Novel early first trimester ultrasound measures in the prediction of miscarriage, small-for-gestational age neonates and maternal hypertensive disorders

Tracey Joy Hanchard

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Discipline of Obstetrics, Gynaecology and Neonatology, Sydney

Faculty of Medicine, University of Sydney

This is to certify that the content of this thesis is my own work that all assistance received in its preparation has been acknowledged.

This thesis has not been submitted elsewhere for any degree or other purpose.

Tracey Hanchard
12th May 2020

This is to certify that the content of the contribution statements in the following chapters of this thesis are, to the best of my knowledge, true and correct.

Clinical Professor Jonathan Hyett (Chapters 1-7):

Associate Professor Ann Quinton (Chapters 1-7)

Dr Bradley de Vries (Chapters 3-7)

Dr Michael Sinosich (Chapters 5 and 6)

ABSTRACT

One of the primary roles of obstetric care is the prediction of adverse fetal and maternal outcomes. Ultrasound is commonly used prior to 11 weeks gestation however there is a paucity of published research investigating the value of ultrasound features at this early stage in the prediction of adverse fetal and maternal outcomes later in pregnancy.

Adverse pregnancy outcomes of small-for-gestational age/intrauterine growth restriction and maternal hypertensive disorders (gestational hypertension and pre-eclampsia) are significant pregnancy complications that often result in poor short- and long-term outcomes for both child and mother. Early prediction coupled with prophylactic intervention has been demonstrated to reduce the prevalence of these outcomes. First trimester miscarriage is common, and while its prediction may not influence the outcome, ultrasound is uniquely situated to significantly impact the clinical management of these women.

Current prediction models for small-for-gestational age and maternal hypertensive disorders are implemented at 11-13^{+6} weeks gestation. In pregnancies considered at an increased risk, therapy with low-dose aspirin has been shown to modify progression of disease if instigated prior to 16 weeks gestation. It is possible that if prediction and therapy occurred at an earlier gestation it would be even more effective than the current regimen.

The research presented in this thesis investigates the potential for a combination of conventional and novel ultrasound measures prior to 11 weeks gestation to predict adverse pregnancy outcomes including miscarriage prior to 12 weeks gestation and the development of small-for-gestational age and maternal hypertensive disorders later in pregnancy.

We found that when measured prior to 11 weeks gestation, less than expected trophoblast volume for gestational age is significantly associated with all three adverse outcomes of interest. In addition, together with maternal characteristics and biochemistry, trophoblast volume measurements may add to the value of current prediction methods for small-for-gestational age and maternal hypertensive disorders and may enable prediction at an earlier gestational age. This adds new knowledge to the field of obstetric medicine and has the potential to influence pregnancy management and improve fetal and maternal outcomes.

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PUBLICATIONS ARISING FROM THIS WORK

Hanchard TJ, de Vries BS, Quinton AE, Sinosich M, Hyett JA. Ultrasound features prior to 11 weeks' gestation and first-trimester maternal factors in prediction of hypertensive disorders of pregnancy. Ultrasound Obstet Gynecol. 2020; 55(5):629-636.

Taylor TJ, Quinton AE, de Vries BS, Hyett JA. Uterine Artery Pulsatility Index Assessment at <11 Weeks' Gestation: A Prospective Study. Fetal Diagn Ther. 2020;47(2):129-137.

Taylor TJ, Quinton AE, de Vries BS, Hyett JA. First trimester ultrasound features associated with subsequent miscarriage: A prospective study. Aust N Z J Obstet Gynaecol. 2019 Oct;59(5):641-648.

Taylor, T, Quinton, A, Hyett, J. The developmental origins of placental function. *AJUM.* 2017 20(4):141-146.

PUBLICATIONS ARISING FROM THIS WORK CURRENTLY UNDER PEER-REVIEW

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PRESENTATIONS ARISING FROM THIS WORK

October 2019	Uterine artery pulsatility index assessment < 11 weeks gestation: A prospective study. **29th World Congress on Ultrasound in Obstetrics and Gynecology, Berlin, Germany.**
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September 2017	Ultrasound markers at 6 – 11 weeks gestational age for the prediction of fetal demise prior to 12 weeks gestation. **27th World Congress on Ultrasound in Obstetrics and Gynecology, Vienna, Austria.**

June 2017	Ultrasound markers at 6 – 11 weeks gestational age for the prediction of fetal demise prior to 12 weeks gestation. **Australasian Sonographers Association International Conference, Brisbane, Australia.**
August 2016	An ultrasound approach to the early prediction of intrauterine growth restriction. **Illawarra and Southern Practice Research Network, Kiama, Australia.**
June 2016	An ultrasound approach to the early prediction of intrauterine growth restriction. **Royal Prince Alfred Hospital: High Risk Obstetrics Department, Sydney, Australia.**

ADDITIONAL INFORMATION

Courses completed in conjunction with this thesis

2018	Categorical Data Analysis	Distinction
	University of Sydney	
2017	Multiple Regression and Stats Computing	High Distinction
	University of Sydney	
2016	Introductory Biostatistics	High Distinction
	University of Sydney	

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AC	abdominal circumference
AFI	amniotic fluid index
αFP	alpha fetoprotein
AUC	area under the curve
βhCG	beta human chorionic gonadotropin
BPD	biparietal diameter
BPS	biophysical profile score
CI	confidence interval
CPR	cerebroplacental ratio
CRL	crown-rump length
CTG	cardiotocography
EFW	estimated fetal weight
Eng	endoglin
FHR	fetal heart rate
FL	femur length
FPR	false positive rate
GDM	gestational diabetes mellitus
GH	gestational hypertension
HC	head circumference
hCG	human chorionic gonadotropin
HELLP	haemolysis, elevated liver enzymes and a low platelet count (syndrome)
IUGR	intrauterine growth restriction

LMP	last menstrual period
MAP	maternal mean arterial pressure
MCA	middle cerebral artery
mg/mmol	milligrams/millimoles per litre
mmHg	millimetres of Mercury
M-Mode	motion-Mode
MoM	multiples of the median
MSD	mean sac diameter
N	number of participants
NIPS	non-invasive prenatal screening
NT	nuchal translucency
PAPP-A	pregnancy – associated plasma protein A
PET	pre-eclampsia
PI	pulsatility index
PIGF	placental growth factor
PP13	placental protein 13
PV	placental volume
RCOG	Royal College of Obstetricians and Gynaecologists
SGA	small-for-gestational age
sEng	soluble endoglin
sFlt-1	soluble fms-like tyrosine kinase-1
SGA	small- for-gestational age
SVEGFR1	soluble vascular endothelial growth factor receptor-1

3D	three-dimensional
TT	trophoblast thickness
TTV	trophoblast volume
UAPI	uterine artery pulsatility index
YS	secondary yolk sac
YSD	yolk sac diameter
VEGF	vascular endothelial growth factor

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CHAPTER 1. LITERATURE REVIEW

INTRODUCTION

One of the primary roles of obstetric care is the prediction of adverse fetal and maternal outcomes with the aim of timely treatment and reduction in associated morbidity and mortality. Intrauterine growth restriction (IUGR) / small-for-gestational age (SGA) and maternal hypertensive disorders (MHD) including gestational hypertension (GH) and pre-eclampsia (PET) are significant pregnancy complications that often result in poor short and long term outcomes for both child and mother (1, 2) .

Early prediction and intervention has been demonstrated to reduce the prevalence of adverse pregnancy outcomes (3, 4) with first trimester prediction currently conducted at 11-13^{+6} weeks gestation. Prediction models at this gestational age include combinations of first trimester ultrasound features, maternal characteristics, biochemistry and mean arterial blood pressure to predict SGA (5), GH (6) and PET (7).

Ultrasound is commonly used prior to 11 weeks gestation to assess pregnancy location, viability, gestational age and to predict miscarriage (8). Despite this, there is a paucity of published research investigating the value of ultrasound features at this early stage in the prediction of adverse fetal and maternal outcomes later in pregnancy. This research will investigate the potential for a combination of conventional and novel ultrasound measures prior to 11 weeks gestation to predict adverse pregnancy outcomes including miscarriage and the development of SGA and MHD later in pregnancy.

These adverse outcomes are thought to have their origins in early placental development (9). For this reason, this review will commence with an overview of the development and structure of the normal placenta including the corresponding ultrasound appearances, explore the requirements of the placenta for normal function, and discuss the implications of placental dysfunction. This will be followed by a description of the uses of ultrasound in the first trimester of

pregnancy and definitions of adverse fetal and maternal outcomes to be addressed in this work; miscarriage prior to 12 weeks gestation, SGA and MHD. The review will continue with a description of the current methods used to diagnose and predict these adverse outcomes and finally introduce the specific aims of the research presented in this thesis.

THE HUMAN PLACENTA

This section has been published in a different format in a peer reviewed journal:

Authors: Taylor, T, Quinton, A, Hyett, J. The developmental origins of placental function. AJUM. 2017 20(4):141-146.

Contributions:

Tracey Hanchard: *Literature review and drafting of the manuscript*

Associate Professor Ann Quinton: *Review of the manuscript*

Clinical Professor Jonathon Hyett: *Review of the manuscript*

INTRODUCTION

This section is a review of the embryological origins and structure of the normal placenta (including the corresponding ultrasound appearances), the requirements of the placenta for normal function and the implications of placental dysfunction.

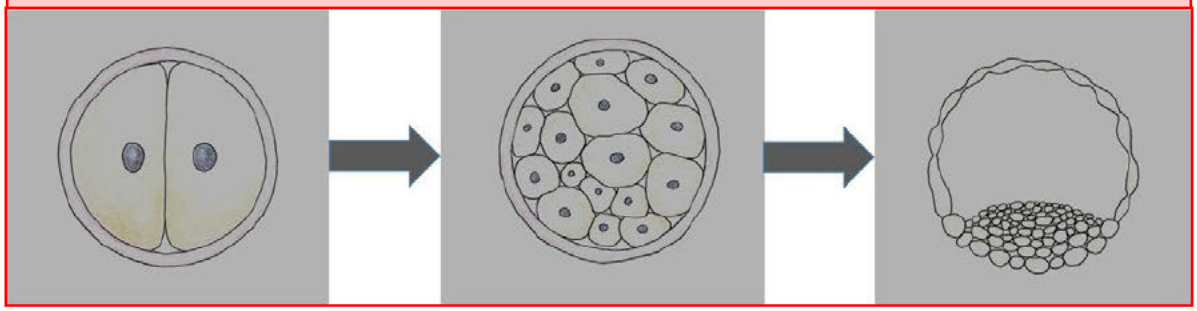
DEVELOPMENT OF THE PLACENTA

There are two main components of the placenta; the fetal component and the maternal component. The development of the fetal component begins at fertilisation and follows a well-documented process through cleavage and implantation into the endometrium, the development of the chorionic sac and villi and establishment of the normal uteroplacental circulation. This process will now be discussed.

Fertilisation

Fertilisation of a single oocyte with a single sperm results in variation of the human species by the restoration of the normal diploid number of chromosomes in the new zygote, determines the gender of the embryo and initiates cleavage. The process of cleavage (Fig 1.1) results in the one cell zygote rapidly dividing. As it divides these new cells are known as blastomeres, a collection of 32 blastomeres is known as a morula (10). As fertilisation normally occurs in the part of the fallopian tube most distant to the uterus cleavage, occurs during its passage towards the uterus and results in the development of the blastocyst.

Figure 1.1. *Cleavage from two cell stage (left) to morula (middle) and blastocyst (right). Modified from [10].*



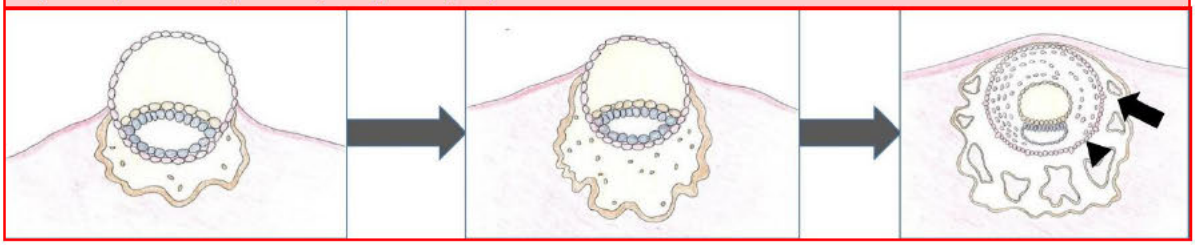
As the blastocyst enters the endometrial cavity, fluid from the uterine glands enters to produce the blastocystic cavity. The fluid separates the blastocyst into two parts – the embryoblast (future embryo) and the trophoblast (the embryonic contribution to the placenta) (10).

Implantation

On approximately day 6 after fertilisation, almost 3 weeks since the beginning of the

last menses, the blastocyst begins to implant into the endometrium. As the trophoblast comes into contact with the endometrium it begins to differentiate into two layers: the outer layer of the syncytiotrophoblast that invades further into the endometrium and the inner layer known as the cytotrophoblast. As the blastocyst invades further into the endometrium the surrounding trophoblast continues to differentiate until the entire blastocyst is surrounded by these two layers (Fig 1.2) (10).

Figure 1.2. *Implantation: early to complete (arrow: syncytiotrophoblast, arrowhead: cytotrophoblast). Modified from [10].*



Chorionic Villi and the Chorionic Sac

By the end of 4 weeks gestational age the blastocyst is surrounded by three layers: the extraembryonic mesoderm (innermost) and the two layers of trophoblast. These three layers collectively form the chorion(10). The chorion surrounds the chorionic sac and it is this that is sonographically known as the gestational sac(11).

As the gestational sac develops the cytotrophoblast continues to proliferate and at the end of the 4th gestational week begin to produce extensions that grow into the surrounding syncytiotrophoblast. These extensions are known as the primary chorionic villi; the beginnings of the placenta(10). These mature into tertiary villi within the next week as they branch, become filled with connective tissue and form capillaries. These capillaries form a network which will become connected to the embryo establishing the circulatory connection with the early placenta (10).

The Endometrium

The maternal component of the placenta develops from the endometrium and its blood supply. The pregnant endometrium is known as the decidua. Using an ultrasound image, figure 1.3 demonstrates the three regions of the

decidua at 6 weeks gestational age; the decidua basalis (yellow) which is deep to the gestational sac and forms the maternal part of the placenta, the decidua capsularis (pink), superficial to the gestational sac and the decidual parietalis (blue), the remainder of the endometrium (10).

The decidua surrounds the implanted gestational sac. As the gestational sac grows the chorionic villi within the decidual capsularis are compressed and degenerate to form the region known as the smooth chorion. The smooth chorion fuses with the decidua parietalis on the opposite side of the endometrial cavity (Fig 1.4). At the same time the villi within the decidua basalis increase and this region becomes known as the villous chorion (10). This is the developing placenta and is demonstrated in figure 4 as the unshaded region of chorion that appears on ultrasound as a thickened hyperechoic region on one side of the gestational sac.

Figure 1.3. *The three regions of decidua basalis (yellow), capsularis (pink), parietalis (blue) at 6 weeks gestational age.*

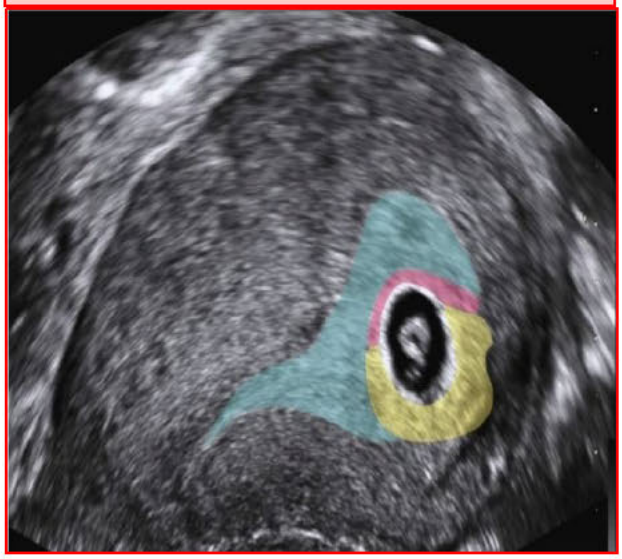
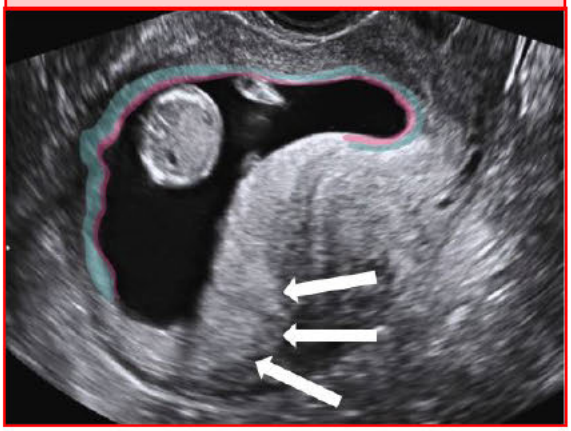


Figure 1.4. *Ultrasound image showing the developing placenta (white arrows) and the relationship between the smooth chorion (pink) and the decidua parietalis (blue) (12 weeks 4 days' gestational age).*



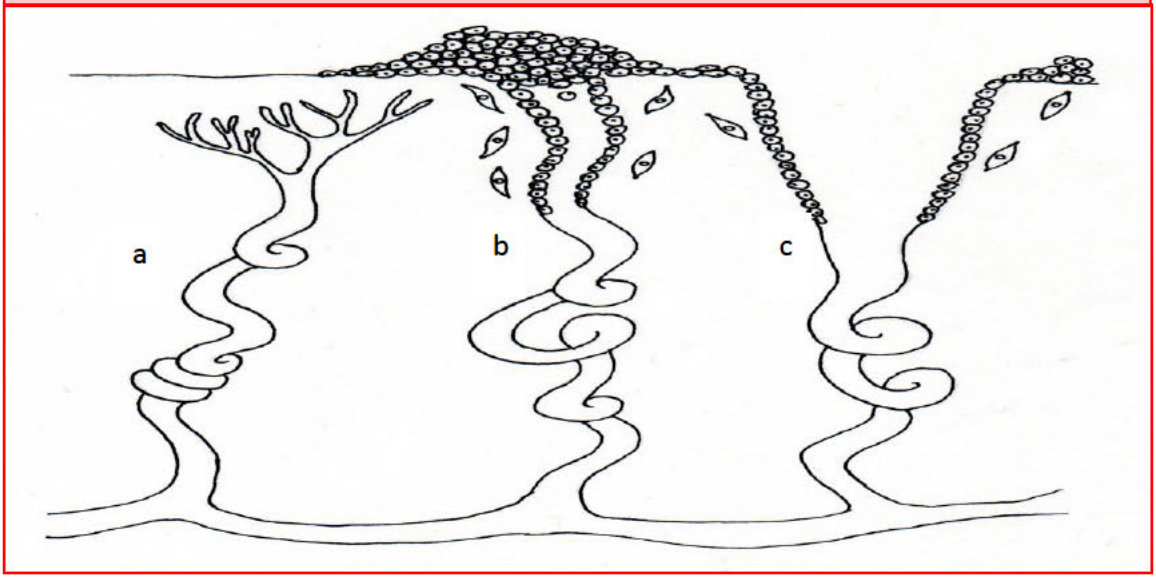
The placental structure is based on the requirements of the uteroplacental circulation. The uteroplacental circulation consists of the maternal supply to the placenta (from the endometrium), the fetal vessels and their communication within the placenta.

The uterus receives its blood supply from the bilateral uterine arteries, branches of the internal iliac arteries. Each uterine

artery branches into progressively smaller arcuate, radial, basal and spiral branches as they penetrate the myometrium into the basal layer of the endometrium (12). Smaller capillaries and venous lacunae are located within the functional layer of the endometrium which is shed each menses.

During pregnancy, physiological changes within the spiral arteries of the endometrium are responsible for adequate perfusion of the placenta and subsequent fetal-maternal circulation. As the syncytiotrophoblast invades the decidual basalis there is a reduction in the media smooth muscle of the spiral arteries, known as spiral artery transformation. Further invasion of the arteries results in their dilatation and loss of elasticity of the smooth muscle fibres (12). These changes result in these arteries being less vasoactive in response to systemic circulatory influences (12). This perpetual state of vasodilation allows unimpeded flow into the intervillous space maximising the maternal side of the fetal-maternal circulation (Fig 1.5) (12).

Figure 1.5. *(a) spiral artery in the non-pregnant decidua. (b) non-transformed spiral artery prior to 8 weeks gestation; (c) normally-transformed spiral artery after 8 weeks gestation. Adapted from [13].*



On the fetal side of the developing placenta the stem chorionic villi anchor the gestational sac to the decidual basalis through the villous chorion. Maternal arteries and veins open into the intervillous space through gaps in the cytotrophoblast allowing exchange of materials between the fetal and maternal

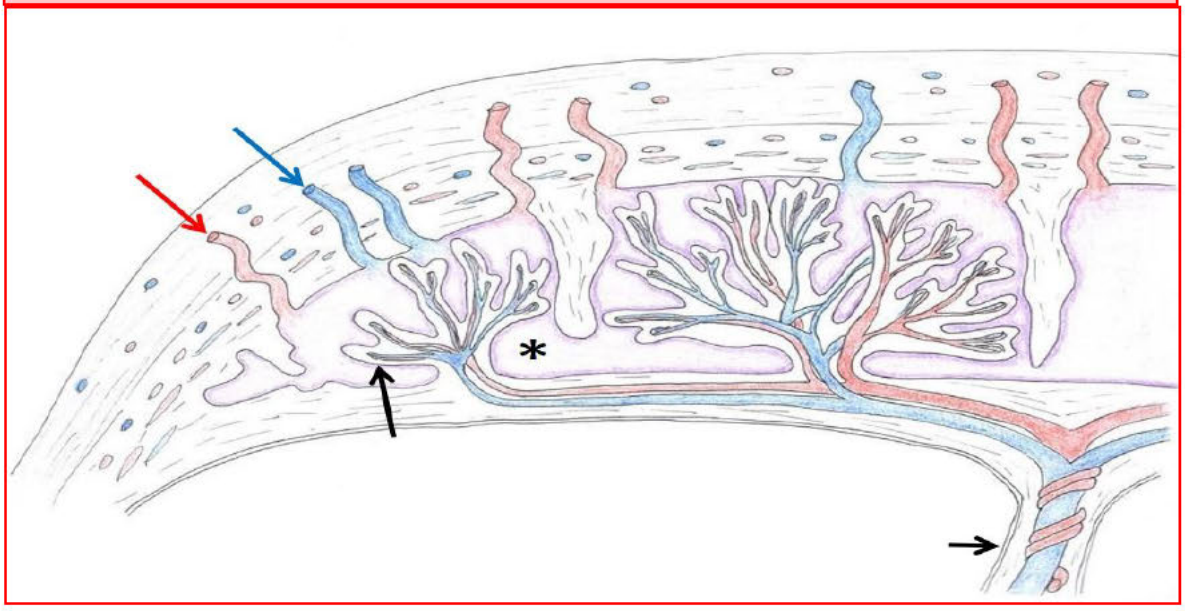
circulations. Continued growth and development sees the enlargement of the intervillous space and it is often discernible on ultrasound (10).

