R. (2007). Previous preeclampsia, preterm delivery, and delivery of a small for gestational age infant and the risk of unexplained stillbirth in the second pregnancy: a retrospective cohort study, Scotland, 1992-2001. *American Journal of Epidemiology*, *165*(2), 194–202. https://doi.org/10.1093/aje/kwj354

Smith, S. D., Dunk, C. E., Aplin, J. D., Harris, L. K., & Jones, R. L. (2009). Evidence for immune cell involvement in decidual spiral arteriole remodeling in early human pregnancy. *The American Journal of Pathology*, *174*(5), 1959–1971. article. https://doi.org/10.2353/ajpath.2009.080995

Snijders, R. J., Noble, P., Sebire, N., Souka, A., & Nicolaides, K. H. (1998). UK multicentre project on assessment of risk of trisomy 21 by maternal age and fetal nuchal-translucency thickness at 10-14 weeks of gestation.

Fetal Medicine Foundation First Trimester Screening Group. *Lancet (London, England)*, *352*(9125), 343–346. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9717920

Spencer, K., Cowans, N. J., Avgidou, K., Molina, F., & Nicolaides, K. H. (2008). First-trimester biochemical markers of aneuploidy and the prediction of small-for-gestational age fetuses. *Ultrasound in Obstetrics {&} Gynecology : The Official Journal of the International Society of Ultrasound in Obstetrics and Gynecology*, *31*(1), 15–19. article. https://doi.org/10.1002/uog.5165

Spencer, K., Cowans, N. J., & Stamatopoulou, A. (2008). ADAM12s in maternal serum as a potential marker of pre-eclampsia. *Prenatal Diagnosis*, *28*(3), 212–216. article. https://doi.org/10.1002/pd.1957

Srinivas, S. K., Edlow, a G., Neff, P. M., Sammel, M. D., Andrela, C. M., & Elovitz, M. a. (2009). Rethinking IUGR in preeclampsia: dependent or independent of maternal hypertension? *Journal of Perinatology : Official Journal of the California Perinatal Association*, *29*(10), 680–4. https://doi.org/10.1038/jp.2009.83

Stampalija, T., Gyte, G. M., & Alfirevic, Z. (2010). Utero-placental Doppler ultrasound for improving pregnancy outcome. *The Cochrane Database of Systematic Reviews*, (9), CD008363. article. https://doi.org/10.1002/14651858.CD008363.pub2

Starzyk, K. A., Salafia, C. M., Pezzullo, J. C., Lage, J. M., Parkash, V., Vercruysse, L., Pijnenborg, R. (1997). Quantitative differences in arterial morphometry define the placental bed in preeclampsia. *Human Pathology*, *28*(3), 353–358. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9042801

Steinberg, G., Lee, C., Rauh-Hain, J. A., Ecker, J., Khankin, E. V, Hsu, C.-D., Lim, K.-H. (2010). Early-pregnancy soluble Fas levels in idiopathic small-for-gestational-age pregnancies. *American Journal of Obstetrics and Gynecology*, *202*(3), 299.e1-7. https://doi.org/10.1016/j.ajog.2009.12.017

Steinberg, G., Lee, C., Rauh-Hain, J. A., Ecker, J., Khankin, E. V, Hsu, C.-D., Lim, K.-H. (2010). Early-pregnancy soluble Fas levels in idiopathic small-

for-gestational-age pregnancies. *American Journal of Obstetrics and Gynecology*, *202*(3), 299.e1-7. https://doi.org/10.1016/j.ajog.2009.12.017

Stepan, H., Krämer, T., & Faber, R. (2007). Maternal plasma concentrations of soluble endoglin in pregnancies with intrauterine growth restriction. *The Journal of Clinical Endocrinology and Metabolism*, *92*(7), 2831–4. https://doi.org/10.1210/jc.2006-2774

Stillbirth Collaborative Research Network Writing Group. (2011). Association between stillbirth and risk factors known at pregnancy confirmation. *JAMA*, *306*(22), 2469–2479. article. https://doi.org/10.1001/jama.2011.1798

Stillbirth Collaborative Research Network Writing Group. (2011). Causes of death among stillbirths. *JAMA*, *306*(22), 2459–2468. article. https://doi.org/10.1001/jama.2011.1823