As the placenta develops and more chorionic villi invade into the decidua basalis placenta septa are formed. These septae divide the placenta into irregular shaped regions known as cotyledons. Each cotyledon contains at least two stem villi and their many branches (13).

Blood leaves the embryo/fetus via the (usually two) umbilical arteries and travel through the umbilical cord to the placenta where they branch into chorionic arteries and subsequently form the arteriocapillary venous system within the chorionic villi. Fetal and maternal blood do not normally co-mingle and are separated by the placental membrane. After exchange of materials within the intervillous space (supplied by the spiral arteries of the decidual basalis) blood is returned to the embryo/fetus via the single umbilical vein(10) (Fig 1.6).

Adequate placental function is central to a successful pregnancy and is reliant on its appropriate early development as discussed above. Aberrations in this development may result in early miscarriage or adverse fetal and maternal outcomes later in pregnancy (9). The following sections will discuss the role of the placenta in pregnancy and the causes and implications of placental dysfunction.

Figure 1.6. *Diagram of the placenta components (red arrow: spiral artery, blue arrow: spiral vein, black arrow: chorionic villi, asterisk: intervillous space, thick black arrow: umbilical cord and vessels). Modified from [10].*



THE ROLE OF THE PLACENTA IN PREGNANCY

Placental function during pregnancy is significantly dependent on the establishment of adequate uteroplacental circulation during the development of the placenta early in pregnancy. This is primarily determined by the processes previously described including adequate invasion of the trophoblast into the decidua and complete transformation of the uterine spiral arteries (9).

The main functional components of the placenta are the chorionic villi with precise regulation of the exchange of waste products and nutrients required to ensure optimal function and fetal growth (14). The role of the placenta is diverse and includes the exchange of gases (oxygen and carbon dioxide), the transport and metabolism of nutrients (carbohydrates, amino acids, lipids), the transfer of water, minerals and vitamins, the removal of waste products (including urea, uric acid and creatinine), immunologic protection for the fetus and the secretion of hormones (including human chorionic gonadotrophin, progesterone, oestrogen, human placental lactogen) (14).

CAUSES OF PLACENTAL DYSFUNCTION

Placental dysfunction can be defined as an abnormality in placental circulation that compromises fetal oxygenation and nutrition (15) and has been linked to placental ischemia and hypoxia which, at least in part, are considered the result of poor trophoblastic invasion and resultant inadequate transformation of the spiral arteries (16).

Transformation of the uterine spiral arteries is essential for adequate placental function in pregnancy. Insufficient transformation of the these vessels results in their failure to convert into low resistance channels (12, 17). This, in turn, results in a variable oxygen supply to the placenta resulting in progressive damage and restriction of nutrients to the developing fetus; the results of which may manifest later in pregnancy (18).

Normal transformation of the spiral arteries has been attributed to immune processes within the decidua (19) followed by the destruction of the smooth muscle within the vessel media by trophoblast cells (20). The molecular mechanisms involved in abnormal transformation of the spiral arteries are

unclear and may include pathologic processes such as endothelial dysfunction, infection and inflammation (21). It has been proposed that endothelial dysfunction may contribute to the shallow invasion of trophoblast into the spiral arteries with activated endothelial cells (those in a proinflammatory / procoagulant state) more resistant to invasion than non-activated cells (22). This results in the failure to completely transform these vessels through their full depth from the decidua into the myometrium (23).

Evidence of non-specific inflammatory changes have been identified in placentae of growth restricted fetuses (24) and have been associated with maternal-fetal infection and, in the absence of infection, been attributed to abnormal maternal-fetal immune reactions (25). While the causal link between placental dysfunction and infection / inflammation has not been established it has been proposed that fetal blood flow is reduced due to occlusion of the fetal stem vessels within the placenta (26).

IMPLICATIONS OF PLACENTAL DYSFUNCTION

Early placental dysfunction has been linked to the adverse pregnancy outcome of miscarriage (27). Manifestations of chronic placental dysfunction include a small fetus, IUGR, MHD and pregnancy complications such as placental abruption, preterm labour and delivery (16). In addition, the small, growth restricted fetus and the mother are at an increased risk of a myriad of disorders later in life (28, 29).

The first evidence of a growth restricted fetus is the diagnosis of small fetal size with ultrasound. All fetuses with an estimated weight of below the 10th centile are considered at risk for IUGR (30). A large proportion of these fetuses are born early and as such are susceptible to the risks of prematurity (31). In addition, they are also vulnerable to a higher prevalence of a myriad of perinatal conditions including oligohydramnios, caesarean delivery, low Apgar scores, acidosis, polycythemia, apnoea, hypoglycemia, hypothermia, sepsis, seizures, stillbirth and neonatal death (32). Approximately 50% of stillbirths are associated with undiagnosed IUGR (33). In the longer term, an infant born small and growth restricted is at an increased risk later in life of developing osteoporosis (28), obesity and some cancers (34), stroke (28, 35),

diabetes (28, 34, 35), hypertension and heart disease (28, 32, 34, 35).

There is also a significant increase in maternal risks both in the short and long term. If the placental dysfunction is associated with PET then short term maternal complications include placental abruption, progression to eclampsia requiring immediate delivery and the development of life-threatening HELLP Syndrome (haemolysis, elevated liver enzymes and a low platelet count)(36). Long term there is an increased risk that subsequent pregnancies will be affected and that the mother will develop cardiovascular disease and metabolic disorders later in life if they have a history of PET or delivering a low birthweight infant (29, 37, 38).

SUMMARY

It is clear from the discussion in this section that the developmental origins of the placenta begin at fertilisation and that the placenta has a significant role to play in the success of every pregnancy and long-term health of both the mother and child. Currently the prediction of miscarriage, SGA and MHD focusses on pregnancies after 11 weeks gestation.

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With the role of ultrasound prior to 11 weeks gestation continually evolving, the earlier prediction of these adverse pregnancy outcomes may be possible, allowing for earlier intervention with the aim of improving outcomes for all potentially affected pregnancies. The following section will discuss the role of ultrasound prior to 11 weeks gestation and introduce both conventional and novel ultrasound measures that can be obtained.

ULTRASOUND IN THE FIRST TRIMESTER OF PREGNANCY

INTRODUCTION

The current role of ultrasound in the first trimester of pregnancy (<11 weeks gestation) is defined as identification of pregnancy location, assessment of fetal number and gestational age and confirmation of viability with heart movement (39). Ultrasound is also used in the assessment of early pregnancy to assess the risk of miscarriage (40).

Conventional ultrasound measures prior to 11 weeks gestation are assessed in all pregnancies with the aim of diagnosing normality. These conventional measures include assessment of maternal uterus and ovaries, gestational sac, YS, embryo, heart movement and amnion (41). Investigation of novel ultrasound measures prior to 11 weeks gestation has been limited. Trophoblast at 5-12 weeks gestation has been reported to be thinner (42) and smaller in volume (43) in pregnancies that subsequently miscarry however there are no identified studies that have addressed the value of trophoblast thickness or volume in the prediction of SGA or MHD. Uterine artery Doppler is currently assessed at 11-13^{+6} weeks gestation for the prediction of SGA and MHD (44) however its value when assessed prior to 11 weeks gestation has not been reported.

This section will first discuss conventional ultrasound measures individually prior to introducing the novel ultrasound measures. This will be followed by a discussion of the published literature addressing their value in the diagnosis and prediction of adverse pregnancy outcomes and identifying specific gaps in the literature that will be addressed in this thesis.

CONVENTIONAL ULTRASOUND MEASURES

Maternal Structures

Any ultrasound examination in the first trimester of pregnancy requires a thorough assessment of the maternal pelvis. The primary organs which should be assessed include the uterus, cervix and ovaries (39). The adnexae and pouch of Douglas should also be evaluated (39).The purpose of this overall assessment is to confirm normality of the reproductive organs and to locate and document any pathological findings that may or may not impact the management and outcome of the pregnancy. In addition, complete

assessment will confirm the number and location of any pregnancies (8, 39).

The structures of an early pregnancy can be visualised by ultrasound in a predictable order. Firstly, the gestational sac appears followed by the YS, the fetus, FHM and lastly, the amnion (11). Beginning with the gestational sac each of these structures will now be discussed.

Gestational Sac

An intrauterine gestational sac is first demonstrable with transvaginal ultrasound at approximately 21 days' post-conception; 5 weeks' gestational age (45). At this stage it measures less than 1ml in volume (46). The gestational sac first appears as a rounded, anechoic (black) structure adjacent to the endometrial surface circumferentially surrounded by a region of hyperechogenicity (brightness) that embryologically corresponds to the developing trophoblast and sonographically is known as the decidual reaction (11) (Fig 1.7).

Visualisation of this early "gestational sac" does not confirm an intrauterine location of the pregnancy. It is possible that a pseudo-gestational sac, similar in appearance to an early gestational sac may form within the uterus in conjunction with an ectopically located true gestational sac (8). Confirmation of this early intrauterine sac as the true gestational sac must be made before any pregnancy risk assessment can be considered. This is achieved with demonstration of the YS (8) (Fig 1.8).

Figure 1.7. *Ultrasound image of an early intrauterine pregnancy (approximately 5 weeks gestation) with the gestational sac deep to the endometrial surface (white asterisk: gestational sac, black asterisk: the decidual reaction, black arrows: the white line between the arrows represents the two adjacent endometrial surfaces).*

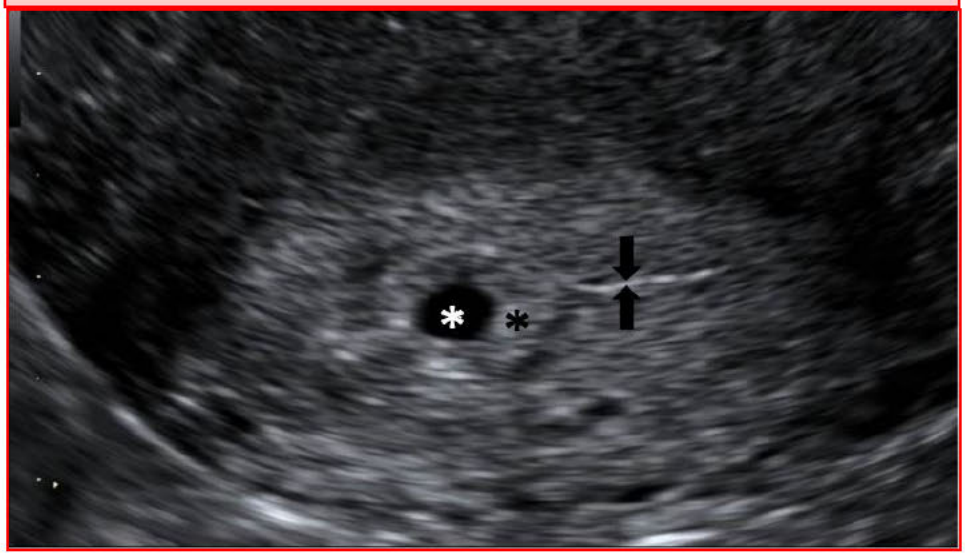
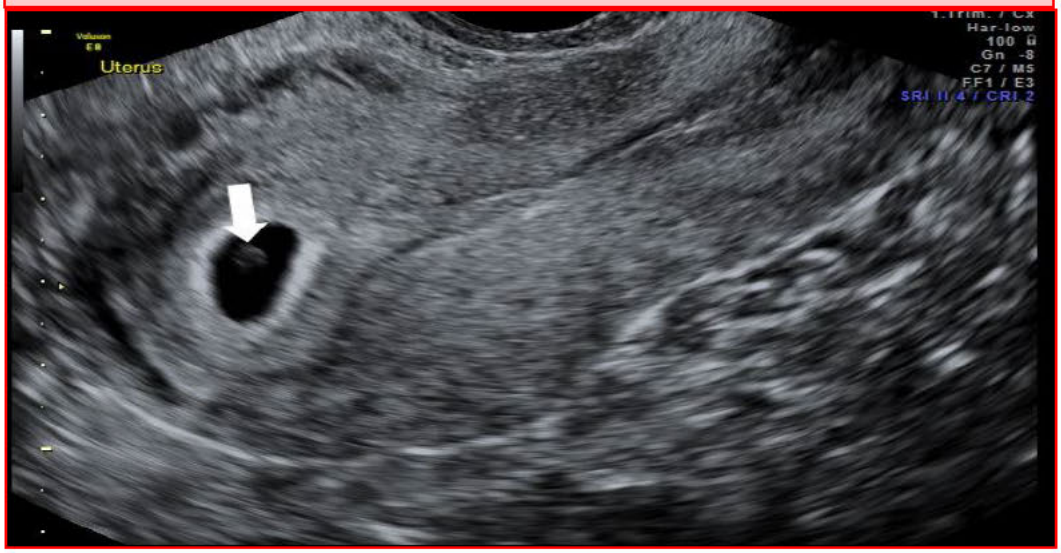
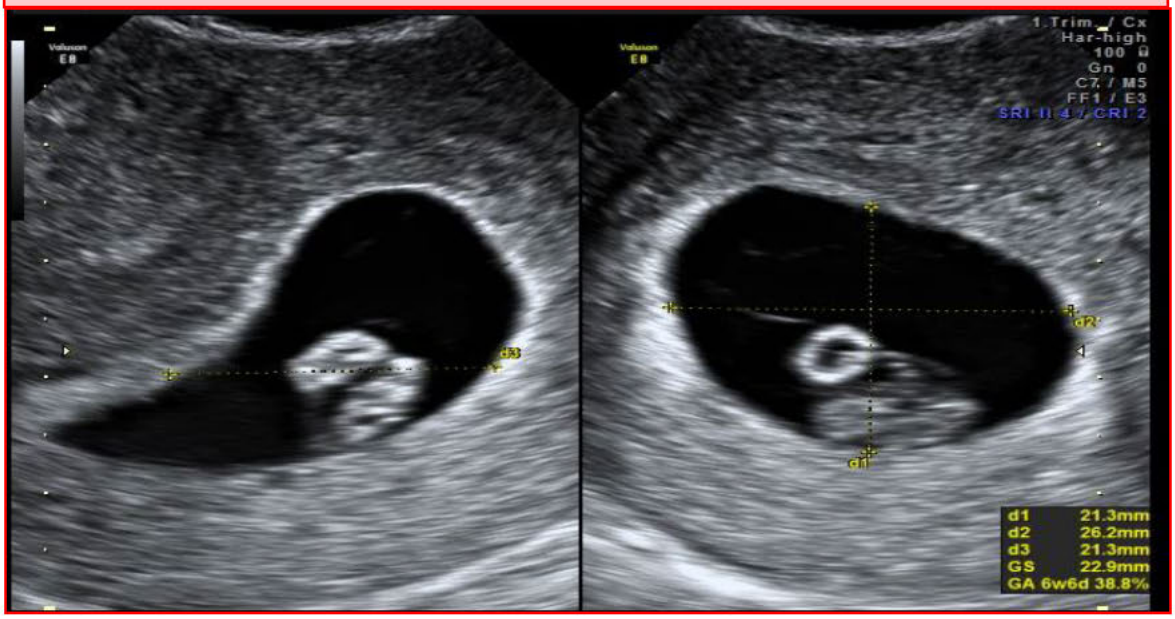


Figure 1.8. *True intrauterine gestational sac (white arrow: yolk sac).*



Once a true intrauterine gestational sac has been confirmed, and prior to demonstration of an embryo, the gestational sac it can be measured to estimate the gestational age of the pregnancy (46, 47). This is achieved by recording two ultrasound images; the first is the longest section of the gestational sac; the second is 90 degrees to the first image. Manual measurements are then recorded in three dimensions (longest diameter (A), perpendicular to this diameter (B) and the widest cross-section (C). The mean sac diameter (MSD) is then calculated automatically by the ultrasound machine using the formula: (A+B+C) /3 (Fig 1.9) (47) and gestational age presented based on published charts (48).

Figure 1.9. *Gestational sac at 6 weeks 6 days: mean sac diameter. d: diameter; GS: mean sac diameter; GA: gestational age in weeks and days.*



The role of the MSD in the diagnosis and prediction of adverse fetal and maternal outcomes will be discussed later in this chapter.

In the research presented in this thesis the MSD will be considered in the prediction models for miscarriage prior to 12 weeks gestation (Chapter 3), SGA (Chapter 5) and MHD (Chapter 6). After demonstration of the gestational sac the next structure to become visible with ultrasound is the YS.

The ultrasound features of the YS will now be discussed.

The secondary yolk sac

The YS is first seen with transvaginal ultrasound on approximately Day 24 post conception; 5 weeks 3 days' gestational age (49). It is the first extraembryonic structure demonstrated with ultrasound within the gestational sac and its presence confirms the pregnancy location as intrauterine (8). The normal YS can be described sonographically as a thin, circular echogenic (bright) structure with anechoic (black) content (Fig 1.10).

The size of the yolk sac can be documented in three ways: the internal diameter (Fig 1.11) (50), the average diameter (51) or the volume (52). Three-dimensional volume assessment of the yolk sac has been shown to offer no additional value to two-dimensional measurements (53).

Figure 1.10. *Normal appearance of the secondary yolk sac (white arrow) at approximately 5 weeks gestational age.*

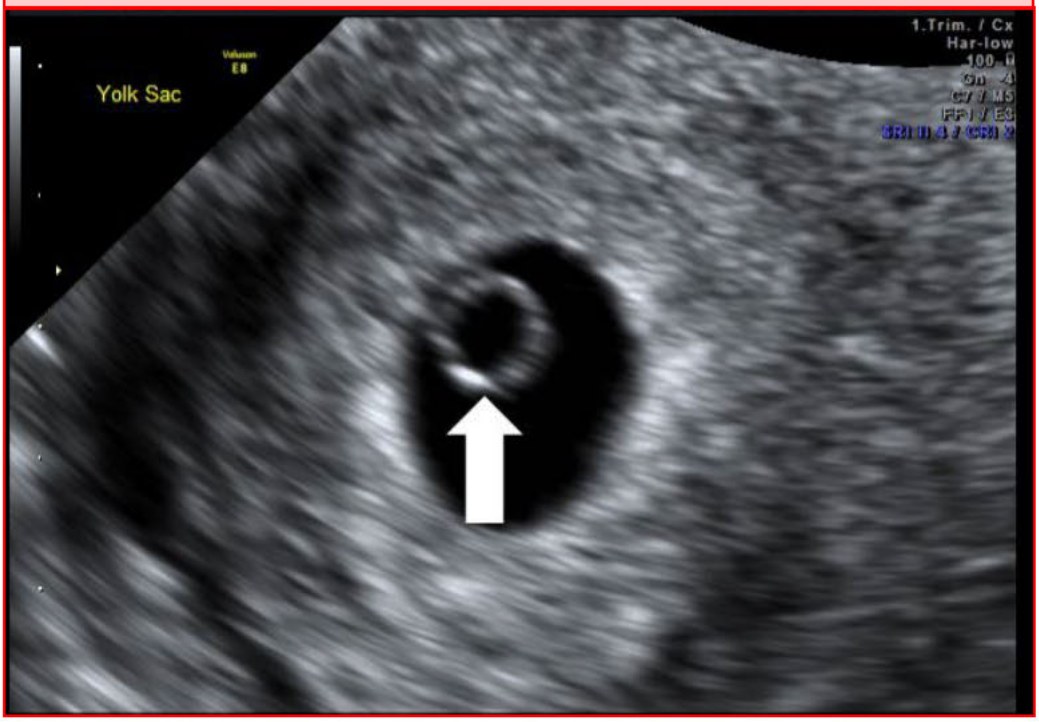
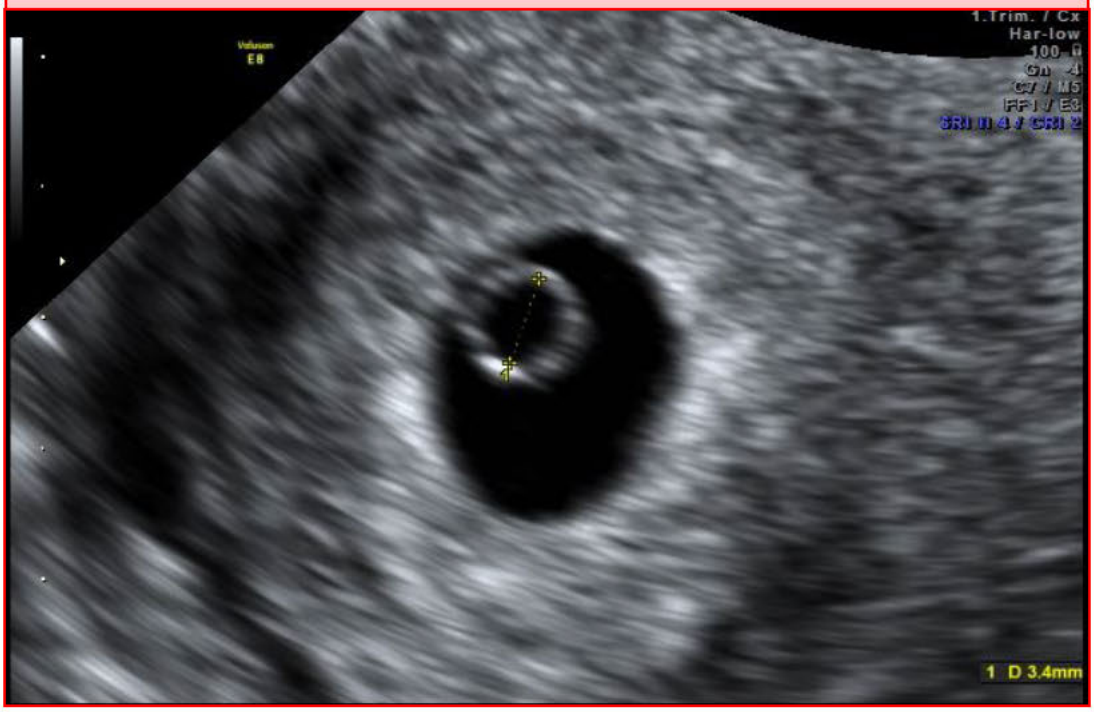


Figure 1.11. *Internal diameter measurement of a normal secondary yolk sac (5-6 weeks gestational age).*



Published research addressing the measurement methodology of the YS mostly compares the size of YS in normal pregnancies to pregnancies which ultimately miscarry. The research presented in this thesis uses the internal diameter of the secondary yolk sac diameter (YSD) as proposed by Lindsay et al. (1992) who presented a simple and reproducible measurement approach comparing a larger cohort of normal and abnormal pregnancies compared to other studies (50-52).

The role of the YSD in the diagnosis and prediction of adverse fetal and maternal outcomes will be discussed further later in this chapter. In the research presented in this thesis the YSD will be considered in the prediction model for miscarriage prior to 12 weeks gestation (Chapter 3).

After demonstration of YS the next structure to become visible with ultrasound is the fetus. The ultrasound features of the fetus will now be discussed.

Embryo

The embryo can be first demonstrated with transvaginal ultrasound between 21-28 days' post conception; 5-6 weeks' gestational age (11). The early embryo is first visualised as a thickening at one margin of the secondary yolk sac when it measures approximately 2mm in length (11, 41). As the pregnancy progresses and the embryo continues to grow it develops into a recognisably human form before the end of the first trimester and from 10 weeks gestational age is known as a fetus (54). Figures 1.12-1.14 demonstrate ultrasound images of an embryo at 7 and 9 weeks and fetus at 11 weeks gestational age respectively.

Figure 1.12. *Embryo at 7 weeks gestational age; mean CRL (white line:10mm [56].*

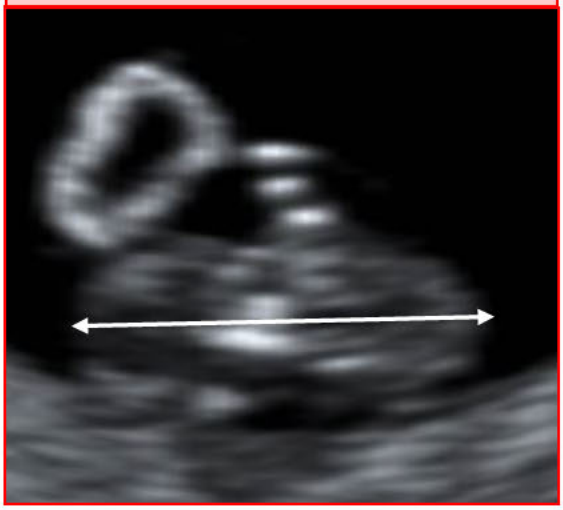


Figure 1.13. *Embryo at 9 weeks gestational age; mean CRL (white line):23mm [56].*

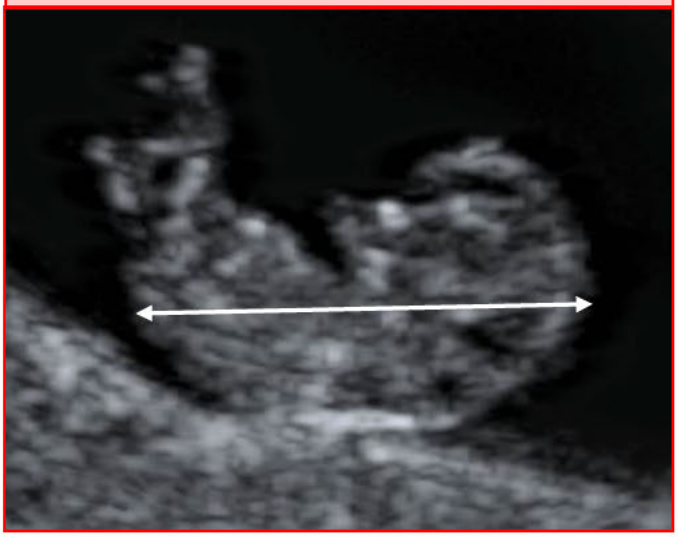
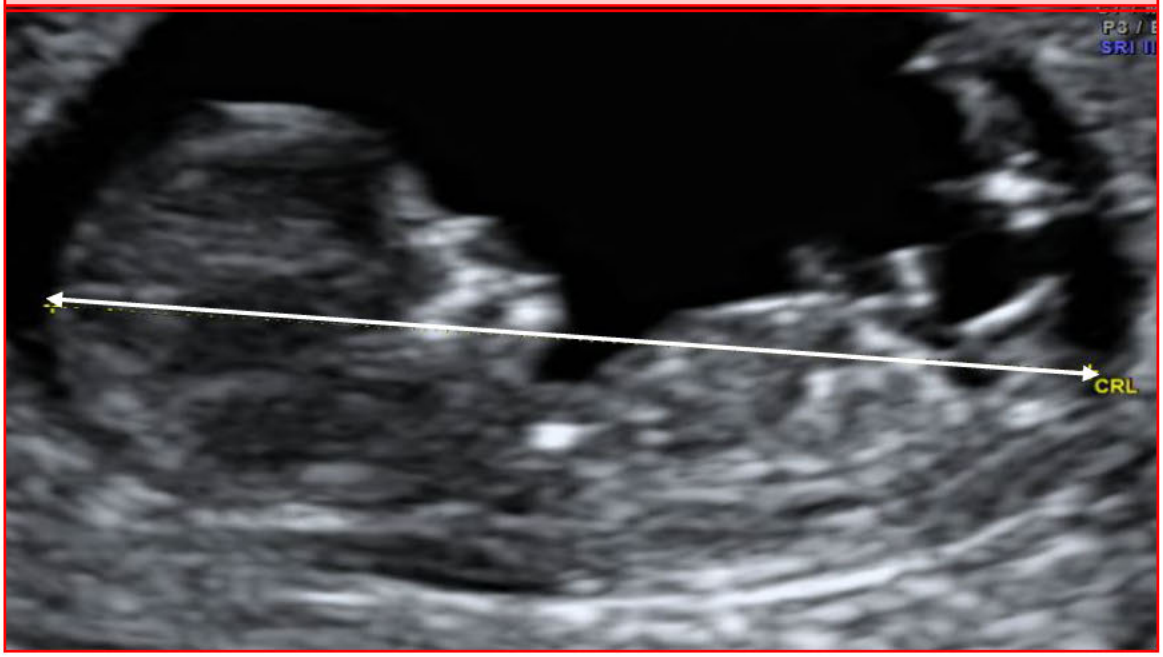


Figure 1.14. *Embryo at 11 weeks' gestational age; mean CRL (white line):42mm [56].*



Using ultrasound, the length of the embryo is referred to as the crown-rump length (CRL). Prior to 18mm, a gestational age of approximately 8 weeks 2 days (55), this measurement represents the length of the linear embryo (Fig 1.15). As the embryo grows its changing shape more precisely conforms to a recognisable human form. When >18mm, the CRL represents the longest length of the curved embryo in a straight line (11) (not the distance from the absolute cranial to caudal margins) (Fig 1.16).

Figure 1.15. *Embryo with a crown rump length of less than 18mm (linear) (white line); less than 8 weeks 2 days gestational age.*

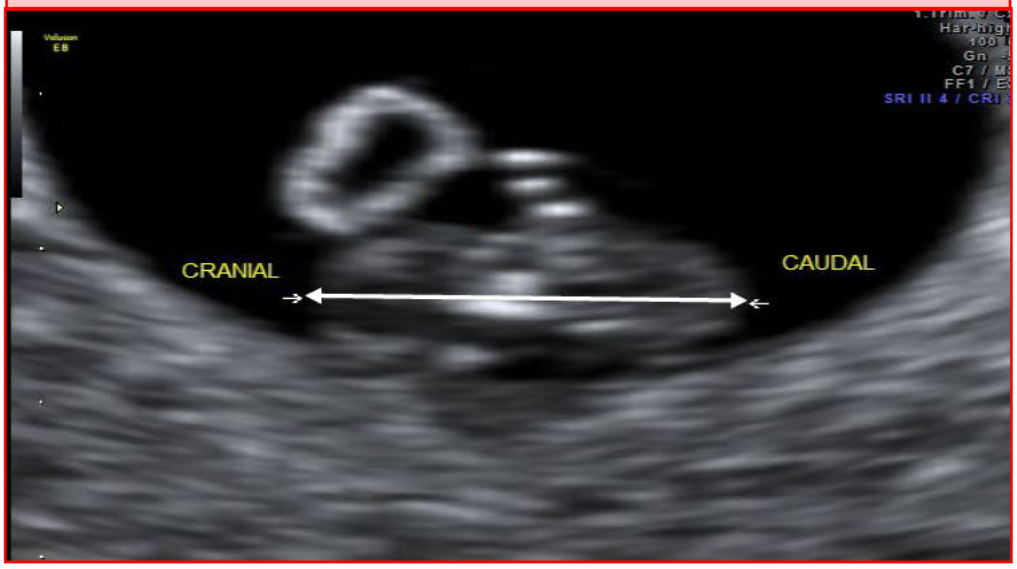
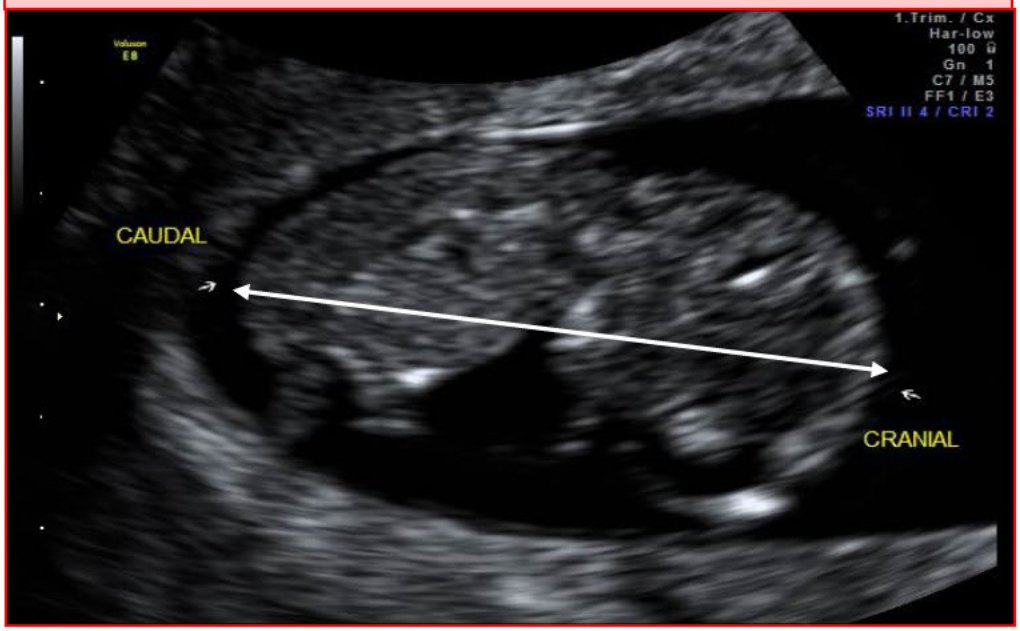


Figure 1.16. *Embryo with a crown rump length (white line) of greater than 18mm (curved); greater than 8 weeks 2 days gestational age.*



The CRL of the embryo has several uses; dating of the pregnancy (46, 56), as one of the criterion for the diagnosis of early pregnancy failure (57) and for the prediction of adverse pregnancy outcomes such as embryonic demise(58), aneuploidy (59-61) and placenta-related disorders later in the pregnancy including IUGR (62).

The use of the CRL for pregnancy dating was first described by Robinson & Fleming (1975)(46). The correlation of gestational age and CRL has been validated by many authors since, with the criteria for gestational age based on CRL in pregnancies of less than 7 weeks redefined by Hadlock et al. (1992) (56). It is these findings that are universally accepted as the most accurate method of pregnancy dating in the first trimester and are currently used in clinical practice around the world (63). For these reasons the research presented in this thesis uses the CRL as measured by ultrasound as a proxy for the gestational age of the pregnancy and will be considered in the diagnosis and prediction of adverse fetal and maternal outcomes. This will be discussed further later in this chapter. In the research presented in this thesis the CRL will be considered in the prediction models for miscarriage prior to 12 weeks gestation (Chapter 3), SGA (Chapter 5) and MHD (Chapter 6).

After demonstration of the fetus the next ultrasound feature to become visible with ultrasound is embryonic heart movement. In clinical practice this is commonly referred to as the fetal heart movement (FHM) and will referred to as such from this point. The ultrasound measurement of FHM will now be discussed.

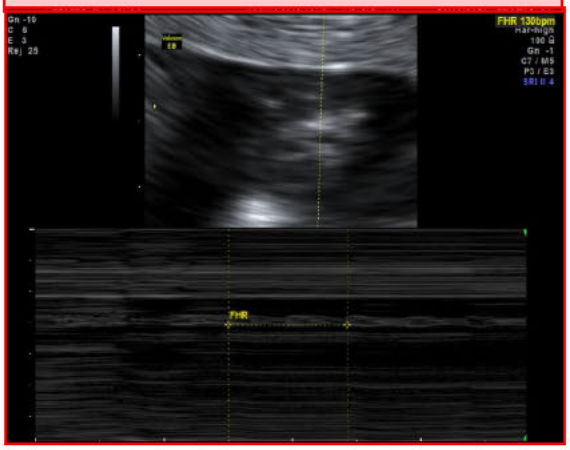
Heart movement

Heart movement begins at Day 21 post-conception at approximately 5 weeks' gestational age, and is demonstrated on ultrasound as a "flickering" in the central region of the embryo on real-time imaging. The fetal heart rate (FHR) can be measured with ultrasound using Motion-Mode (M-Mode). M-Mode enables the ultrasound machine to detect the motion of the heart and the heart rate can be measured from the captured image (Fig 1.17).

Heart rate increases with advancing gestational age from approximately "110bpm at 6 weeks to a maximum of 175bpm at 9 weeks" (48). After this age the FHR steadily decreases to approximately 160-170bpm (64). Published ranges are available that detail

the expected heart rate at different gestational ages within the first trimester (48) however these are not used to predict or establish gestational age in clinical practice (8).

Figure 1.17. *M-Mode trace of fetal heart movement with rate (130 beats per minute) measured in the first trimester.*



In a normally progressing pregnancy, following the detection of the embryo, heart movement should be demonstrated by the time the CRL reaches 7mm. Failure to demonstrate heart movement when the CRL is greater than or equal to 7mm is diagnostic of a failed pregnancy (57).

The role of the FHR in the diagnosis and prediction of adverse fetal and maternal outcomes will be discussed later in this chapter. In the research presented in this thesis the FHR will be considered in the prediction models for miscarriage prior to 12 weeks gestation (Chapter 3).

After demonstration of the FHR, the next ultrasound feature to become visible with ultrasound is the amnion. The ultrasound appearances of the amnion will now be discussed.

Amnion

Subsequent to the appearance of an embryo and heart movement the amnion is first demonstrated with transvaginal ultrasound at approximately 38 days' post conception; 7.5 weeks' gestational age (11). It is seen within the gestational sac as a thin membrane that surrounds the embryo forming the amniotic cavity (inside the amnion) and excluding the secondary yolk sac within the extraembryonic coelom (outside the amnion). Figures 1.18-1.20 demonstrates the ultrasound appearance of the amnion at 7, 9 and 11 weeks gestational age respectively.

Figure 1.18. *Amnion at 7 weeks.*

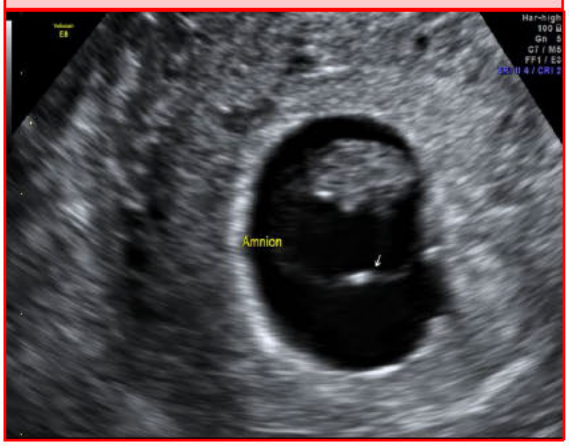


Figure 1.19. *Amnion at 9 weeks.*

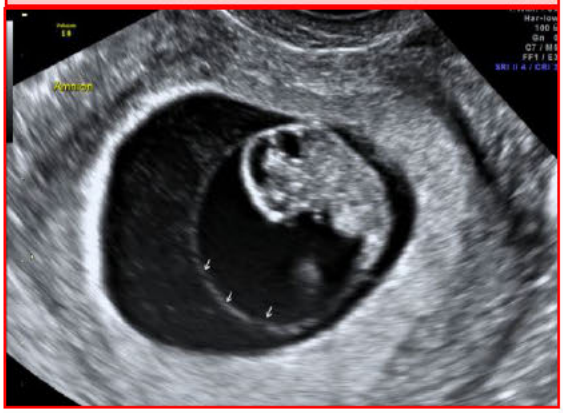
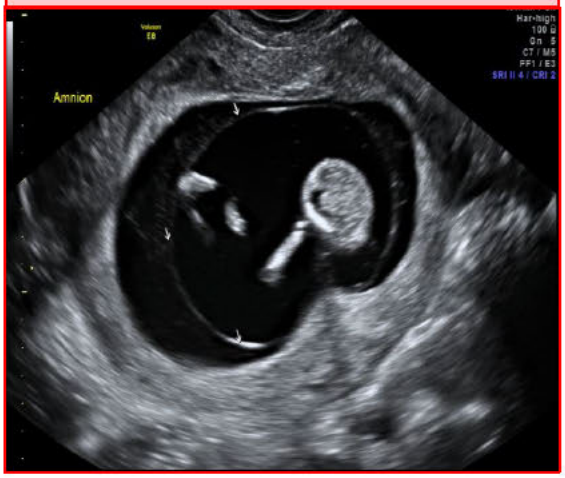


Figure 1.20. *Amnion at 11 weeks' gestational age.*



The amnion represents a normal developmental landmark with visualisation serving several purposes including confirmation of a free-moving embryo within the amniotic cavity excluding rare abnormalities such as limb-body wall complex. In a multiple pregnancy the number of amnions has important implications for management and prognosis and normal amniotic fluid volume suggests adequate diffusion of fluids from the uterine cavity (prior to 11 weeks' gestational age) and fetal renal function after 11 weeks' gestational age (10).

This section has presented the conventional ultrasound measures made prior to 11 weeks gestation including assessment of the maternal structures, gestational sac, secondary yolk sac, embryo, FHR and amnion. The following section will introduce novel ultrasound measures of trophoblast thickness and volume and uterine artery Doppler that will later be investigated to determine if they add value to conventional measures in the prediction of adverse pregnancy outcomes.

NOVEL ULTRASOUND MEASURES

Trophoblast thickness

As discussed earlier in this chapter the placenta develops from the layers of trophoblast (within the chorion) surrounding the early gestational sac (10). The chorion is demonstrable on first trimester ultrasound as the region of hyperechogenicity circumferentially surrounding the gestational sac (the decidual reaction) (11). The inner margin of the trophoblast is clearly delineated due to its boundary with the gestation sac (containing anechoic fluid). The decidua is less echogenic than the adjacent trophoblast allowing for clear demonstration of the outer boundary of the trophoblast. It is this decidual reaction that for the purposes of the research in this thesis will be called 'trophoblast' (Fig 1.21).

Measurement of trophoblast thickness was described by Bajo et al. (2000) in a study designed to assess the evolution of pregnancy based on the trophoblast thickness measured from 5 to 12 weeks gestational age in 592 low-risk pregnancies (42). This well-designed study describes measuring the thickness of the trophoblast thickness at the cord insertion site or adjacent to the secondary yolk sac if the cord insertion site cannot be identified. Results demonstrated that a discrepancy of ≥3mm between trophoblast thickness and gestational age in weeks was predictive of subsequent miscarriage with a sensitivity of 82% (42).