Surkan, P. J., Stephansson, O., Dickman, P. W., & Cnattingius, S. (2004). Previous preterm and small-for-gestational-age births and the subsequent risk of stillbirth. *The New England Journal of Medicine*, *350*(8), 777–785. article. https://doi.org/10.1056/NEJMoa031587

Sydor, J. R., & Nock, S. (2003). Protein expression profiling arrays: tools for the multiplexed high-throughput analysis of proteins. *Proteome Science*, *1*(1), 3. article. https://doi.org/10.1186/1477-5956-1-3

Teasdale, F. (n.d.). Idiopathic intrauterine growth retardation: histomorphometry of the human placenta. *Placenta*, *5*(1), 83–92. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/6728834

Thadhani, R. (2004). First Trimester Placental Growth Factor and Soluble Fms-Like Tyrosine Kinase 1 and Risk for Preeclampsia. *Journal of Clinical Endocrinology & Metabolism*, *89*(2), 770–775. https://doi.org/10.1210/jc.2003-031244

Thomas, C. P., Andrews, J. I., & Liu, K. Z. (2007). Intronic polyadenylation signal sequences and alternate splicing generate human soluble Flt1 variants and regulate the abundance of soluble Flt1 in the placenta. *FASEB Journal : Official Publication of the Federation of American*

Societies for Experimental Biology, *21*(14), 3885–95. https://doi.org/10.1096/fj.07-8809com

Thompson, J. M., Clark, P. M., Robinson, E., Becroft, D. M., Pattison, N. S., Glavish, N., Mitchell, E. A. (2001). Risk factors for small-for-gestational-age babies: The Auckland Birthweight Collaborative Study. *Journal of Paediatrics and Child Health*, *37*(4), 369–375. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11532057

Thorp, J. A., Walsh, S. W., & Brath, P. C. (1988). Low-dose aspirin inhibits thromboxane, but not prostacyclin, production by human placental arteries. *American Journal of Obstetrics and Gynecology*, *159*(6), 1381–1384. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/3061295

Tidwell, S. C., Ho, H. N., Chiu, W. H., Torry, R. J., & Torry, D. S. (2001). Low maternal serum levels of placenta growth factor as an antecedent of clinical preeclampsia. *American Journal of Obstetrics and Gynecology*, *184*(6), 1267–1272. article. https://doi.org/10.1067/mob.2001.113129

Toth, P., Li, X., Rao, C. V, Lincoln, S. R., Sanfilippo, J. S., Spinnato, J. A., & Yussman, M. A. (1994). Expression of functional human chorionic gonadotropin/human luteinizing hormone receptor gene in human uterine arteries. *The Journal of Clinical Endocrinology and Metabolism*, *79*(1), 307–315. article. https://doi.org/10.1210/jcem.79.1.8027246

Troe, E. J. W. M., Raat, H., Jaddoe, V. W. V, Hofman, A., Looman, C. W. N., Moll, H. A., Mackenbach, J. P. (2007). Explaining differences in birthweight between ethnic populations. The Generation R Study. *BJOG : An International Journal of Obstetrics and Gynaecology*, *114*(12), 1557–1565. article. https://doi.org/10.1111/j.1471-0528.2007.01508.x

Tsatsaris, V., Goffin, F., Munaut, C., Brichant, J.-F., Pignon, M.-R., Noel, A., Foidart, J.-M. (2003). Overexpression of the soluble vascular endothelial growth factor receptor in preeclamptic patients: pathophysiological consequences. *The Journal of Clinical Endocrinology and Metabolism*, *88*(11), 5555–5563. article. https://doi.org/10.1210/jc.2003-030528

Tul, N., Pusenjak, S., Osredkar, J., Spencer, K., & Novak-Antolic, Z. (2003). Predicting complications of pregnancy with first-trimester maternal serum free-betahCG, PAPP-A and inhibin-A. *Prenatal Diagnosis*, *23*(12), 990–996. article. https://doi.org/10.1002/pd.735

van Kleffens, M., Groffen, C., Lindenbergh-Kortleve, D. J., van Neck, J. W., González-Parra, S., Dits, N., Drop, S. L. (1998). The IGF system during fetal-placental development of the mouse. *Molecular and Cellular Endocrinology*, *140*(1–2), 129–135. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9722180