Figure 1.21. *Trophoblast appearances at 7 weeks gestational age. white arrowheads: inner margin of trophoblast; black arrowheads: outer margin of*

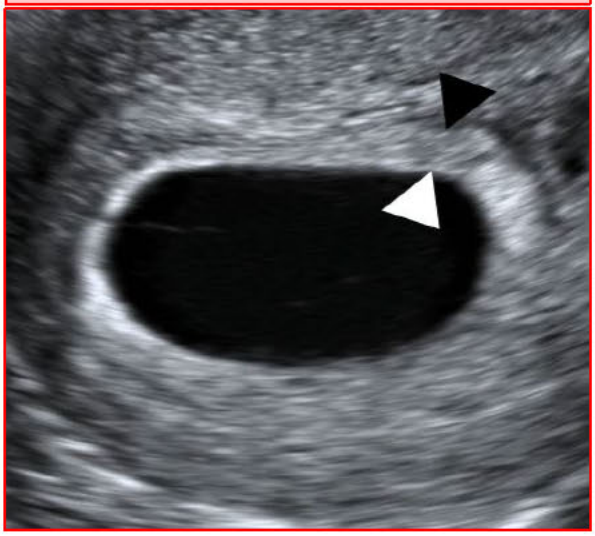
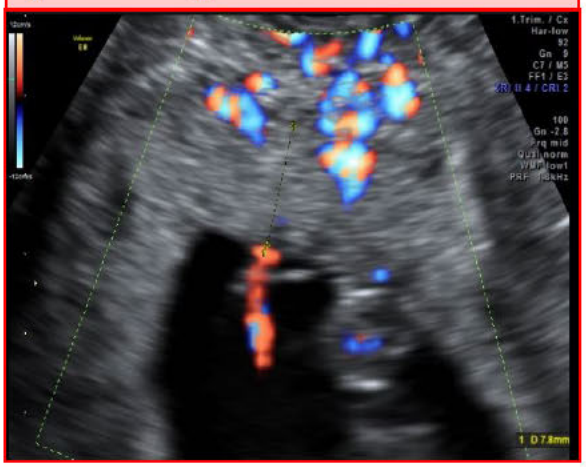


Figure 1.22 demonstrates measurement of trophoblast thickness (at the cord insertion site) at 8 weeks 2 days gestational age. In contrast, an earlier study investigating the difference in trophoblast thickness at less than 10 weeks gestational age between pregnancies that resulted in a live birth (n=77) and those that subsequently

miscarried (n=36) found no significant difference between the two groups (65),

Figure 1.22. *Trophoblastic thickness (7.8mm) measures at the cord insertion site (white arrowhead) at 8 weeks 2 days gestational age.*



Trophoblast volume

Trophoblast volume is assessed with the use of three-dimensional (3D) ultrasound imaging. Virtual Organ Computer-Aided Analysis (VOCAL) software (3-dimensional Sonoview; GE Healthcare) was first described in the measurement of placental volume at 11-13^{+6} weeks gestation (66) and is used off-line after the examination to calculate the required volumes for analysis.

The VOCAL method of volume calculation begins with a 3D volume sweep of the uterus that includes the outer margins of the trophoblast. After this sweep is taken the 3D data is stored for off-line assessment. A feature of VOCAL software is the option for the operator to select the rotation angle that the volume data is rotated through for each step of the measurement process. Selecting a 15-degree rotation angle will result in 12 sections being individually traced by the operator for the volume to be calculated. In comparison, selecting a 30-degree rotation angle results in fewer sections to be traced (six) whilst a 12-degree rotation angle results in a greater number of sections (15).

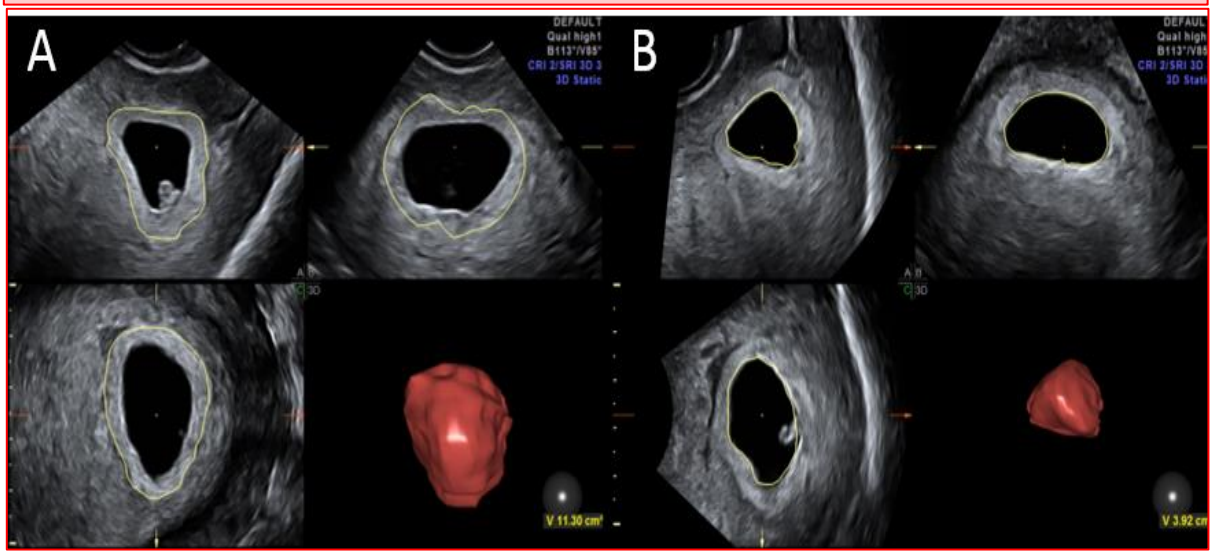
A number of studies have compared of volume measurements calculated by different rotation angles. Nowak et al. (2008) found a high correlation between placental volumes measured at 7-10 weeks gestational age with 12 and 30-degree rotation angles, with an intraclass co-efficient (ICC) of 0.994 (95%CI: 0.997-0.9. In addition, good intra- and interobserver reproducibility of trophoblast volume measurements has been demonstrated at a similar gestational age with a mean intraobserver difference of 0.2mls representing an ICC 0.979 (95%CI: 0.943-0.995); p=0.603 and a mean interobserver difference of 0.2mls representing an ICC 0.971 (96%CI: 0.920-

0.990); p=0.743 respectively (67). These studies are limited by their small sample sizes however similar findings in a larger study by Wegrzyn et al. (2005) showed similar results for placental volume measurements calculated with a 15-degree rotation angle at 11-13^{+6} weeks gestation; mean intraobserver difference: 0.6mls; mean interobserver difference: 0.7mls (66). Due to the smaller number of measurement sections required and the similar reproducibility in the volumes measured the decision was made to use a 15-degree rotation angle (12 sections in total) using the sagittal plane as reference (66) for the calculation of trophoblast volume in this research.

Acquisition of the 3D volume during the ultrasound examination takes less than 10 seconds and the volume is stored for later off-line analysis. After the examination is complete the outer margin of the trophoblast is manually traced by the operator in each of the 12 sections. The software calculates the resulting total pregnancy volume at the completion of the rotations (Fig 1.23a). Secondly, the inner margin of the trophoblast is manually traced using the same method and the resulting gestational sac volume is calculated by the software (Fig 1.23b). The trophoblast volume is calculated using the formula:

Trophoblast volume (millilitres) = total pregnancy volume - gestational sac volume.

Figure 1.23. *Trophoblastic volume measurement; (a) total pregnancy volume (b) gestational sac volume.*



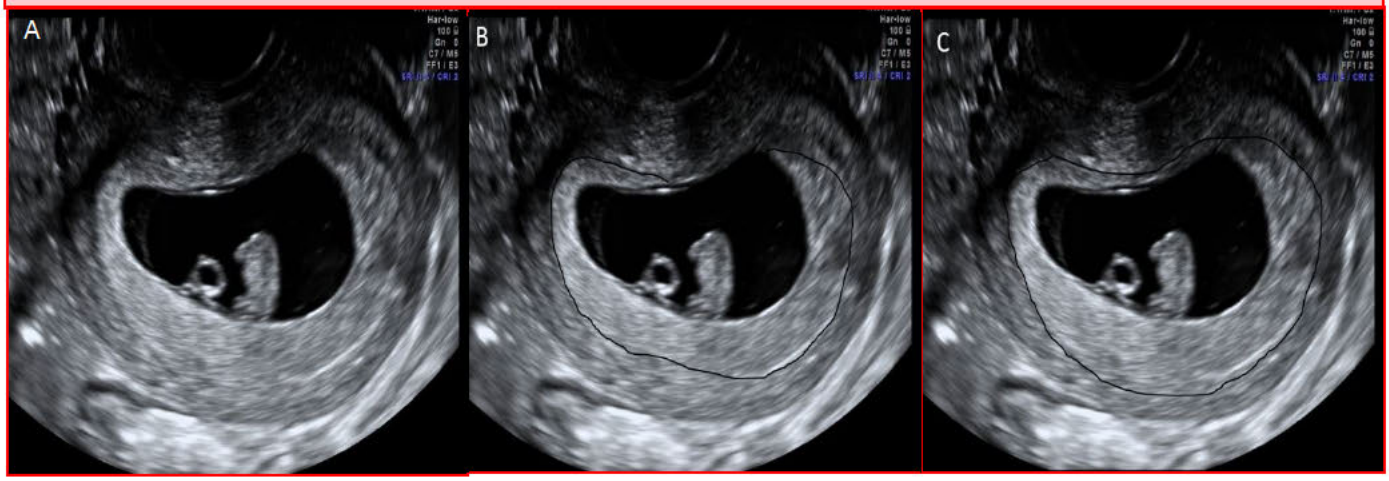
There have been few studies assessing trophoblast volume in its entirety prior to 11 weeks gestation. One prospective study using the VOCAL technique of volume measurement compared trophoblast volume at 6-12 weeks gestation between 112 normal pregnancies and 56 pregnancies which ultimately miscarried. Sequential scanning of these pregnancies resulted in 380 measurements of trophoblast volume over this gestational age range; normal outcome (n=273) and miscarriage (n=107). Comparison of Z-scores demonstrated that trophoblast volume in pregnancies that miscarried was significantly smaller compared to those that did not (-1.28 +/-1.23 versus -0.09 +/-0.99; p <0.01) (43). The clinical significance of this difference in the prediction of miscarriage is discussed in Chapter 3 of this research. Chapters 5 and 6 investigate the potential value of trophoblast volume prior to 11 weeks gestation in the prediction of SGA and MHD respectively.

The majority of previous studies have focussed on the calculation of placental volume rather than the trophoblast as a whole (68, 69). In addition, most of this work has assessed pregnancies after 11 weeks gestation (70-73) with only small studies investigating placental volume prior to this age (67, 74). The placental margins early in pregnancy can be difficult to define on ultrasound, as found in a small study of 37 pregnant women between 7- and 10-weeks gestation where Nowak et al. (2008) stated that the ability to correctly identify the placenta prior to 10 weeks was a limitation of their work (74). This was addressed by the authors with the use of a transvaginal transducer to increase the resolution of the ultrasound images. To address this limitation, the research presented in this thesis measures the volume of the entire trophoblast, eliminating the need to clearly identify the early placental boundaries (Figure 1.24 (a-c)) with the aim of providing a more reliable and reproducible volume estimation.

The translatability of trophoblast volume into clinical practice may be limited to services with dedicated 3D transvaginal transducers and access to VOCAL software. Despite these limitations the skills needed for trophoblast volume calculation are easily acquired by sonographers of all levels of expertise, there is negligible change in patient scanning time and only two to three

minutes of off-line analysis are required. In the research presented in this thesis trophoblast thickness and volume will be considered in the prediction models for miscarriage prior to 12 weeks gestation (Chapter 3), SGA (Chapter 5) and MHD (Chapter 6). The following section will discuss the novel use of uterine artery Doppler prior to 11 weeks gestation.

Figure 1.24. *(a) Ultrasound image of an intrauterine pregnancy at 8 weeks gestational age; (b) the same image with placental boundaries "estimated" (black line); (c) The same image with the outer boundaries of the entire trophoblast clearly defined (black line).*



Uterine Artery Doppler

As discussed earlier in this chapter the uterus receives its blood supply from the bilateral uterine arteries (12). Doppler ultrasound can be used in these vessels to demonstrate normal or restricted blood flow to the uterus (75). The process of placentation normally involves 'transformation' of the spiral arteries within the endometrium from high resistance/low flow to low resistance/ high flow which is complete by 16 weeks (76). Failure to develop this low resistance circulation is recognised as a key feature of placental insufficiency and has been linked to the development of adverse fetal and maternal outcomes later in pregnancy (77, 78).

Doppler studies have shown that uterine artery flow in all human pregnancies is altered from the 8th week of gestation (79). Prior to this gestational age researchers have shown that the spiral arteries are occluded by invading

trophoblast that restrict perfusion of the developing pregnancy (80) with many concluding that this is necessary to protect the developing fetus from very high level of oxygen at this stage (81, 82).

Despite changes in uterine artery blood flow being demonstrated from 8 weeks gestation previous studies have focused on the Doppler assessment of uterine artery flow from 11 weeks' gestational age onwards (83). These studies report that an increased resistance to flow is associated with an increased risk of placental-related adverse outcomes including PET (84) and SGA/IUGR (85). No studies have been identified in the literature investigating the use of uterine artery Doppler prior to 11 weeks gestation and its relationship with adverse maternal and fetal outcomes, including miscarriage.

The method of Doppler assessment of the uterine arteries is well-documented with a transabdominal approach at 11 to 13^{+6} weeks gestation (86) and is readily extrapolated to the transvaginal approach prior to 11 weeks (87). Colour Doppler is used to identify each uterine artery lateral to the internal os of the cervix and spectral Doppler is used to obtain a representative uterine artery waveform. The sample gate size should be set to 2mm to cover the entire vessel and the insonation angle should be less than 30 degrees to optimise the quality of the waveform. At least three consecutive waveforms are recorded and the pulsatility index (PI) is calculated either by manual tracing of the waveform or with automatic measurement by the ultrasound machine (Fig 1.25) (86). The PI is defined by the formula:

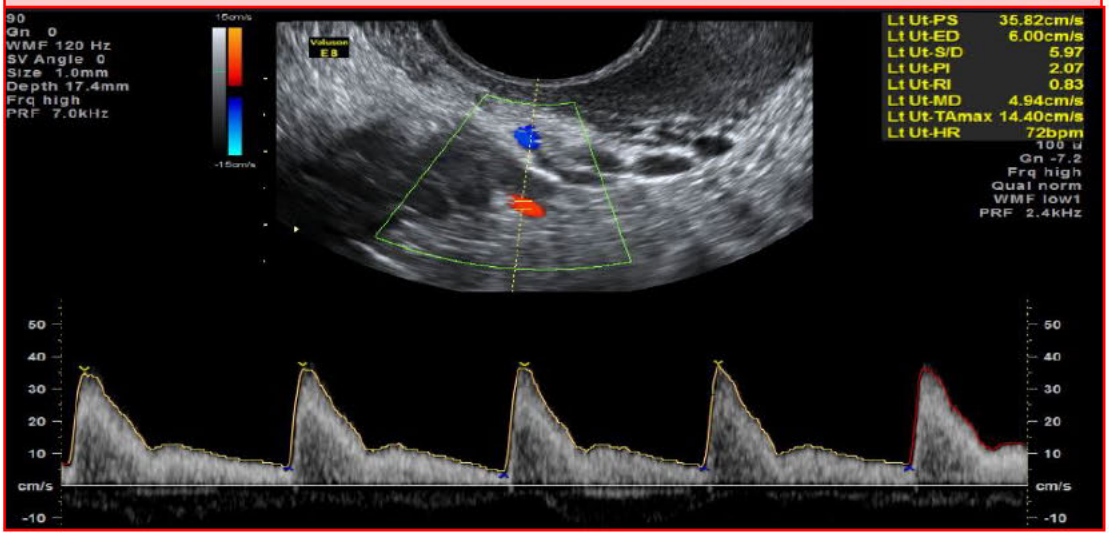
$$\frac{\textit{peak systolic velocity - end diastolic velocity}}{\textit{time averaged velocity}}$$

Statistically significant lower resistance Doppler waveforms have been demonstrated in the uterine artery closest to the location of developing placenta (χ^2=7.00; p value not reported) (88). Calculating the meanUAPI (right UAPI + left UAPI divided by 2) compensates for the differences in laterality improving the reliability and reproducibility of uterine artery Doppler quantitative measures used in prediction models for adverse pregnancy outcomes (89).

The role of the uterine artery Doppler in the prediction of adverse fetal and maternal outcomes will be discussed later in this chapter. In the research presented in this thesis uterine artery Doppler will be

considered in the prediction models for miscarriage prior to 12 weeks gestation (Chapter 3), SGA (Chapter 5) and MHD (Chapter 4 & 6).

Figure 1.25. *Normal left uterine artery waveform at 7 weeks gestational age with automatic measurement of the pulsatility index. Lt Ut-PI, left uterine artery pulsatility index.*



SUMMARY

This section has presented the conventional and novel measures that can be assessed with ultrasound prior to 11 weeks gestation. It is clear that there is a paucity in research addressing the value of the novel trophoblast measures of thickness and volume and uterine artery Doppler in the prediction of adverse pregnancy outcomes. The research presented in this thesis will address these gaps in the literature with the following section presenting the definitions and diagnostic criteria for the adverse pregnancy outcomes to be considered: miscarriage prior to 12 weeks gestation, SGA and MHD.

ADVERSE FETAL AND MATERNAL OUTCOMES

INTRODUCTION

The previous sections have discussed the role of the placenta in a successful pregnancy and introduced the conventional and novel measures which can be assessed with ultrasound prior to 11 weeks gestation. Placental-related adverse pregnancy outcomes in the short-term include miscarriage prior to 12 weeks gestation and in the long-term SGA and MHD. The previous discussion has also outlined the paucity of research available, particularly of the novel ultrasound measures, at this early gestation in the prediction of adverse pregnancy outcomes. These novel measures indirectly assess the success of early placental development as adverse outcomes have been linked to abnormal measurements early in pregnancy (42, 43). For these reasons, the research presented in this thesis will investigate the role of ultrasound prior to 11 weeks gestation in the prediction of miscarriage prior to 12 weeks gestation and the development of SGA and MHD later in pregnancy. For each of these adverse pregnancy outcomes the following sections will discuss the definitions and the current role of ultrasound in diagnosis and prediction.

MISCARRIAGE PRIOR TO 12 WEEKS GESTATION

First trimester miscarriage is common, affecting one in five pregnancies (90). Women symptomatic of miscarriage are universally anxious and looking for reassurance as to the current and expected ongoing health of their pregnancy (91). The prediction of miscarriage may be useful in counselling and guiding the short-term management of early pregnancies (92).

DEFINITION

Miscarriage is defined by the Royal College of Obstetricians and Gynaecologists as the spontaneous loss of a pregnancy prior to viability (until 24 weeks gestation) (93). For the purposes of the research in this thesis a pregnancy loss prior to 12 weeks gestation is referred to as a miscarriage.

THE ROLE OF ULTRASOUND IN DIAGNOSIS OF MISCARRIAGE

Ultrasound is considered the gold-standard in the diagnosis of early pregnancy loss. With transvaginal

ultrasound the criteria for the diagnosis of a pregnancy loss are:

1. A MSD ≥25 mm without an embryo or yolk sac or;
2. A CRL of ≥7 mm with no heart movement (57).

In the event these criteria cannot be met at the first ultrasound then a repeat scan is required for confirmation of miscarriage:

- First scan: no visible embryo (with or without visible yolk sac) with a MSD ≥12 mm. Repeat scan (no less than 7 days after the first scan) a miscarriage is confirmed if there is no embryo with heart movement visible;
- First scan: no visible embryo (with or without visible yolk sac) with a MSD ≤12mm. Repeat scan (no less than 14 days after the first scan) a miscarriage is confirmed if there is no heart movement and the MSD has not doubled;
- First scan: embryo (irrespective of crown-rump length) without heart movement. Repeat scan (no less than 7 days after the first scan) a miscarriage is confirmed if there is still no heart movement visible (94).

THE ROLE OF ULTRASOUND IN PREDICTION OF MISCARRIAGE

The ultrasound appearances discussed above for the *diagnosis* of miscarriage early in pregnancy are well-established (57, 95). In contrast, ultrasound appearances are less specific for the *prediction* of miscarriage (96). The following section will firstly discuss the role of each of the conventional and novel ultrasound measures introduced above for the prediction of miscarriage. This will be followed by a discussion of current prediction models using a combined approach for miscarriage prediction and how the research in this thesis will address the gaps identified in the literature.

CONVENTIONAL ULTRASOUND MEASURES

Gestational sac

The role of the MSD in the diagnosis of pregnancy loss has been discussed earlier in this chapter. In summary, the diagnosis of miscarriage can be made when the MSD is ≥25 mm without an embryo or yolk sac (95). If the gestational sac is smaller than 25mm in mean diameter the diagnosis of pregnancy failure cannot be made with a single ultrasound examination due to the significant overlap

between viable and non-viable pregnancies at this size (57, 97). A repeat scan no less than 7 days later should be performed (94). No growth in the MSD is then considered strongly suggestive of a non-viable pregnancy in the absence of embryonic structures (57).

A reduction in MSD has been reported to be associated with subsequent miscarriage. In a large, retrospective study of 5,427 cases (729 with miscarriage and 4,698 with normal outcomes) assessed by ultrasound at 6-10 weeks gestation, Papaioannou *et al*. (2011) reported that the risk of miscarriage was inversely related to gestational sac diameter (odds ratio 0.84). They suggested that a smaller than expected MSD (based on the CRL) implies reduced amniotic or coelemic fluid and is likely to predict impaired placentation and that this in turn may lead to miscarriage or placenta-related complications later in pregnancy (96).

In contrast, Odeh et al. (2012) measured the gestational sac as a whole and its components; the amniotic and coelemic cavities, separately (98). In this series of patients presenting with vaginal bleeding between 6 and 12 weeks gestation they found a chorionic cavity volume (MSD – volume of the amniotic sac) <1.8ml able to predict miscarriage prior to 20 weeks with sensitivity 89.5% and specificity 42.9%. Despite this seemingly good sensitivity, the small number of participants (n=90) and number of miscarriages (14/90) and technical limitations of calculating the volume of the amniotic sac may have contributed to the reported poor discriminatory area under the ROC c-statistic of 0.65. Individual measures of MSD and amniotic sac volume showed no significant differences between pregnancies that miscarried prior to 20 weeks and those which delivered after 24 weeks (98).

Other studies have demonstrated that in the presence of a measurable embryo with heart movement the gestational sac size can be compared to the CRL and used to predict both adverse fetal and maternal outcomes. A smaller than expected gestational sac (compared to CRL) has a high association with subsequent miscarriage (99-101).

In addition to the MSD other ultrasound features of the gestational sac have been investigated in the prediction of miscarriage including an irregular shape, absent double-decidual sign and presence of a fluid-fluid level, although all have been found to have a low sensitivity in the prediction of an abnormal pregnancy

(102). Although assessment of the size and shape of the gestational sac will identify a high proportion of pregnancies that fail to progress beyond the first trimester, these measures are not specific enough in isolation to be of significant predictive value (97).

Secondary yolk sac

The role of the YSD in the diagnosis of pregnancy loss has been discussed earlier in this chapter. In summary, a secondary yolk sac (YS) should be demonstrated in a normal pregnancy when the MSD reaches 8mm (103). This was revised to 20mm with 100% sensitivity and specificity by Rowling et al. (1997) (104). Current criteria state that in the absence of a demonstrable YS the ultrasound diagnosis of pregnancy failure should only be considered with a MSD ≥25 mm(57).

The YS has also been reported to be useful in the prediction of miscarriage. A YSD of below the 5th centile (96) or above the 95th centile for gestational age (Fig 1.26)(50, 51, 96) has been associated with an increased chance of miscarriage. Each of these studies demonstrates a high odds ratio; 1.88 (96) and high specificity; 95.3% (50) and 99.0% (51) for the prediction of miscarriage. Of these, the studies including a smaller numbers of pregnancies report conflicting sensitivity; 15.6% (50) and 68.7% (51) and positive predictive values; 44.4% (50) and 91.6% (51) for the prediction of miscarriage in the first trimester. This limits the clinical value of yolk sac size as a sole predictor of abnormal pregnancy outcome (105, 106). Its use in combination with other sonographic features may be of benefit in the prediction of early pregnancy failure.

Studies investigating yolk sac volume demonstrated no significant difference between pregnancies that subsequently miscarried and those that continued (52, 53). Conversely an irregularly-shaped YS (Fig 1.27) has also been associated with early miscarriage (50, 105-107).

Figure 1.26. *Enlarged secondary yolk sac.*

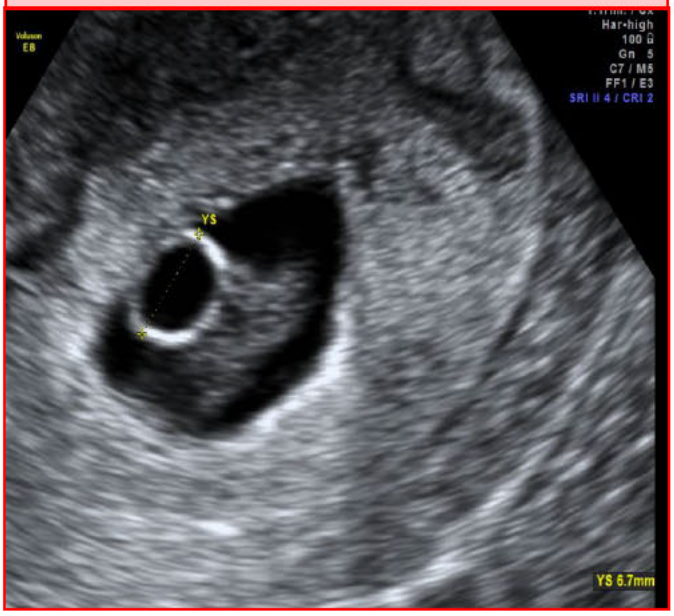
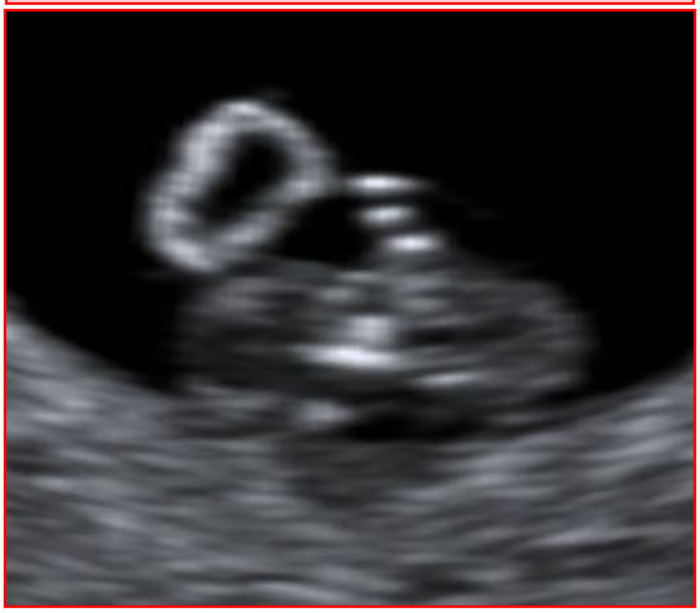


Figure 1.27. *Irregularly shaped secondary yolk*



Embryo

The role of the size of the embryo (CRL) in the diagnosis of pregnancy loss has been discussed earlier in this chapter. In summary, current guidelines state that in the presence of a CRL ≥7mm with absent heart movement a diagnosis of early pregnancy failure can be made (57). When the CRL measures less than 7mm, the absence of heart movement should be viewed with caution and the scan repeated in no less than 7 days to assess for progression of the pregnancy (94). Demonstration of no interval growth or a CRL of 7mm or greater without demonstrable heart movement is then considered diagnostic of early pregnancy failure (57, 94). This caution is warranted as a small CRL, even in the context of "certain dates"; may be due to late ovulation or inaccurate reporting of LMP (48). If these pregnancies with smaller than expected CRL are simply re-dated, we may be providing false reassurance of a normally progressing pregnancy. Progress ultrasound after a few weeks interval may be useful to assess for normal growth and re-dating could be considered at this time.

Studies have demonstrated that a negative discrepancy in CRL compared with expected gestational age is associated with a significantly increased risk of subsequent miscarriage (58, 108). These prospective studies demonstrated

that the risk of miscarriage increased as the CRL decreased below the expected

mean (108) particularly when the CRL was less than 18mm (58). While the clinical utility of the results of these studies may be limited due to their small sample size, high-risk populations and use of last menstrual period to date pregnancy, they highlight the importance of a known gestational age when using CRL to predict adverse pregnancy outcomes. It is for this reason the research presented in this thesis uses CRL rather than clinical history to determine gestational age.

Heart movement

The role of fetal heart movement in the diagnosis of pregnancy loss has been discussed earlier in this chapter. A low fetal heart rate has previously been associated with an increased risk of first trimester miscarriage; a retrospective study including 729 pregnancies reported that the risk of miscarriage was inversely related to FHR (96). A heart rate of below the 5th centile for gestational age was demonstrated in approximately 24% of pregnancies that subsequently miscarried compared to 5% of pregnancies with a normal outcome (96). This is particularly evident in pregnancies between 6 and 8 weeks' gestational age (64, 109, 110)). In clinical practice, any embryonic heart rate in the first trimester less than 100bpm is considered bradycardic and indicates that a repeat scan should be performed to ensure the progression of the pregnancy (94).There is no literature suggesting that tachycardia at less than 11 weeks' gestational age is associated with adverse pregnancy outcomes.

While the FHR has been shown to be lower than expected in circumstances where there is subsequent miscarriage prior to 12 weeks gestation this has not been found to be of sufficient predictive value in isolation to guide clinical management (64). The research in this thesis addresses the role of FHR in combination with other first trimester measures in the prediction of subsequent miscarriage.

The differing methodologies of these ultrasound measures in studies investigating conventional prediction of miscarriage makes direct comparison difficult. The larger, prospective (96) studies in low-risk populations for miscarriage; no vaginal bleeding (50) and FHM at the initial scan (64) are more likely to be translatable into routine clinical practice compared to smaller (51), retrospective (98) studies in high-risk populations (94, 96). These differences in study design may be reflected in

conflicting results in terms of sensitivity for the prediction of miscarriage. This, together with the paucity of more recent research is a catalyst for further investigation.

NOVEL ULTRASOUND MEASURES

Trophoblast thickness and volume

Histological studies have demonstrated that approximately two-thirds of miscarriages are due to defective placentation with reduced trophoblast invasion into the decidua of the uterus (27). As discussed earlier in this chapter the trophoblast is easily demonstrated with ultrasound however, despite this, there is little published literature addressing the usefulness of trophoblast thickness or volume in the prediction of miscarriage.

In one large, prospective study, Bajo et al. (2000) describe a discrepancy of greater than 3mm between 'trophoblastic thickness' and the embryonic length as having a high sensitivity (82%) and specificity (93%) for the prediction of spontaneous abortion (42). In contrast, Nyberg et al. 1986 found a thin decidual reaction (trophoblast) of less than 2mm had a sensitivity of 28% and specificity of 99% for the prediction of subsequent miscarriage (111).

Previous research that has investigated the relationship between placental volume and adverse pregnancy outcomes has predominantly measured placental volume from 11 weeks gestational age (69, 71, 72, 77, 112-115). This may be due, at least in part, to convenience as the majority of pregnancies are scanned at $11\text{-}13^{+6}$ weeks gestation for the purposes of aneuploidy screening (83). In addition, the placental margins are much easier identified with ultrasound after this time (74). In consequence there are few studies addressing the value of trophoblast (or placental) volume measured prior to 11 weeks for the prediction of miscarriage.

One study by Reus *et al.* (2013) demonstrated a significant reduction in the trophoblast volume in pregnancies between 6 and 12 weeks gestation that miscarried compared to pregnancies that resulted in a live birth (43). This study used serial measurements from a sample of 112 pregnancies with normal outcomes compared with measurements from 56 pregnancies that miscarried. No definition of miscarriage was provided, and it is unclear if the miscarriages occurred prior

12 weeks or later in pregnancy (43). The inclusion of "empty sac" miscarriages (those not containing an embryo) in their data may have also overestimated the significance of their findings (43). In contrast, with the aim of increasing the clinical validity and usefulness of the results the research presented in this thesis limits inclusion to pregnancies demonstrating fetal heart movement at the time of enrolment.

Uterine artery Doppler

As discussed earlier in this chapter uterine artery Doppler waveforms change dramatically through pregnancy. In summary, prior to 12 weeks gestation researchers have shown that the spiral arteries are occluded by invading trophoblast to restrict perfusion of the developing pregnancy (80) with an increase in perfusion found in pregnancies that had miscarried (116). Despite a decrease in meanUAPI being reflective of an increase in uterine blood flow, no studies were identified investigating the use of uterine artery Doppler prior to 11 weeks gestation or its relationship with miscarriage prior to 12 weeks gestation. It is proposed by the research presented in this thesis that uterine artery Doppler prior to 11 weeks gestation may be useful in the prediction of subsequent miscarriage (Chapter 3).

COMBINED APPROACH TO THE PREDICTION OF MISCARRIAGE

Models for the prediction of miscarriage predominantly include a combination of maternal factors and ultrasound measures within their reported algorithms. Papaioannou *et al*. (2011) combined maternal factors (age, race, smoking, vaginal bleeding) and ultrasound parameters (CRL, FHR, gestational sac diameter, YSD) in a large retrospective study (n= 792 miscarriages; 4698 normal outcomes) reporting an area under the curve (AUC) of 0.88 for the prediction of miscarriage (96). Similar results were reported by Stamatopoulos *et al*. (2015) when using a combination of maternal factors (age, vaginal clots) and ultrasound parameters (CRL, FHR, logarithm [gestational sac volume/CRL]) (AUC:0.89) (117).

No studies were identified that considered the novel ultrasound measures of trophoblast thickness, trophoblast volume or meanUAPI either in isolation or in conjunction with conventional ultrasound measures. For this reason, the research presented in this thesis will investigate the value of both

conventional and novel ultrasound measures individually and in combination in the prediction of miscarriage prior to 12 weeks gestation (Chapter 3).

SUMMARY

This section of the review has discussed the role of ultrasound measures in the first trimester in the prediction of miscarriage prior to 12 weeks gestation. The value of conventional measures (MSD, YSD, CRL and FHR) is well represented in the literature however there is a clear paucity of research addressing the role of novel ultrasound measures (trophoblast thickness and volume and meanUAPI). It is the aim of the research presented in this thesis to investigate the potential of both conventional and novel ultrasound measures to improve the prediction of miscarriage prior to 12 weeks gestation. These results are presented in Chapter 3.

SMALL-FOR-GESTATIONAL AGE AND MATERNAL HYPERTENSIVE DISORDERS

INTRODUCTION

The aim of prediction of adverse pregnancy outcomes is the early identification of women and fetuses with an increased risk of being affected to facilitate intervention with the aim of reducing the morbidity and mortality associated with the condition of interest (118). In terms of early prediction models for SGA and MHD, the aim is to be able to intervene with the intention of decreasing the amount of affected pregnancies (119), optimising the care of those still affected (119) and targeting future research into preventative interventions (120). The following section will discuss the current ultrasound methods used for the prediction of SGA and MHD.

Current ultrasound methods of SGA diagnosis have been reported to miss approximately 40% of affected pregnancies (121). Maternal hypertensive disorders are more easily diagnosed, however like SGA, are currently not treatable in-utero after diagnosis with the only cure being delivery of the fetus (122). This often results in significantly early delivery (123) and increased morbidity for both the mother and child (124). Prediction of these adverse outcomes provides the opportunity for early intervention with aspirin which has been

shown to significantly decrease the incidence of both SGA and MHD and improve management of those pregnancies still affected (125, 126).

Beginning with SGA the following sections will individually define SGA and MHD and discuss the role of ultrasound in their diagnosis and prediction. This will be followed by a combined discussion of the current predictive models used in clinical practice.

SMALL-FOR-GESTATIONAL AGE

DEFINITION

Definitions of normal fetal/newborn size based on the estimated fetal weight (EFW) or birthweight (BW) vary widely with different research groups and clinicians using different standards. Commonly used thresholds for the diagnosis of SGA are an EFW or BW below the 10th, 5th or 3rd centile (28, 127, 128).

The definition of SGA as an EFW or BW below the 10th centile is reported by Maulik, 2006 and is recommended for clinical use by various national governing bodies (128-132). The Royal Australian & New Zealand College of Obstetricians & Gynaecologists provide no local guidelines deferring to the Canadian definition of EFW below the 10thcentile (133). This threshold of below the 10th centile identifies a group of fetuses at a higher risk of poor perinatal outcome despite including fetuses that are phenotypically small (134).

It can be argued that lower thresholds for SGA are more appropriate for use; below the 5th centile (28) or below the 3rd centile (127) with their comparatively lower sensitivity off-set by their higher specificity for diagnosis (28). It is important to note that whatever threshold is used, it will exclude fetuses whose weight falls within the normal range but have failed to reach their individual growth potential (134). These infants and fetuses are at the same risk as those with a weight below the 10th centile (135).

For the purpose of the research presented in the thesis SGA is defined as a BW <10th centile for gestational age.

THE ROLE OF ULTRASOUND IN THE DIAGNOSIS OF THE SMALL-FOR-GESTATIONAL AGE FETUS

INTRODUCTION

Prenatal diagnosis of SGA with ultrasound requires a well-defined gestational age, accurate fetal measurements, calculation of an EFW and comparison of the observed EFW to which is expected (30). As fetuses which are identified as SGA are not necessarily pathologically small, pregnancy management decisions are made by taking serial fetal measurements to demonstrate interval growth and through the identification of functional changes and consequences associated with IUGR (136). These functional changes are demonstrated by the use of ultrasound and maternal and fetal Doppler techniques including increased uterine artery pulsatility (137, 138), increased umbilical artery pulsatility (134), decreased middle cerebral artery (MCA) pulsatility (139), increased ductus venosus pulsatility (140), a decrease in the amniotic fluid volume (141) and fetal behavioural changes (loss of breathing and gross body movements, loss of heart rate reactivity) (142).

ACCURATE KNOWLEDGE OF GESTATIONAL AGE

If the date of the first day of the last menstrual period (LMP) is known, then that is often used to calculate the expected gestational age. This approach is limited as the actual date of conception (and hence the age of the fetus) varies from woman to woman depending on the length of the menstrual cycle (143).

In the research presented in this thesis the ultrasound measurement of fetal CRL at inclusion in the study is used as a proxy for gestational age as this is considered the most accurate method of dating a pregnancy between 5 and 11 weeks gestation (143).

Ultrasound assessment of fetal size

Ultrasound assessment of fetal size relies on standard biometric measurements and the calculation of EFW. The EFW is then compared to charts of expected fetal size during gestation to determine if the fetus is appropriately grown, large- or small- for gestational age. The following section will discuss the standard biometric

measurements and estimation of fetal weight.

Fetal measurements routinely assessed and recorded by ultrasound in the second and third trimesters include the fetal head: biparietal diameter (BPD) and head circumference (HC) (Fig 1.28), the fetal abdominal circumference (AC) (Fig 1.29) and femur length (FL) (Fig 1.30) (144).

Figure 1.29. *Biparietal diameter (BPD) and Head Circumference (HC).*

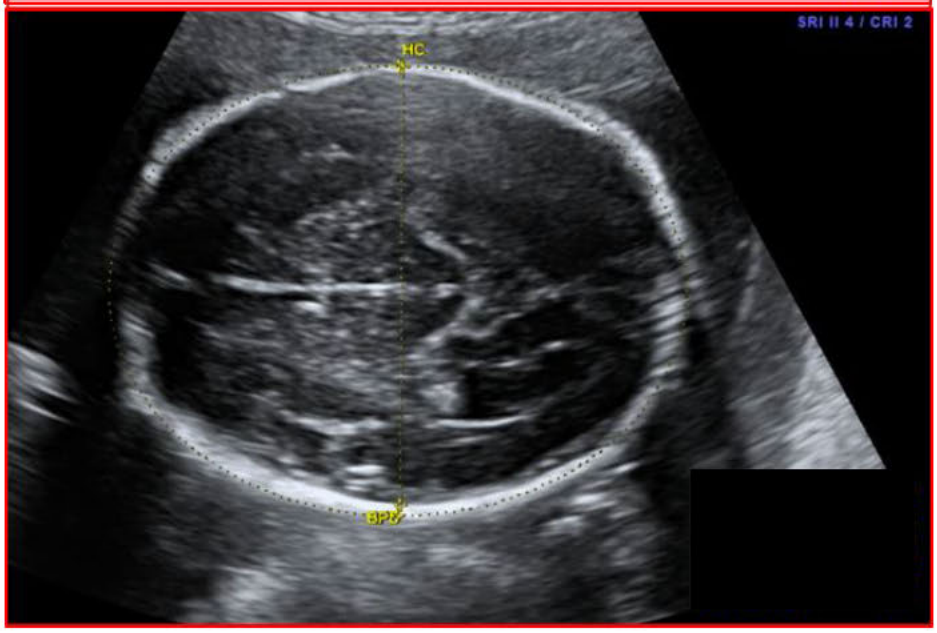


Figure 1.29. *Abdominal circumference (AC).*

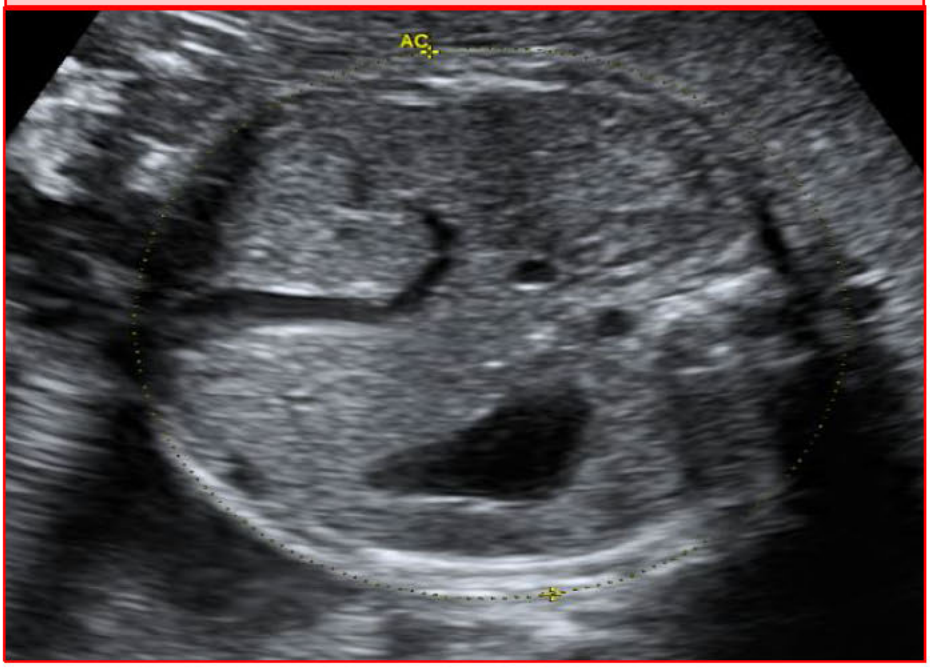
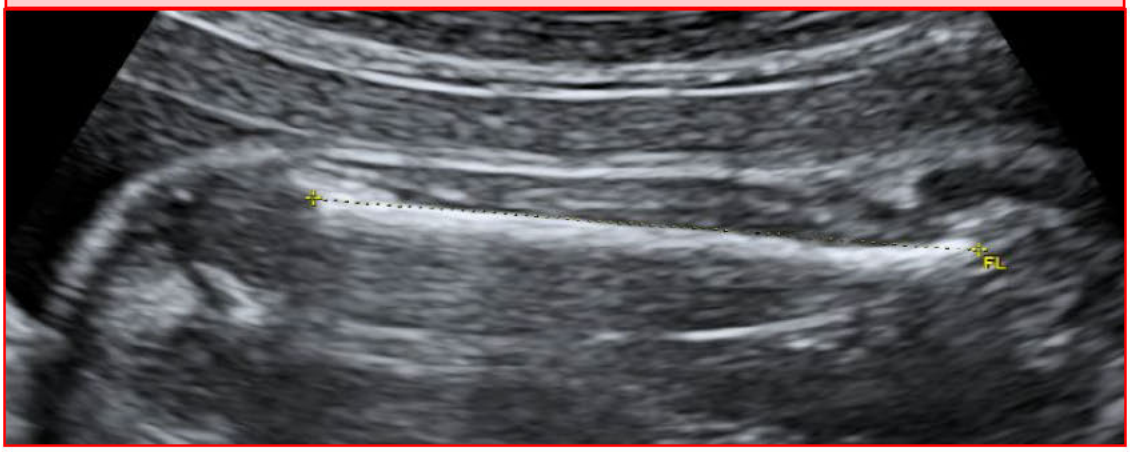


Figure 1.30. *Femur length (FL).*



Based on these measurements the EFW is calculated to facilitate comparison with the expected fetal size for gestational age. There are over 20 different formulae described in the literature that can be used to estimate fetal weight based on fetal biometric measurements. None of these formulae have achieved an accuracy high enough to recommend clinical use (144). It is therefore up to local practice to choose the formula used.