Vergani, P., Cozzolino, S., Pozzi, E., Cuttin, M. S., Greco, M., Ornaghi, S., & Lucchini, V. (2008). Identifying the causes of stillbirth: a comparison of four classification systems. *American Journal of Obstetrics and Gynecology*, *199*(3), 319.e1--4. article. https://doi.org/10.1016/j.ajog.2008.06.098

Villar, J., Carroli, G., Wojdyla, D., Abalos, E., Giordano, D., Ba'aqeel, H., World Health Organization Antenatal Care Trial Research Group. (2006). Preeclampsia, gestational hypertension and intrauterine growth restriction, related or independent conditions? *American Journal of Obstetrics and Gynecology*, *194*(4), 921–931. article. https://doi.org/10.1016/j.ajog.2005.10.813

von Dadelszen, P., Watson, R. W., Noorwali, F., Marshall, J. C., Parodo, J., Farine, D., Rotstein, O. D. (1999). Maternal neutrophil apoptosis in normal pregnancy, preeclampsia, and normotensive intrauterine growth restriction. *American Journal of Obstetrics and Gynecology*, *181*(2), 408–414. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10454692

Wallace, A. E., Fraser, R., & Cartwright, J. E. (2012). Extravillous trophoblast and decidual natural killer cells: a remodelling partnership. *Human Reproduction Update*, *18*(4), 458–471. article. https://doi.org/10.1093/humupd/dms015

Wallenburg, H. C., Dekker, G. A., Makovitz, J. W., & Rotmans, P. (1986). Low-dose aspirin prevents pregnancy-induced hypertension and pre-eclampsia in angiotensin-sensitive primigravidae. *Lancet (London,*

England), *1*(8471), 1–3. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2867260

Wallner, W., Sengenberger, R., Strick, R., Strissel, P. L., Meurer, B., Beckmann, M. W., & Schlembach, D. (2007). Angiogenic growth factors in maternal and fetal serum in pregnancies complicated by intrauterine growth restriction. *Clinical Science (London, England : 1979)*, *112*(1), 51–7. https://doi.org/10.1042/CS20060161

Weiner, C. P., & Robinson, D. (1989). Sonographic diagnosis of intrauterine growth retardation using the postnatal ponderal index and the crown-heel length as standards of diagnosis. *American Journal of Perinatology*, *6*(4), 380–383. article. https://doi.org/10.1055/s-2007-999622

Wen, S. W., Goldenberg, R. L., Cutter, G. R., Hoffman, H. J., Cliver, S. P., Davis, R. O., & DuBard, M. B. (1990). Smoking, maternal age, fetal growth, and gestational age at delivery. *American Journal of Obstetrics and Gynecology*, *162*(1), 53–58. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/2301517

Wikström, A.-K., Stephansson, O., & Cnattingius, S. (2011). Previous preeclampsia and risks of adverse outcomes in subsequent nonpreeclamptic pregnancies. *American Journal of Obstetrics and Gynecology*, *204*(2), 148.e1--6. article. https://doi.org/10.1016/j.ajog.2010.09.003

Wikström, A.-K., Svensson, T., Kieler, H., & Cnattingius, S. (2011). Recurrence of placental dysfunction disorders across generations. *American Journal of Obstetrics and Gynecology*, *205*(5), 454.e1-8. https://doi.org/10.1016/j.ajog.2011.05.009

World Health Organisation, Promoting Optimal fetal development, 2006 http://www.who.int/nutrition/publications/fetomaternal/9241594004/en/

Wolfe, H. M., Gross, T. L., & Sokol, R. J. (1987). Recurrent small for gestational age birth: perinatal risks and outcomes. *American Journal of Obstetrics and Gynecology*, *157*(2), 288–293. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/3618674

Wölter, M., Röwer, C., Koy, C., Reimer, T., Rath, W., Pecks, U., & Glocker, M. O. (2012). A proteome signature for intrauterine growth restriction derived from multifactorial analysis of mass spectrometry-based cord blood serum profiling. *Electrophoresis*, *33*(12), 1881–1893. article. https://doi.org/10.1002/elps.201200001

Wright, D., Kagan, K. O., Molina, F. S., Gazzoni, A., & Nicolaides, K. H. (2008). A mixture model of nuchal translucency thickness in screening for chromosomal defects. *Ultrasound in Obstetrics {&} Gynecology : The Official Journal of the International Society of Ultrasound in Obstetrics and Gynecology*, *31*(4), 376–383. article. https://doi.org/10.1002/uog.5299