Comparisons of the accuracy of EFW based on formulae using various combinations of fetal measurements suggest that the Hadlock B formula provides the best comparison with actual birthweight over most gestational ages (145-147). Due to the possibility that fetal head moulding may influence the HC, it has been suggested that the Hadlock C formula excluding HC (Fig 1.31) be used having demonstrated a reported accuracy compared to birthweight of within 7.5% (one standard deviation) (148). During pregnancy the EFW as calculated by ultrasound measurements is compared to an expected standard based on gestational age (149). This provides a description of fetal size in centiles, with an EFW of <10th centile considered to be SGA.

In Australia, multiple charts are available for use and, similar to the formula for calculating the estimated fetal weight, the choice of chart choice is based on local preference and convention.

Figure 1.31. *Hadlock C formula for the estimation of fetal weight [148]. BW, current estimated fetal weight; AC, abdominal circumference; BPD, biparietal diameter; FL, femur length.*

$$\log(10)\,BW = 1.335 - 0.0034(AC) \times (FL) + 0.0316(BPD) + 0.0457(AC) + 0.1623(FL)$$

Prior to 1999 the charts used in Australia were based on local populations in Melbourne (150-153), Western Australia (154) and New South Wales (155). The first national Australian based standards for birthweight was published by Roberts & Lancaster (1999) (Fig 9) (156). Despite this, there is no explanation as to why a Hadlock chart that provides non-gender specific centile ranges from the early second trimester to term was based on a small sample of 392 pregnancies (157). It remains the most commonly used chart in clinical practice in Australia (158).

A recently published single centre study from New South Wales, Australia presented by Joseph et al. (2018) developed gender differentiated growth charts based on data from 80,550 spontaneous live births between 26 and 42 weeks (149). This study compared birthweights of spontaneous and iatrogenic births finding that iatrogenic pre-term infants weighed significantly less than their spontaneous birth counterparts (149). In addition, median and 10th centile birthweights derived from the spontaneous cohort closely resembled previously defined ultrasound-based curves (149, 157). The authors concluded that birthweight reference charts based on spontaneous rather than iatrogenic births more accurately represented normal growth patterns and outcomes and proposed that these charts are likely to be more accurate than existing charts for the identification of fetuses at risk of being SGA (149).

As discussed earlier, for the purposes of the research presented in this thesis SGA is defined as a BW below the 10th centile. Due to the geographical proximity of the data presented by Joseph et al. (2018) and the similarities to the population in the data used in this thesis, the formulae presented by Joseph et al. (2018) for derivation of the 10thcentile (Fig 1.32) will be used to determine the 10th centile used in this work to define SGA (149).

Figure 1.32. *Formula for the calculation of the 10th centile birthweight for gestational age [149]. GA, gestational age in weeks.*

Female =

$-14524.63 + 2087.97 \times GA - 112.927 \times GA2 + 2.73430 \times GA3 - 0.023592 \times GA4$

Male =

$-61436.50 + 7763.86 \times GA - 367.562 \times GA2 + 7.75998 \times GA3 - 0.060402 \times GA4$

CONVENTIONAL ULTRASOUND MEASURES

As discussed earlier in this chapter the conventional ultrasound measures assessed prior to 11 weeks gestation include MSD, YSD, CRL and FHR. Previously, the use of these measures in the prediction of SGA has predominantly been assessed after 11 weeks gestation (62, 73). There was no research identified as to the value of these measures in the prediction of MHD. The following section will discuss the limited research in the value of gestational sac volume and CRL in the prediction of SGA.

Gestational sac

There is a paucity of published literature addressing the value of the gestational sac size prior to 11 weeks gestation in the prediction of SGA. No studies prior to 11 weeks gestation could be identified. A small, prospective study conducted at 11-13^{+6} weeks gestation reported that a decrease of 100mm^3 in gestational sac volume (compared to CRL) resulted in a mean decrease in birthweight of 220g (β=0.20; p=0.013) (73).

Embryo

It is well documented that a smaller than expected CRL at 10-14 weeks gestation is associated with an increased risk of SGA at birth (62). While variations in methodologies and reporting make direct comparison difficult between studies researchers have demonstrated an increased risk of SGA with a CRL of 2-6 days less than expected at 13 weeks (RR: 3.0; 95%CI:2.0-4.4) (159), a decreased risk of SGA with a 1 day increase in CRL at 10^{+3}-13^{+6} weeks (OR:0.87; 95%CI:0.81-0.94) (62), and that a CRL Z-score (for gestational age) at 11-14 weeks is a significant independent predictor of BW Z-score; β=0.17; p=0.001 (160) and AUC of 0.59; p<0.0001 (161). Despite these statistically significant results the modest value of CRL as predictor for (AUC of 0.59) is not sufficient for it to be used in isolation for SGA prediction (161).

No studies addressing the use of CRL prior to 10 weeks gestation in the prediction of SGA were identified. The use of CRL in combined predictive models for SGA will be considered in Chapter 5.

NOVEL ULTRASOUND MEASURES

Novel ultrasound measures that can be assessed prior to 11 weeks gestation include trophoblast thickness and volume and the meanUAPI. The following section will discuss the current literature addressing the value of these measures in the prediction of SGA and MHD.

Trophoblast thickness and volume

As discussed earlier in this chapter the placenta is derived from the layers of trophoblast surrounding the early gestational sac (10). The margins of the trophoblast can be defined from as early as five weeks gestation however no published literature could be identified investigating trophoblast thickness prior to 11 weeks gestation and any relationship with pregnancy outcome.

Beginning at the 8th week of pregnancy the trophoblast adjacent to the decidua basalis thickens forming the placenta (10). Despite this early beginning the boundaries of the placenta are difficult to delineate with ultrasound prior to 10 weeks gestation (74) and the majority of studies have focussed on the assessment of placenta volume after 11 weeks for the prediction of SGA and MHD (68, 69, 112, 162, 163) rather than the volume of the trophoblast as a whole.

Comparison between studies conducted at 11-13^{+6} weeks gestation addressing the association between placental volume (PV) and SGA is difficult due to differences in the definition for SGA (<5th or <10th centile) (68, 112, 162) and different reporting measures including PV (68, 69, 112, 163), placenta quotient (PQ) which is placental volume/CRL, (77, 112, 113, 163) and standardised placental volume (SPV) measured as placental volume/CRL (162).

Compared to pregnancies with normal outcomes, the PV at 11-13 weeks gestation has been reported as significantly lower in neonates with a BW <5th centile (PV multiples of the median (MoM) of 0.88 compared to 1.00 in pregnancies with a normal outcome; $p<0.0001$) (68). Similarly, PV MoM at 11-13 weeks has been reported as lower in neonates with a BW <10th centile (PV MoM 0.79 vs. 1.00 in pregnancies with a normal outcome; $p<0.001$) (69).

In contrast, other studies conducted at a similar gestational age have not demonstrated a significant difference in PV between outcome groups (112, 163). These differences are possibly accounted for by

smaller sample sizes and differences in reporting values for PV; MoM in the groups reporting significant results (68, 69) and mm^3 in groups reporting non-significant findings (112, 163). In contrast to PV, reports that the PQ is lower in pregnancies that subsequently develop SGA compared to pregnancies with normal outcomes (Table 1.1) are reasonably consistent (162, 163). Comparison of studies reporting predictive values report varying sensitivities for the prediction of SGA with PQ of 22% (false positive rate (FPR) 9%) (77) and 44% (FPR 10%)(162). In contrast one study showed no significant difference in PQ between normal and SGA pregnancies (112). A single study reports good results for the prediction of SGA <10^{th} centile using SPV however this is mathematically identical to the PQ (162).

In summary, no studies have been identified addressing the use of trophoblast thickness or volume prior to 11 weeks gestation in the prediction of SGA. The use of these measures in combined predictive models for SGA will be considered in Chapter 5.

Table 1.1. *Placental Quotient at 10^{+6} to 14 weeks gestation in the prediction of small-for gestational age <10^{th} centile.*

Author	Gestation (weeks)	N	Variable	Results
Odeh et al. [112]	10^{+6}-13^{+6}	308	PQ* (mean)	No statistically significant difference between groups
Odibo et al. [163]	11-14	388	PQ* (mean)	0.64 vs. 0.80 (p=0.06)
Hafner et al. [73]	11-12	2489	PQ* (<10^{th} centile)	sensitivity 27.1% specificity 90%
Schuchter et al. [77]	11-14	380	PQ* (<10^{th} centile)	sensitivity 22%, FPR 9%
Collins et al. [162]	11-13^{+6}	143	PQ** (mean) SPV*** (mean)	PQ and SPV lower in SGA PQ, p=0.003; SPV, p<0.001 SPV prediction of SGA sensitivity 67%, FPR 20% sensitivity 44%, FPR 10%

FPR false positive rate, N number of participants, PQ placental quotient, SPV standardised placental volume. * PQ= placental volume/CRL, ** PQ=CRL/ placental volume), ***

Uterine Artery Doppler

As discussed earlier in this chapter the uterus receives its blood supply from the bilateral uterine arteries, branches of the internal iliac arteries. Each uterine artery branches into progressively smaller arcuate, radial, basal and spiral branches as they penetrate the myometrium into the basal layer of the endometrium. Smaller capillaries and venous lacunae are located within the functional layer of the endometrium which is shed each menses (12). Uterine artery Doppler has long been assessed during pregnancy to demonstrate normal or restricted blood flow to the uterus. It was first used in the later second and early third trimesters to show the presence or absence of the expected transformation from a high-resistance to low-resistance waveform by 26 weeks' gestation (79).

The advent of first trimester screening provided an earlier opportunity in routine pregnancy care when the uterine artery could be assessed and innumerable studies have addressed the use of uterine artery blood flow at 11-14 weeks' gestation and its relationship to and usefulness in the prediction of many adverse pregnancy outcomes (83). A higher resistance to flow (defined as an increase in the PI) has been linked to defective placentation and an increased risk of placenta-related pregnancy complications such as SGA (77, 78, 85).

Despite an increased meanUAPI at 11-14 weeks gestation demonstrating a low sensitivity and positive predictive values across studies it is still considered a good predictor for subsequent development of SGA (Table 1.2) due to its consistently high specificity and negative predictive values (77, 78, 85). A recent meta-analysis has confirmed these results; in a pooled cohort of 55974 women found a similarly low sensitivity and high specificity for UAPI at 11-14 weeks gestation to predict SGA (15% and 93% respectively) (83).

A normal range of uterine artery PI has been published that demonstrates good reproducibility and reliability at 11-14 weeks' gestation (164) however their utility outside of the specified ranges cannot be assumed. There is no identified research addressing the value of uterine artery Doppler prior to 11 weeks. More research is needed assessing any correlation between uterine artery Doppler PI earlier in the first trimester and adverse fetal and maternal outcomes is required. This is addressed in this thesis in Chapters 3-6.

Table 1.2. *Uterine pulsatility index (mean >95th centile) at 11-14 weeks gestation for the prediction of small-for-gestational age.*

Study	Sensitivity	Specificity	PPV	NPV	FPR
Pilalis et al. (2007) [85]	17.8*		14.0*		
	9.6**		19.3**		
Gomez et al. (2002) [164]	24.3*	95.4*	16.9*	97.0*	
Schuchter et al. (2001) [77]	25.0**				8.4**
Martin et al. (2001) [78]	11.7**	95.6**	21.9**	91.1**	

PPV positive predictive value, NPV negative predictive value, * <5th centile,

** <10th centile. All values expressed as %,

SUMMARY

This section has described the paucity of published literature on the use of ultrasound measures prior to 11 weeks gestation in the prediction of SGA. The research presented in this thesis aims to investigate the predictive value of the novel ultrasound measures trophoblast thickness and volume and uterine artery Doppler in the prediction of these adverse pregnancy outcomes. The following section will define MHD and discuss the published literature of the use of ultrasound measures prior to 11 weeks gestation in its prediction.

MATERNAL HYPERTENSIVE DISORDERS

DEFINITION

The maternal hypertensive disorders considered in this research are GH and PET. Blood pressure is measured in millimetres of Mercury (mmHg) and creatine is measured in milligrams/millimoles per litre (mg/mmol).

Gestational hypertension is defined as the new onset of hypertension (>140 mmHg systolic or >90 mmHg diastolic) after 20 weeks gestation (165).

Pre-eclampsia is defined as the new onset of hypertension (>140 mmHg systolic or >90 mmHg diastolic) after 20 weeks gestation in conjunction with one or more complications (165). These complications may include urine protein/creatinine ≥30 mg/mmol, maternal organ dysfunction or uteroplacental dysfunction (165). Maternal organ dysfunction may include renal insufficiency (serum or plasma creatinine >90µmol/L), liver involvement (elevated transminases with or without right epigastric or right upper quadrant pain), neurological complications (altered mental status, stroke, blindness, hyperreflexia, severe headache), haematological complications (165). Uteroplacental dysfunction refers to evidence of IUGR in the fetus (165).

THE ROLE OF ULTRASOUND IN THE DIAGNOSIS OF MATERNAL HYPERTENSIVE DISORDERS

The diagnosis of MHD is based on the clinical features discussed above. Ultrasound may add to the diagnosis of pre-eclampsia through its ability to demonstrate SGA and poor fetal growth (165) however the significant role of ultrasound is in prediction of MHD (166).

THE ROLE OF ULTRASOUND IN THE PREDICTION OF MATERNAL HYPERTENSIVE DISORDERS

CONVENTIONAL ULTRASOUND MEASURES

As discussed earlier in this chapter the conventional ultrasound measures assessed prior to 11 weeks gestation include MSD, YSD, CRL and FHR. There was no evidence identified as to the value of these measures in the prediction of MHD. This clear gap in the literature will be addressed by the research presented in this thesis in Chapter 6.

NOVEL ULTRASOUND MEASURES

Novel ultrasound measures that can be assessed prior to 11 weeks gestation include trophoblast thickness and volume and the meanUAPI. The following section will discuss the current literature addressing the value of these measures in the prediction of MHD.

Trophoblast thickness and volume

As discussed earlier in this chapter the placenta is derived from the layers of trophoblast surrounding the early gestational sac (10). The margins of the trophoblast can be defined from as early as 5 weeks gestation however no published literature could be identified investigating trophoblast thickness prior to 11 weeks gestation and any relationship with pregnancy outcome.

Beginning at the 8th week of pregnancy the trophoblast adjacent to the decidua basalis thickens forming the placenta (10). Despite this early beginning the boundaries of the placenta are difficult to delineate with ultrasound prior to 10 weeks gestation (74) and the majority of studies have focussed on the assessment of placenta volume (PV) after 11 weeks for the prediction of MHD (68, 69, 112, 162, 163)

rather than the volume of the trophoblast as a whole.

The PV has been reported to be smaller but not statistically significantly different in pregnancies that subsequently develop MHD (69, 112, 163). These studies have a low incidence of PET within their cohorts. Effendi et al. (2014) reports a 2.2% incidence of PET (69) while in comparison a group reporting a significantly decreased PV in pregnancies that later developed PET (delta value: -1.540, $t=4.636$, $p<0.003$) reported a 4.5% incidence of PET, possibly explaining these conflicting results (114). In contrast to PV, the PQ has been reported as being lower for MHD compared to pregnancies with normal outcomes (Table 1.3).

Conflicting results have been reported on the significance of PQ in the prediction of MHD later in pregnancy. Hafner et al. (2006) report a sensitivity of 38.5% (specificity 90%) for the prediction of PET (72) and Schuchter et al. (2001) report a sensitivity 22% (FPR 9%) for the development of GH (77). Others report no statistically significant difference in PQ between outcome groups for either GH (163) or PET (112). These conflicting results may be due to heterogeneity in the number of adverse outcomes between the different cohorts.

Despite the inconsistencies between reports these results suggest an association between placental size and the development of MHD (72). As mentioned earlier in this section the placental margins are difficult to define prior to 10 weeks gestation (74) and for this reason the research presented in chapter 6 will discuss the value of the more easily measured trophoblast thickness and volume prior to 11 weeks gestation.

Table 1.3. *Placental Quotient at 10^{+6} to 14 weeks gestation in the prediction of maternal hypertensive disorders.*

Author	Gestation (weeks)	N	Variable	Out-come	Results
Odeh et al. [112]	10^{+6}-13^{+6}	308	PQ* (mean)	GH	No difference between groups (p>0.05)
Odibo et al. [163]	11-14	388	PQ* (mean)	GH PET	0.70 vs. 0.80 (p=0.03) 0.80 vs. 0.80 (P=0.89)
Hafner et al. [73]	11-12	2489	PQ* (<10th centile)	GH	sensitivity 38.5% specificity 90%
Schuchter et al. [77]	11-14	380	PQ* (<10th centile)	GH	sensitivity 22% FPR 9%

FPR false positive rate, GH gestational hypertension, N number of participants, PET pre-eclampsia, PQ placental quotient. * PQ= placental volume/CRL, ** PQ=CRL/ placental volume)

Uterine Artery Doppler

As discussed earlier in this chapter the uterus receives its blood supply from the bilateral uterine arteries, branches of the internal iliac arteries. Each uterine artery branches into progressively smaller arcuate, radial, basal and spiral branches as they penetrate the myometrium into the basal layer of the endometrium. Smaller capillaries and venous lacunae are located within the functional layer of the endometrium which is shed each menses (12). Uterine artery Doppler has long been assessed during pregnancy to demonstrate normal or restricted blood

flow to the uterus. It was first used in the later second and early third trimesters to show the presence or absence of the expected transformation from a high-resistance to low-resistance waveform by 26 weeks' gestation (79).

The advent of First Trimester Screening provided an earlier opportunity in routine pregnancy care when the uterine artery could be assessed and innumerable studies have addressed the use of uterine artery blood flow at 11-14 weeks' gestation and its relationship to, and usefulness in, the prediction of many adverse pregnancy outcomes (83). A higher resistance to flow (documented as an increase in the PI has been linked to defective placentation and an increased risk of placenta-related pregnancy complications such as MHD (77, 78, 85).

Despite an increased meanUAPI at 11-14 weeks gestation demonstrating a low sensitivity and positive predictive values across studies it is still considered a good predictor for subsequent development of PET (Table 1.4) due to its consistently high specificity and negative predictive values (77, 78, 85, 114, 167, 168). A recent meta-analysis has confirmed these results; in a pooled cohort of 55974 women found a similarly low sensitivity and high specificity for UAPI at 11-14 weeks gestation to predict PET (20% and 93% respectively) (83).

A normal range of uterine artery PI has been published that demonstrates good reproducibility and reliability at 11-14 weeks' gestation (164). These ranges have been established using polynomial linear regression and their utility outside of the specified ranges cannot be assumed. There is no identified research addressing the value of uterine artery Doppler prior to 11 weeks with more research needed assessing any correlation between uterine artery Doppler PI earlier in the first trimester and adverse fetal and maternal outcomes is required. This is addressed in this thesis in Chapters 3-6.

Table 1.4. *Uterine pulsatility index (mean >95th centile) at 11-14 weeks gestation for the prediction of pre-eclampsia.*

Study	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Rizzo et al. (2008) [114]				
all PET				
PET + delivery <32w	50.0	96.7	44.4	97.6
	66.7	95.9	22.2	99.4
Pilalis et al. (2007) [85]				
all PET				
PET + delivery <34w	21.4		5.3	
	33.3		3.5	
Parra et al. (2005) [167]				
all PET				
PET + delivery <35w	25.0	95.2	10.3	98.3
	66.6	95.1	6.8	99.8
Martin et al. (2001) [78]				
all PET				
PET + delivery <32w	27.0	95.4	11.0	98.4
PET + delivery <34w	60.0	95.1	3.9	99.9
	50.0	95.1	4.5	99.8

PET pre-eclampsia, PPV positive predictive value, NPV negative predictive value.

SUMMARY

This section has described the paucity of published literature of the use of ultrasound measures prior to 11 weeks gestation in the prediction of MHD. The research presented in this thesis aims to investigate the predictive value of the novel ultrasound measures trophoblast thickness and volume and uterine artery Doppler in the prediction of this adverse pregnancy outcome. The final section in this chapter will present the current combined approaches used at 11-14 weeks gestation to predict SGA and MHD

with a summary of the value of investigating these measures prior to 11 weeks gestation.

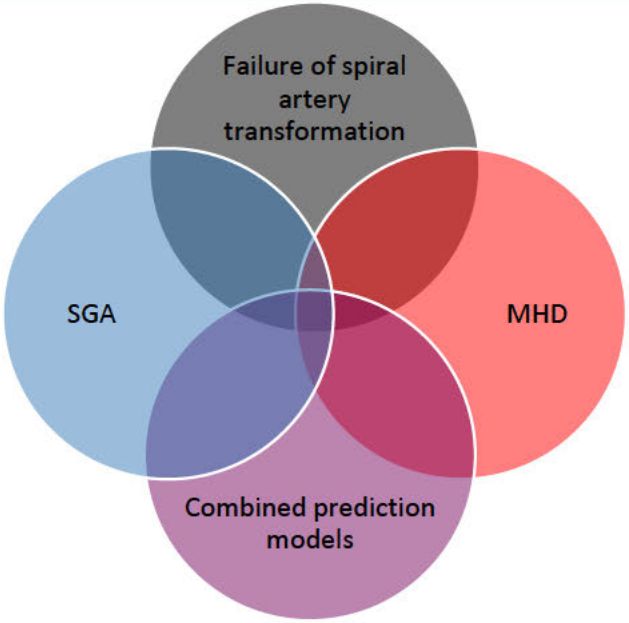
CLINICAL RISK FACTORS AND MATERNAL SERUM BIOCHEMISTRY FOR THE PREDICTION OF SMALL-FOR-GESTATIONAL AGE NEONATES AND MATERNAL HYPERTENSIVE DISORDERS

INTRODUCTION

Small-for-gestational age fetuses (with or without IUGR) and MHD are both characterised by impaired transformation of the maternal spiral arteries resulting in their failure to convert to the low-resistance vasculature required for adequate placental function during pregnancy (17). Current prediction models for both SGA and MHD (in particular PET) use a combination of clinical history, maternal serum biochemistry and uterine artery Doppler at 11-13^{+6} weeks gestational age to define those at an increased risk of complications of placental dysfunction later in pregnancy (5-7, 120). Most models focus on the prediction of early-onset and pre-term PET however histological studies have shown that pregnancies complicated by PET and those which are SGA (but without PET) both show evidence of impaired placentation (9, 17, 169, 170). Therefore, it would be reasonable to assume that risk factors for MHD/PET and SGA are present earlier and can be assessed earlier than 11-13^{+6} weeks gestation.

The research presented in this thesis is based on this principle of impaired placentation early in gestation increasing the risk of SGA and MHD in later pregnancy. For this reason, the prediction of these pregnancy outcomes prior to 11 weeks gestation (Figure 1.33) will, from this point, be considered together.

Figure 1.33. Venn diagram demonstrating association between failure of spiral artery transformation, small-for-gestational age, maternal hypertensive disorders and combined prediction models.



It has been shown that early intervention with low-dose aspirin prior to 16 weeks' gestation is effective in reducing the prevalence of PET (3, 171) and therefore any successful prediction model needs to be implemented prior to this gestation so that the results can be used to the greatest advantage. The value of low-dose aspirin in the prevention of SGA is less clear. Good quality studies suggest minimal changes in the incidence of SGA following aspirin therapy however these all instigate therapy over a range of gestational ages up to 32 weeks (172-174). Studies focussing on the instigation of aspirin therapy prior to 16 weeks gestation show more promising results however these are marred by small sample sizes and more research in this area is required (175, 176).

Prediction of risk for SGA and MHD has been traditionally combined with aneuploidy screening at 11-13^{+6} weeks gestation (119, 177). Combination of screening at this time is cost-effective and, with low-dose aspirin therapy instigated prior to 16 weeks gestation, has the potential to significantly improve pregnancy outcome in those screened 'high-risk' (178). The value of a 11-13^{+6} week ultrasound is being questioned following the introduction of non-invasive

prenatal aneuploidy screening (NIPS). There is a possibility that ultrasound to confirm location, plurality and viability of a pregnancy conducted *prior* to NIPS at 10-11 weeks (179) may replace the 11-13^{+6} week ultrasound. As aspirin prophylaxis against SGA and PET becomes significantly less effective after 16 weeks (3), the loss of risk assessment at that gestational age has the potential to impact the success of prediction and prevention. Risk assessment for SGA and PET earlier than 11 weeks has the potential to alleviate this issue however there has been little research in this area, with no large prospective studies identified.

Current prediction models at 11-13^{+6} weeks gestational age use a combination of clinical risk factors, maternal serum biochemistry and uterine artery Doppler (180). The following section will begin by presenting the individual components used in current prediction models for SGA and MHD at 11-13^{+6} weeks gestation including clinical history, maternal serum biochemistry and uterine artery Doppler. Finally, current combined prediction models for SGA and MHD will be presented with a discussion of how the research presented in this thesis may add to the value of these models.

CLINICAL RISK FACTORS

The development of SGA and MHD in the second and third trimesters of pregnancy has been associated with a variety of clinical risk factors/conditions. These risk factors can be grouped as being of maternal, fetal or placental in origin (181). While the risk factors for SGA and MHD overlap, there are differences, and each will be presented separately.

Small-for-gestational age

Maternal risk factors for SGA are summarised in Table 1.5. These include clinical, obstetric and family histories and current medical status and represent a woman's *a-priori* risk for the development of SGA (180). In addition, fetal factors including multiple gestations, aneuploidy, genetic syndromes and infection also contribute to an increased risk of SGA (181). Placental related risk factors for SGA include previa, abruption, infarction, haemangioma, accreta, circumvellate and confined mosaicism (181). Other risk factors include intrauterine bleeding, marginal/velamentous cord insertions, placental mosaicism and uterine malformations (21, 182).

Table 1.5. *Maternal risk factors for the development of the small-for-gestational age fetus.*

CLINICAL	OBSTETRIC	FAMILY	CURRENT MEDICAL STATUS
advanced maternal age [183] maternal age <20 years [184] ethnicity [37]	nulliparity [183]	family history of IUGR [183]	Blood disorders: thrombophilia [183] anemia [183]
drug use [183]	previous gestational hypertension [183]		autoimmune disorders (SLE, anti-phospholipid syndrome) [183]
malnutrition [184]	previous PET or IUGR [183]		chronic cardio-pulmonary disease [182]
smoking [183]	previous IUFD [183]		Current or chronic hypertension [181]
socioeconomic status [183]	high altitude living [183]		pre-existing diabetes [183]
maternal weight [183]			renal disease [181]

PET pre-eclampsia, IUGR intrauterine growth restriction, IUFD intrauterine fetal death, SLE systemic lupus erythematous.

Maternal Hypertensive Disorders

Prediction of MHD has predominantly focussed on the prediction of PET (183). This is most likely due to the lack of treatment options for the disorder and the significant risk of adverse fetal and maternal outcomes have been discussed earlier in this chapter. In contrast, GH is treatable with anti-hypertensive medications and therefore less risk of adverse pregnancy outcomes. The research in this study addresses the prediction of MHD as a single entity as it felt that an increased risk of MHD predicted early in pregnancy has the potential to influence pregnancy management and may result in the reduction of adverse fetal and maternal outcomes.

Maternal risk factors for PET are summarised in Table 1.6. Similar to the risk factors for SGA, these include clinical, obstetric and family histories and current medical status and represent a woman's *a-priori* risk for the development of PET (184). The most significant factor for the prediction of PET is having a previous pregnancy affected by the disorder; meta-analysis demonstrating that women who have pre-eclampsia in a previous pregnancy have over seven times the risk of its recurrence (RR:7.19; 95%CI: 5.85 to 8.83) (183).

Table 6. *Maternal risk factors for the development of pre-eclampsia.*

CLINICAL	OBSTETRIC	FAMILY	CURRENT MEDICAL STATUS
advanced maternal age [188]	nulliparity [188]	family history of PET [185]	pre-existing diabetes [185]
	previous PET [185]		chronic hypertension
obesity [188] maternal height [189]	partner changes between pregnancies [167]		renal disease [185]
ethnicity [190]			autoimmune disorders [188]
PET pre-eclampsia			

Assessment of clinical risk factors alone has not been found to be an effective screening test for SGA or PET (44, 177, 185). This is thought to be due to factors including heterogeneity between individuals and the magnification of risk conferred by a combination of risk factors (3). The value of clinical risk factor assessment lies in providing an individual *a-priori* risk for adverse outcome that can be improved by the addition of other variables in combined prediction models (44) (Chapter 5 & 6).

MATERNAL SERUM BIOCHEMISTRY

There are many maternal serum biomarkers with encouraging results for the prediction of adverse pregnancy outcomes, particularly SGA and MHD. The research presented in this thesis focusses on the value of conventional and novel ultrasound measures prior to 11 weeks gestation in the prediction of SGA and MHD. Currently the most common maternal biomarkers used in first trimester predictive algorithms for these outcomes are measured at 10-14 weeks gestation. The following section will discuss the maternal biomarkers currently in use at this gestational age and demonstrate that many can be measured prior to 10 weeks gestation (186-189) and therefore in conjunction with an early ultrasound examination prior to NIPS. It is hypothesised that the value of ultrasound measurements in the prediction of these adverse pregnancy outcomes presented in Chapters 5 & 6 will encourage further research addressing the value of including these measurements and maternal biomarkers into predictive algorithms earlier in gestation than is currently possible.

Many studies have investigated the use of biochemical markers during pregnancy as a predictor of SGA and MHD (190). A meta-analysis of novel biomarkers for predicting IUGR included the review of 53 studies with 39 974 women and 37 different biomarkers found that individually each showed a sufficiently low predictive accuracy not to be considered helpful in the prediction of later placental insufficiency (190). The value of maternal biochemistry in the prediction of SGA or MHD appears to lie in combination with maternal clinical risk factors and other parameters in combined prediction models.

A consistent theme in the biomarker literature is the importance of angiogenesis-related biomarkers; both proangiogenic, antiangiogenic and the

balance between the two. The most common of these biomarkers will now be discussed.

Proangiogenic Biomarkers

The most commonly investigated proangiogenic markers are vascular endothelial growth factor (VEGF) and placental growth factor (PIGF). VEGF plays a major role in endothelial cell proliferation and vascular permeability (191). PIGF is thought to work together with VEGF promoting angiogenesis and supporting endothelial function (192).

In the first and second trimesters decreased serum level of these proangiogenic markers has been associated with the subsequent development of SGA and PET (193-195). Vascular endothelial growth factor below the expected median for gestational age, measured in the early second trimester, has demonstrated a RR of 8.79 (95%CI:1.86-14.59) for subsequent development of SGA (196) and 1.43 (95%CI: 0.64-3.18) for PET (196).

Reports of the value of PIGF early in the second trimester for the prediction of adverse pregnancy outcomes have demonstrated varying results (Table 1.7). Despite these varying results a reduction in serum levels of PIGF has also been found from as early as 10 weeks in women who subsequently developed a SGA fetus (without PET) (188) and 12 weeks in women subsequently developing PET (186) and these levels are often presented as a significant contributor to combined prediction models for SGA and PET (Chapters 5 & 6).

Table 1.7. *Placental growth factor in the second trimester associated with the development of pre-eclampsia.*

Thadhani et al.. 2004 [197]	relative risk 2.4	95% CI: 1.4-4.6
Bersinger et al.. 2005 [196]	relative risk 3.7	95%CI: 1.4-8.93
Lambert et al. 2009[195]	sensitivity 50%	false positive rate 2%
Kusanovic et al. 2009[193]	sensitivity 70%	specificity 70%
Ghosh et al 2013 [194]	sensitivity 84%	specificity 78%

Antiangiogenic Biomarkers

The most commonly investigated antiangiogenic markers are soluble fms-like tyrosine kinase-1 (sFlt-1) and soluble endoglin (sEng). Soluble fms-like tyrosine kinase-1 has been found to inhibit proangiogenic factors VEGF and PIGF by promoting endothelial dysfunction leading to hypertension and renal disease (192). Measured at 4-18 weeks gestation sFlt-1 has been associated with an increased risk of the development of SGA (OR: 2.1; 95%CI: 0.9-5.0) (187). In the second trimester sFlt-1 has been shown to be a good predictor of the subsequent development of PET and when used in conjunction with sEng at 21 weeks' gestation has 100% sensitivity and 93% specificity in the prediction of pre-term PET (197). Like sEng, increasing levels of sFlt-1 are considered indicative of worsening PET(198).

Soluble endoglin plays an important role in the maintenance of normal vascular physiology (192) demonstrating anti-angiogenic properties due to its capability of inducing endothelial dysfunction and down regulation of vascular dilatation. This in turn has the effect of increasing vascular permeability predisposing to the development of hypertension (192). Babies with a BW <10th centile for gestational age/SGA have been found to have significantly increased sEng levels from 10 weeks gestation (188). In contrast, and most likely due to the wide gestational age range , a study measuring sEng at 4-18 weeks gestation found only a minimal change in risk of the development of SGA (OR: 1.0; 95%CI: 0.4-2.9) (187). However, later in the second trimester, increased levels of sEng have consistently been found to be associated with pre-term PET (197, 199, 200) with the severity of the dysfunction increasing as serum levels increase (199).

Proangiogenic/antiangiogenic ratios

In the second trimester an increased PIGF/sFlt-1 ratio has been reported to be associated with an increased risk of SGA later in pregnancy (OR: 4.7; 95%CI: 1.5-14.1). The PIGF/sEng ratio in the second trimester has been found to be better at the prediction of pre-term PET than the individual levels (197) with one author reporting a 100% sensitivity and 98-99% specificity (193).

In the first trimester, a significant decrease in the PIGF/sEng ratio has been found from 10 weeks' gestation in pregnancies subsequently diagnosed with

SGA (p=0.0008), preterm PET (p=0.0341), and term PET (p<0.0001) (188).

Other Biomarkers

In addition to angiogenesis-related biomarkers other groups have also been proposed as potentially useful. These include both endothelial function/oxidative-stress related biomarkers and placental protein/hormone-related biomarkers (190). The most commonly reported biomarkers from these groups include homocysteine, PAPP-A, human chorionic gonadotropin (hCG), alpha-fetoprotein (α-fp) and placental protein 13 (PP13).

Homocysteine

Homocysteine is required for normal cell and tissue growth as it is dependent on folate and vitamins B6 and B12 for its metabolism (192). The importance of serum homocysteine levels has been debated in the literature with differing opinions emerging (136, 201, 202).

It has been argued that increased levels of homocysteine at 20 weeks' gestation is associated with pregnancy loss and PET but not GH or SGA fetuses (136, 190). The same association was not found with serum levels at 16 weeks' gestation (201) however Bergen et al. (2012) disagrees finding an association of increased homocysteine levels prior to 18 weeks' gestation with decreased placental weight and birthweight and an increased risk of a SGA fetus (OR: 1.7; 95%CI: 1.2-2.4; p=0.006) (202).

Pregnancy associated plasma protein-A

Pregnancy associated plasma protein-A is a product of the placenta and the decidua that promotes trophoblast invasion (203, 204). It has been found to be useful in both the first and second trimesters in the prediction of placenta-related adverse outcomes later in pregnancy (205, 206).

At 10-14 weeks gestation a decrease in PAPP-A MoM as little as 0.97 (95%CI: 0.90-1.04) is associated with a BW of below the 10th centile (p=0.002) and a PAPP-A MoM of 0.90 (95%CI: 0.80-1.01) with an increased risk of GH (p=0.001) (207). As the MoM decreases the risk of adverse outcomes increases with a study by Yaron, et al. (2002) reporting PAPP-A MoM of ≤0.5Mom is associated with a relative risk of 3.30 (96%CI: 1.87-5.80) of development of SGA (p<0.0001) and a relative risk of 6.09 (95%CI: 2.2-16.9) for GH when the MoM is ≤0.25MoM (p=0.005) (208). Similarly, Moslemi et al. (2012) reported a

PAPP-A level less than the 5th centile for gestation is associated with a 2.9 (95%CI:2.0-4.1) increase in odds of developing SGA/IUGR and a 2.3 (95%CI:1.6-3.3) increase in odd of developing PET later in pregnancy (206).

Placental protein 13

Placental protein 13 (PP13) is considered an important part of blastocyst implantation and spiral artery remodelling aiding in the binding of the placenta and endometrium (192). Maternal serum levels of PP13 have been found to be low in the first trimester in women who subsequently develop PET (209) (Table 1.8) .

Table 1.8. *Reported sensitivity & specificity of placental protein 13 in the prediction of pre-eclampsia.*

PP13	SENSITIVITY	SPECIFICITY
Gonen et al. 2008[189] 6-10 weeks	80%	20% false positive rate PET
Romero et al. 2008 [209] 8-13 weeks	80%	44% (all PET) 84% (PET <34 weeks)
Chafetz et al. 2007[210] 9-12 weeks	90%	79% PET

Human chorionic gonadotropin

Human Chorionic Gonadotropin is a glycoprotein hormone produced by the syncytiotrophoblast within the chorionic villi of the placenta (210). Free beta-Human Chorionic Gonadotropin (free βhCG) is a subunit of hCG commonly measured in conjunction with First Trimester Screening for aneuploidy (211). A study by Ong et al. (2012) reported that free βhCG measured at 10-14 weeks gestation was below the 10th centile in approximately 15% of pregnancies that subsequently developed GH or IUGR within their cohort. This poor predictive result is consistent with other studies which demonstrated no association between free βhCG levels in the first trimester and birthweight (212), SGA (211) or MHD (213).

Alpha-fetoprotein

Alpha-fetoprotein is a plasma protein produced by the embryonic yolk sac and the fetal liver (214). Serum αFP levels have conventionally been used as a screening test for congenital disabilities and chromosomal abnormalities (215, 216) and have also been hypothesised as having value in the prediction of adverse pregnancy outcomes including SGA and PET (217, 218).

Waller et al. (1996) reported than an αFP level of below the 25th or above the 75th centile at 15-19 weeks gestation is associated with a 2.3 times increased risk of SGA compared to interquartile αFP levels ($p<0.01$) (217) and women with increased α-fp levels have a 10.5 times increased risk of developing PET ($p<0.001$) (217). A more recent study showed less encouraging results with an odds ratio for SGA of 0.9 (95%CI: 0.5-1.6) with an elevated αFP alone (218) however in combination with low PAPP-A levels this significantly improves to 8.5 (95%CI: 3.6-20.0) (218).

SUMMARY

There are many maternal serum biomarkers with encouraging results for the prediction of adverse pregnancy outcomes, particularly SGA and PET. This section has provided a summary of the most common biomarkers currently used at 11-13^{+6} weeks gestation. Risk assessment for adverse pregnancy outcomes prior to 11 weeks gestation has the potential to alleviate the possibility of an early ultrasound examination prior to NIPS replacing risk prediction for aneuploidy and SGA/PET at 11-$^{13+6}$ weeks gestation.

The majority of the participants recruited for the research presented in the thesis subsequently underwent first trimester screening for aneuploidy. This included the analysis of maternal serum levels of free βhCG, PAPP-A, PIGF and αFP at 10-13^{+6} weeks gestation. Due to cost restraints and the ease of access to these biomarker levels, the analysis presented in Chapters 5 & 6 will consider these four biomarkers in the prediction models for SGA and PET. Further research addressing the value of including these ultrasound measurements and maternal biomarkers into predictive algorithms prior to 11 weeks gestation will still be required.

COMBINED APPROACHES FOR THE PREDICTION OF SMALL-FOR-GESTATIONAL AGE NEONATES AND MATERNAL HYPERTENSIVE DISORDERS

Using maternal factors alone has a reported prediction rate for SGA of 34% (219), for all PET of 35% (220, 221), pre-term PET of 40-49% (177, 220, 221) and 38% of term PET (177). Various combinations of biomarkers have been proffered as the most accurate and specific for the prediction of adverse pregnancy outcomes. In the first trimester, proposed combinations include PAPPA and PP13 (206), PIGF/Eng and PIGF/soluble vascular endothelial growth factor receptor-1 (SVEGFR1) (188), PIGF and PAPPA (177).

Others advocate the inclusion of uterine artery PI into prediction models for PET. The most widely used model for the prediction of PET at 11-13^{+6} week's gestation uses a combination of maternal factors (age, weight, height, body-mass index, racial origin, medical history, smoking status and obstetric history, maternal mean arterial blood pressure, maternal biochemistry (PIGF and PAPP-A) and uterine artery PI. With a 10% false-positive rate the reported detection rate for PET (<37 weeks gestation) is 75% (95%CI:70-80) with less predictive value for PET (>37 weeks gestation); detection rate 47% (95%CI:44-51)(177). Comparable results are seen in the model designed by Scazzocchio et al. 2013 based on maternal factors, uterine artery Doppler, maternal blood pressure, and PAPP-A implemented at 11-13^{+6} days gestation with a reported detection rate of 80.8% for early PET and 39.6% for late PET (10% FPR) (222).

The Fetal Medicine Foundation provide a prediction model specific to SGA that can be used 11-13^{+6} days gestation with a 73% detection rate for small-for-gestational age fetuses requiring delivery before 37 weeks' gestation and 46% for those delivering at term (219). Improvement in this detection rate was found in the SGA prediction model designed by Crovetto et al. 2016 using a combination of maternal factors, maternal mean arterial blood pressure, mean uterine artery PI and maternal serum biochemistry (PIGF and sFlt-1) implemented at 11-13^{+6} days gestation with a reported detection rate of 86% for early IUGR and 66% for late IUGR (10% FPR) (5).

The addition of serum αFP to maternal characteristics at 11-13 weeks has been reported to improve the detection rate of PET <32 weeks from 44% (95%CI: 28-62) to 53% (95%CI: 35-70) for a FPR of 5% (184). This improvement in detection rate is modest and the wide and overlapping confidence intervals may limit the clinical usefulness of maternal characteristics and αFP alone. More research is needed into the value of including αFP in models with larger numbers of combined features.

Maternal characteristics included in prediction models were age, weight, height, parity, racial origin, chronic hypertension, diabetes mellitus, systemic lupus erythematous, antiphospholipid syndrome, history of SGA or PET, method of conception, smoking status and a history of renal disease (68, 177, 180, 185, 221-223). A summary of predictive values (defined by the AUC) of various combined models for SGA and PET at 11-14 weeks gestation is presented in Tables 1.9 and 1.10 respectively.

SUMMARY

These combined models are the basis of the prediction for placental-based pregnancy complications in many settings. Combined with First Trimester Screening (FTS) for aneuploidy the window of assessment for ultrasound scanning is currently from 11-13^{+6} days. The more recent advent of NIPS and its availability from 10 weeks' gestation may move this "screening window" earlier in pregnancy as many women are presenting for scans prior to undertaking NIPS (179). As NIPS effectively replaces FTS from an aneuploidy perspective there is potential for the woman not to present for another scan until morphological assessment at 18-20 weeks (179).

Reference ranges for uterine artery Doppler have not been established prior to 11 weeks gestation, in addition, this shift to assessment prior to 11 weeks gestation presents the opportunity to add the additional parameters into a prediction model. This includes features of an early pregnancy which are clearer and easier to define than at later gestations.

The research presented in this thesis will investigate the combined value of maternal characteristics, serum biochemistry, conventional and novel ultrasound measures prior to 11 weeks gestation in the prediction of SGA and MHD later in pregnancy.

Table 1.9. *Combined prediction models at 11-14 weeks gestation for the prediction of small-for-gestational age.*

Predictors	n	Outcome	AUC (95%CI)
Maternal characteristics Placental volume (MoM) PAPP-A (MoM) 11-13 weeks GA [70]	3104 (144 SGA)	SGA <5th centile	Maternal characteristics only 0.678 (0.631-0.26) All combined 0.706 (0.660-0.753)
Maternal characteristics CRL, NT, PAPP-A (MoM) BhCG (MoM) 11-14 weeks GA [230]	4702	SGA <5th centile	Maternal characteristics only 0.677 (0.64-0.71) All combined 0.730 (0.69-0.76)
Maternal characteristics PAPPA, PIGF, UAPI, MAP 11-13 weeks GA [191]	62052 (3168 SGA)	SGA <5th with delivery < 37 weeks	Maternal characteristics only 0.727 (0.724-0.731) All combined 0.822 (0.819-0.825)
Maternal characteristics MAP, UAPI, PIGF, sFlt-1 11-13^{+6} weeks GA [181]	9150 (979 SGA)	SGA <10th centile	Maternal characteristics only 0.645 (0.657-0.700) All combined 0.678 (0.657-0.700)

AUC area under the curve, BhCG Beta human chorionic gonadotropin, CI confidence interval, CRL crown-rump length, GA gestational age, MAP maternal mean arterial pressure, MoM multiples of the median, N number of participants, PAPP-A pregnancy – associated plasma protein A, PIGF placental growth factor, sFlt-1 soluble fms-like tyrosine kinase-1, SGA small-for-gestational age, UAPI uterine artery pulsatility index.