Yaron, Y., Heifetz, S., Ochshorn, Y., Lehavi, O., & Orr-Urtreger, A. (2002). Decreased first trimester PAPP-A is a predictor of adverse pregnancy outcome. *Prenatal Diagnosis*, *22*(9), 778–82. https://doi.org/10.1002/pd.407

Yasmeen, S., Wilkins, E. E., Field, N. T., Sheikh, R. A., & Gilbert, W. M. (2001). Pregnancy outcomes in women with systemic lupus erythematosus. *The Journal of Maternal-Fetal Medicine*, *10*(2), 91–96. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11392599

Yasuda, M., Takakuwa, K., Tokunaga, A., & Tanaka, K. (1995). Prospective studies of the association between anticardiolipin antibody and outcome of pregnancy. *Obstetrics and Gynecology*, *86*(4 Pt 1), 555–559. article. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/7675379

Yu, C. K. H., Khouri, O., Onwudiwe, N., Spiliopoulos, Y., Nicolaides, K. H., & Fetal Medicine Foundation Second-Trimester Screening Group. (2008). Prediction of pre-eclampsia by uterine artery Doppler imaging: relationship to gestational age at delivery and small-for-gestational age. *Ultrasound in Obstetrics {&} Gynecology : The Official Journal of the International Society of Ultrasound in Obstetrics and Gynecology*, *31*(3), 310–313. article. https://doi.org/10.1002/uog.5252

Zetterström, K., Lindeberg, S. N., Haglund, B., & Hanson, U. (2006). Chronic hypertension as a risk factor for offspring to be born small for gestational age. *Acta Obstetricia et Gynecologica Scandinavica*, *85*(9), 1046–50. https://doi.org/10.1080/00016340500442654

Zetterström, K., Lindeberg, S. N., Haglund, B., & Hanson, U. (2006). Chronic hypertension as a risk factor for offspring to be born small for gestational age. *Acta Obstetricia et Gynecologica Scandinavica*, *85*(9), 1046–50. https://doi.org/10.1080/00016340500442654

Zhang, X., Mumford, S. L., Cnattingius, S., Schisterman, E. F., & Kramer, M. S. (2010). Reduced birthweight in short or primiparous mothers: physiological or pathological? *BJOG : An International Journal of Obstetrics and Gynaecology*, 1–7. https://doi.org/10.1111/j.1471-0528.2010.02642.x

Zhang, X., Platt, R. W., Cnattingius, S., Joseph, K. S., & Kramer, M. S. (2007). The use of customised versus population-based birthweight standards in predicting perinatal mortality. *BJOG : An International Journal of Obstetrics and Gynaecology*, *114*(4), 474–477. article. https://doi.org/10.1111/j.1471-0528.2007.01273.x

Zhong, Y., Tuuli, M., & Odibo, A. O. (2010). First-trimester assessment of placenta function and the prediction of preeclampsia and intrauterine growth restriction. *Prenatal Diagnosis*, *30*(4), 293–308. https://doi.org/10.1002/pd.2475

Zhou, Y., Damsky, C. H., & Fisher, S. J. (1997). Preeclampsia is associated with failure of human cytotrophoblasts to mimic a vascular adhesion phenotype. One cause of defective endovascular invasion in this syndrome? *The Journal of Clinical Investigation*, *99*(9), 2152–2164. article. https://doi.org/10.1172/JCI119388

Zygmunt, M., Hahn, D., Münstedt, K., Bischof, P., & Lang, U. (1998). Invasion of cytotrophoblastic JEG-3 cells is stimulated by hCG in vitro. *Placenta*, *19*(8), 587–93. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9859862

Zygmunt, M., Hahn, D., Münstedt, K., Bischof, P., & Lang, U. (1998). Invasion of cytotrophoblastic JEG-3 cells is stimulated by hCG in vitro. *Placenta*, *19*(8), 587–93. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/9859862

Zygmunt, M., Herr, F., Keller-Schoenwetter, S., Kunzi-Rapp, K., Münstedt, K., Rao, C. V, Preissner, K. T. (2002). Characterization of human chorionic gonadotropin as a novel angiogenic factor. *The Journal of Clinical Endocrinology and Metabolism*, *87*(11), 5290–5296. article. https://doi.org/10.1210/jc.2002-020642