Table 1.10. *Combined prediction models at 11-14 weeks gestation for the prediction of pre-eclampsia.*

Predictors	n	AUC (95%CI)
Maternal characteristics 11-13 weeks GA [228]	120,492 (2704 with PET)	All PET 0.7562 PET with delivery < 37 weeks 0.7920 PET with delivery < 34 weeks 0.8106 (CI's not provided)
Maternal characteristics UAPI (early PET) PAPP-A (late PET) $11\text{-}13^{+6}$ weeks GA [229]	5170 (136 with PET)	PET <34 weeks GA: 0.96 (0.94 – 0.98) PET >34 weeks GA: 0.710 (0.658-0.763)
Maternal characteristics, MAP, UAPI, PAPP-A, PIGF [178]	35,948 (1058 with PET)	Maternal characteristics only PET <37 weeks GA 0.800 PET >37 weeks GA 0.745 All combined: PET < 37 weeks GA 0.907 PET >37 weeks GA 0.796 (CI's not provided)

AUC area under the curve, CI confidence interval, GA gestational age, MAP maternal mean arterial pressure, N number of participants, PAPP-A pregnancy – associated plasma protein A, PIGF placental growth factor, UAPI uterine artery pulsatility index.

CONCLUSION

This review of the literature indicates a paucity of knowledge on the value of ultrasound features early in the first trimester in the prediction of adverse pregnancy outcomes. It is on this foundation that the aims of this research are based. The following section will discuss the aims of the research presented in this thesis and why they are important.

SUMMARY: WHAT THIS RESEARCH IS ABOUT AND WHY IT IS IMPORTANT

This research is a prospective longitudinal cohort study focussing on the underlying physiological principles of effective trophoblastic development being reflected in the thickness and volume of the early placenta and uterine artery blood flow prior to 11 weeks gestation.

Hypothesis

Novel ultrasound measures prior to 11 weeks gestation have the potential to improve the of prediction of miscarriage prior to 12 weeks, SGA and MHD.

Specific Aims

To investigate the value of:

1. Conventional and novel first trimester ultrasound measures prior to 11 weeks in the prediction of miscarriage prior to 12 weeks gestation (Chapter 3).
2. meanUAPI measured prior to 11 weeks in the prediction of SGA & MHD (Chapter 4).
3. Conventional and novel ultrasound measures prior to 11 weeks in the prediction of SGA neonates (Chapter 5).
4. Conventional and novel ultrasound measures prior to 11 weeks in the prediction of MHD (Chapter 6).

Anticipated Outcomes

It is anticipated that the novel first trimester ultrasound measures being investigated could be used either in isolation or in combination with each other and / or current methods of risk assessment for SGA and MHD to identify those pregnancies at risk earlier than is currently available with intervention offered earlier in the pregnancy. This in turn, has potential to inform pregnancy management and improve outcomes for those considered at an increased risk.

Results from this study may also be able to influence and encourage future research into the measures available to assess the trophoblast that could result in the development of new approaches that further improve the outcomes for these pregnancies.

Chapter 2. Materials and methods

Contributions:

Tracey Hanchard: *Drafting of the manuscript*

Associate Professor Ann Quinton: *Review of the manuscript*

Clinical Professor Jonathon Hyett: *Review of the manuscript*

INTRODUCTON

This chapter describes the research design and methods used for the research presented in this thesis. Originally designed to assess the combined value of conventional and novel ultrasound measures prior to 11 weeks gestation in the prediction of SGA and MHD, interim analysis of the data provided insights for the value of these measures in the prediction of miscarriage prior to 12 weeks gestation (Chapter 3). Chapter 4 investigates the value of meanUAPI in the prediction of SGA and MHD and Chapters 5 & 6 presented combined prediction models for SGA and MHD respectively.

The research results presented in this thesis are from the data collected from a prospective cohort presenting for an obstetric ultrasound prior to 11 weeks gestation at a single private obstetric ultrasound practice in Wollongong, NSW between February 2016 and July 2018.

Derived from this work are four separate papers presented in Chapters 3-6. Many of the features are common between papers and these will be discussed is the remainder of this chapter. Design features that vary between papers will be discussed in the subsequent relevant chapters.

ETHICS APRROVAL

Ethical approval was provided by NSW Health Human Research and Ethics Committee (SLHD Protocol No. X15-0323) on 16th November 2015. This approval is applicable to all research presented in Chapter 3-6.

SAMPLE SIZE CALCULATION

The long-term goal of the research was to assess the value of early ultrasound

measures in the prediction of SGA and MHD. As discussed in Chapter 1, SGA is defined as a BW of below the 10th centile (128); therefore 10% of all babies born should be SGA. It was estimated that approximately 100 cases of SGA (requiring 1000 participants) would be required for an appropriate number of variables to be considered in the proposed prediction models (224).

Allowing for participants who would be subsequently excluded or lost to follow-up 1200 participants were recruited from February 2016 and July 2018 with all pregnancies due to deliver prior to mid-February 2019.

ROLE OF PHD CANDIDATE

The role of myself, Tracey Hanchard in this thesis included research planning, data collection, statistical analysis and writing of the manuscript.

RECRUITMENT & DATA COLLECTION

Women presenting for an ultrasound examination <11 weeks gestation were given the study information sheet to read whilst waiting for their scan. Once the scan had commenced and the patient reassured that the pregnancy was viable the woman was given the opportunity to ask questions about the research and asked if they would like to participate.

If the woman agreed to participate, informed consent was obtained and the novel ultrasound measures recorded. Following the examination, the women were asked to complete the participant questionnaire and the participant data collection sheet was completed. De-identified patient data was entered into an SPSS database for later analysis.

Relevant information from subsequent scans was added to the data collection sheet and database as it became available. Birth data was accessed either directly from medical records or a centralised database depending on which local facility the baby was delivered. All data collection forms and electronic data were stored as per University of Sydney policy (225).

ELIGIBILITY CRITERIA

Eligibility criteria included:

- consent for a transvaginal scan with capture of additional ultrasound images and measurements (taking <5 minutes),
- the presence of a single intrauterine pregnancy,

- a CRL measuring less than 45mm, and
- demonstration of a fetal heart beat.

Participants were subsequently excluded from analysis if:

- consent for participation was withdrawn, or
- pregnancy outcomes details could not be collected.

In addition, participants with continuing pregnancies at 12 weeks gestation were subsequently excluded if:

- fetal aneuploidy was diagnosed,
- fetal structural anomalies were diagnosed,
- there was pregnancy loss prior to 20 weeks gestation,

or

- stillbirth or neonatal death (after 20 weeks gestation).

METHODOLOGY

At first presentation (<11 weeks gestation) transvaginal ultrasound examination was conducted with a GEVolusonE8 or E10 ultrasound machine (GEHealthcare, Milwaukee, WI) equipped with a 5-to 9-MHz 3-dimensional transducer.

Conventional ultrasound measures recorded for the purposes of the research were MSD, YSD, CRL and FHR. Novel ultrasound measures recorded were trophoblast thickness and bilateral UAPI. A 3D volume of the entire pregnancy was recorded at the time of the scan for subsequent off-line calculation of the trophoblast volume. The precise method used for each of these ultrasound measures have been presented in previous sections of Chapter 1. Participants who subsequently presented for FTS at 11-13^{+6} weeks gestation had measurements of the UAPI recorded for comparison with measurements recorded at the initial scan. Maternal serum biochemistry results for these participants were also recorded.

STATISTICAL ANALYSIS

Preliminary data analysis for each paper included data-checking for outliers, incorrect entries and missing values.

Each chapter presented in this thesis was conducted at a different stage of data collection. Each chapter presented in this thesis was conducted at a different stage of data collection. Maternal characteristics, medical history and current pregnancy characteristics in the various pregnancy outcome groups are summarised in Table 2.1.

Table 2.1. *Maternal characteristics, medical history and current pregnancy characteristics in the pregnancy outcome groups: normal outcome (normotensive with birthweight ≥10th centile) (n=1019); miscarriage <12 weeks (n=50); birthweight <10th centile (n=73); pregnancy-induced hypertension (n=49) and pre=eclampsia (n=6).*

Maternal Characteristic	Normal outcome (n=1019)	Miscarriage <12 weeks (n=50)	BW <10th centile (n=73)	PIH (n=49)	PET (n=6)
Age (years)	32 (16-44)	33 (21-43)	31 (19-40)	31 (21-43)	29 (23-37)
Weight (kilograms)	72 (36-146)	68 (57-77)	69 (48-98)	81 (51-136)	90 (55-129)
Height (centimetres)	166 (140-186)	166 (163-174)	163 (145-189)	164 (150-180)	160 (145-179)
GA at enrolment in study (weeks)	7^{+4} (5^{+3}-11^{+2})	6^{+6} (6^{+5}-7^{+2})	7^{+3} (5^{+3}-10^{+6})	7^{+5} (5^{+5}-10^{+2})	7^{+5} (5^{+3}-11^{+2})
GA at delivery (weeks)	39^{+0} (26^{+3}-42^{+0})	N/A	38^{+4} (30^{+2}-41^{+6})	38^{+2} (35^{+0}-42^{+0})	35^{+1} (30^{+2}-39^{+6})
Number of pregnancies	2.5 (1-10)	2.5 (1-6)	2.1 (1-6)	2.1 (1-6)	1.5 (1-3)
Pre-existing medical conditions					
None	860 (84.3)	43 (86.0)	61 (83.6)	38 (77.6)	6 (100.0)
Hypothyroidism	81 (7.9)	4 (8.0)	9 (12.3)	7 (14.3)	-
Psychological	20 (2.0)	-	-	3 (6.1)	-
Asthma	22 (2.2)	2 (4.0)	-	-	-
Epileptic	6 (<1.0)	-	-	-	-
Grave's Disease	2 (<1.0)	-	-	-	-
Hashimoto's thyroiditis	1 (<1.0)	-	1 (1.4)	-	-
Metabolic Syndrome	1 (<1.0)	-	-	1 (2.0)	-
Factor 5 Leiden	1 (<1.0)	-	-	-	-
APS	1 (<1.0)	-	-	-	-
Blood clots	5 (<1.0)	-	-	-	-
SLE/Lupus	2 (<1.0)	-	-	-	-
Multiple	17 (1.7)	1 (2.0)	2 (2.7)	-	
Current Medications					
None	858 (84.2)	43 (86.0)	59 (80.8)	38(77.6)	6 (100.0)
Thyroxine	78 (7.7)	4 (8.0)	8 (11.0)	7 (14.3)	-
Anti-depressants	29 (2.8)	1 (2.0)	-	2 (4.1)	-
Diabetes	12 (1.2)	-	-	1 (2.0)	-
Asthma	14 (1.4)	1 (2.0)	1 (1.4)	-	-
Anti-epileptics	3 (<1.0)	-	-	-	-
Multiple	25 (2.5)	1 (2.0)	5 (6.9)	1 (2.0)	
Smoking status					
Non-smoker	998 (97.9)	50 (100.0)	70 (95.9)	49 (100.0)	5 (83.3)
Smoker	21 (2.1)	-	3 (4.1)	-	1 (16.7)
Diabetic Status					
None	926 (90.9)	49 (98.0)	69 (94.5)	40(81.6)	6 (100.0)
Type I	4 (<1.0)	1 (2.0)	-	1 (2.0)	-
Type II	6 (<1.0)	-	-	-	-
Gestational	83 (8.1)	-	4 (5.5)	8 (16.3)	-
Menstrual cycle					
28 days	450 (44.2)	22 (44.0)	36 (49.3)	29 (59.2)	3 (50.0)
< 28 days	116 (11.4)	3 (6.0)	7 (9.6)	3 (6.1)	-
> 28 days	201 (19.7)	10 (20.0)	12 (16.4)	8 (16.3)	2(33.3)
Variable	252 (24.7)	15 (30.0)	18 (24.7)	9 (18.4)	1 (16.7)
Method of Conception					
Spontaneous	937 (91.9)	44 (88.0)	59 (80.8)	44(89.8)	6 (100.0)
Ovulation induction	56 (5.5)	4 (8.0)	8 (11.0)	4 (8.2)	-
IVF (no induction)	24 (2.4)	2 (4.0)	6 (8.2)	1 (2.0)	-
IVF (donor)	2 (<1.0)	-	-	-	-
Indication for ultrasound					
None	306 (30.0)	15 (30.0)	323 (31.5)	9 (18.4)	1
Dating	516 (50.1)	20 (40.0)	30 (41.1)	26 (53.1)	5
Viability	139 (13.6)	13 (26.0)	17 (23.3)	10 (20.4)	-
Previous miscarriage	44 (4.3)	1 (2.0)	-	2 (4.1)	-
Pain	8 (<1.0)	1 (2.0)	-	2 (4.1)	-
Exclude plurality	3 (<1.0)	-	1 (1.4)	-	-
Pre-NIPS	3(<1.0)	-	-	-	-
Uterine anomaly	-	-	2 (2.8)	-	-

Data are provided as mean (range) or n *(%). BW, birthweight; GA, gestational age; APS, Antiphospholipid Syndrome; IVF, in-vitro fertilisation; P.V., per vaginal; NIPS, non-invasive prenatal screening.*

All data analyses were conducted using using SAS OnDemand for Academics **(**SAS release: 9.04.01M5P09132017, 2017 or IBM SPSS Statistics for Windows, Version 23.0 (2015). Armonk, NY: IBM Corp.

Chapters 3, 5 and 6 include the application of binary logistic regression with predictive models proposed for miscarriage prior to 12 weeks gestation (Chapter 3), small-for-gestational age neonates (Chapter 5) and maternal hypertensive disorders (Chapter 6). For the purposes of these models parameters found to be not linearly related with the logit-outcome were categorised into two groups above and below the median. The use of the median as the cut-off had the advantage of dividing each variable into two equal sized groups. These parameters were then analysed as categorical rather than continuous variables within the logistic regression model. Parameters linearly related to the logit outcome (maternal height) were included in the model as continuous variables.

Specific techniques used varied for each aim of the research are presented in Chapters 3-6.

Chapter 3. First trimester ultrasound features in the prediction of miscarriage.

Addressing the first aim of this research, this chapter discusses the predictive value of conventional and novel first trimester ultrasound measures in the prediction of miscarriage.

This chapter has been published in a different format in a peer reviewed journal.

Citation:

Taylor TJ, Quinton AE, de Vries BS, Hyett JA. First trimester ultrasound features associated with subsequent miscarriage: A prospective study. Aust N Z J Obstet Gynaecol. 2019 Oct;59(5):641-648.

Contributions:

***Tracey Hanchard**: research planning, data collection, statistical analysis and drafting of the manuscript*

***Associate Professor Ann Quinton:** research planning and review of the manuscript*

***Dr Bradley de Vries**: statistical advice and review of the manuscript*

***Clinical Professor Jonathon Hyett:** research planning and review of the manuscript.*

Relevance:

Miscarriage during the first trimester of pregnancy is common and easily diagnosed with ultrasound. Prediction of miscarriage using ultrasound is less accurate. This chapter demonstrates that a reduction in TV and meanUAPI measured prior to 11 weeks gestation is significantly associated with subsequent miscarriage prior to 12 weeks gestation. These measures, in conjunction with a reduction in MSD and FHR are of good predictive value for miscarriage and may be useful in counselling and guiding the short-term management of early pregnancies

Chapter 3. First trimester ultrasound features associated with subsequent miscarriage.

INTRODUCTION

First trimester miscarriage is common, affecting one in five pregnancies (90). Women are increasingly offered an ultrasound scan early in the first trimester to assess location, plurality, gestational age and pregnancy viability. These women are universally anxious and looking for reassurance as to the current and expected ongoing health of their pregnancy (91).

First trimester ultrasound appearances are well-established for the *diagnosis* of miscarriage (57). In contrast, ultrasound appearances are less specific for the *prediction* of miscarriage. Although assessment of the size and shape of the gestational sac and yolk sac will identify a high proportion of pregnancies that fail to progress beyond the first trimester, these measures are not specific enough to be of predictive value (97, 226). Similarly, fetal heart rate is frequently reduced in circumstances where there is fetal demise prior to 12 weeks gestation, but this has also not been found to be of sufficient predictive value to guide clinical management (64).

Predictive value may be improved by including other ultrasound markers. The potential value of assessing trophoblast appearance and uterine artery Doppler has been demonstrated at 11-14 weeks gestation. A limited amount of data is available on trophoblast thickness and placental volume, reported to be thinner / smaller in pregnancies that miscarry (42). Uterine artery Doppler changes are widely reported from 11 weeks gestational age, with an increase in mean uterine artery pulsatility index (meanUAPI) reported in pregnancies that have an adverse outcome (84, 85). The aim of this study was to determine if ultrasound appearances including mean sac diameter (MSD), yolk sac diameter (YSD), fetal heart rate (FHR), trophoblast thickness (TT), trophoblast volume (TV) and bilateral uterine artery pulsatility index (UAPI) in pregnancies prior to 9 weeks gestation, are of value in determining progress beyond 12 weeks.

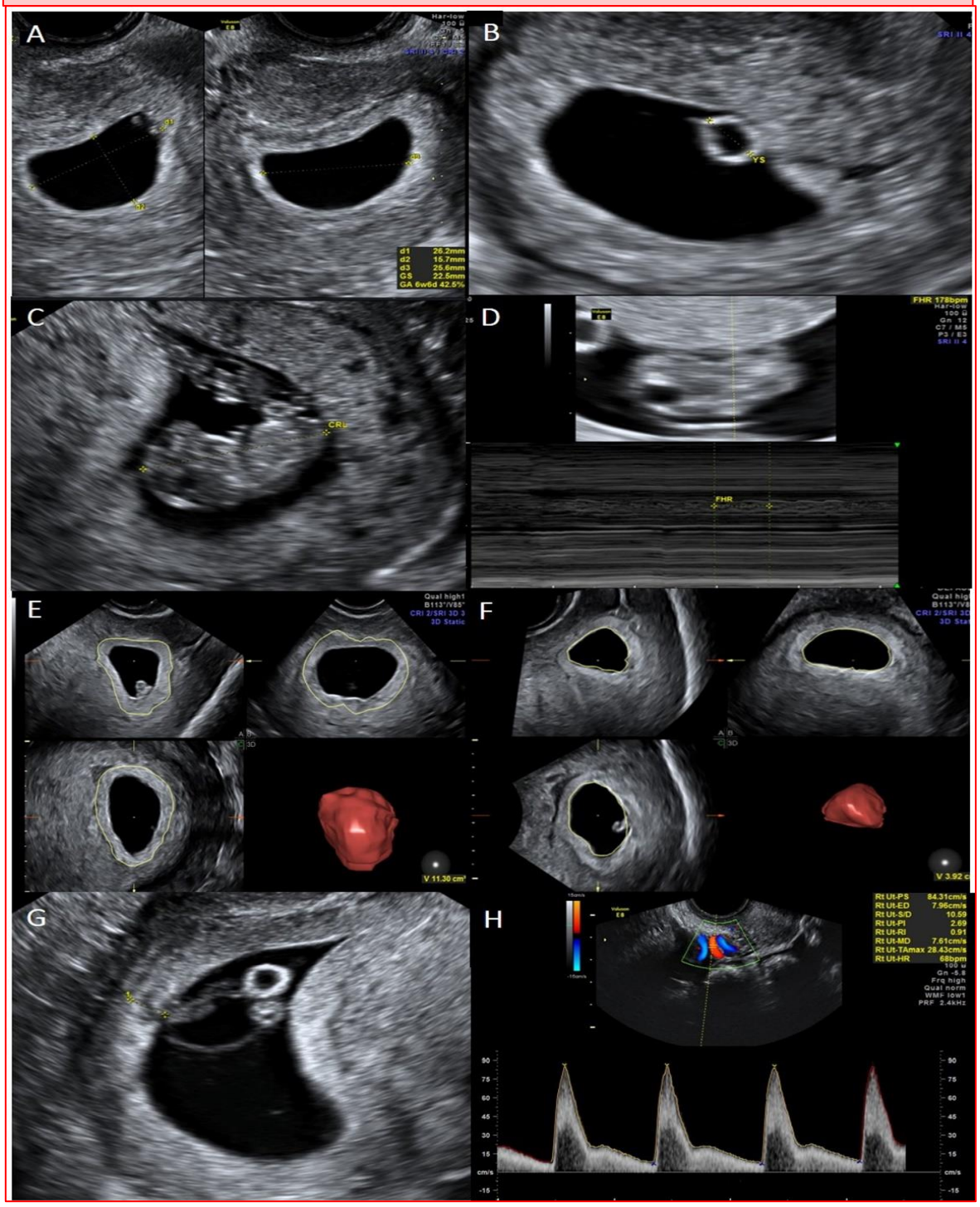
MATERIALS AND METHODS

This was a prospective cohort study designed to assess the predictive value of early ultrasound measurements in the assessment of adverse pregnancy outcomes, including miscarriage by the end of the first trimester (12 weeks) of pregnancy. Consecutive patients that attended for an early (<9 weeks) ultrasound scan at a single private obstetric ultrasound practice were enrolled in the study between February 2016 and May 2018. Eligibility criteria included consent for a transvaginal scan with capture of additional ultrasound images and measurements (taking <5 minutes), the presence of a single intrauterine pregnancy, a crown rump length measuring less than 21mm in length, demonstration of a fetal heart beat, absence of per-vaginal (PV) bleeding and availability of pregnancy outcome data. All participants provided written informed consent. Fetal ultrasound exposure was maintained as low as reasonably achievable by minimising output power, mechanical and thermal indices.

Transvaginal ultrasound examination was conducted with a GEVolusonE8 ultrasound machine (GEHealthcare, Milwaukee, WI) equipped with a 5-9MHz 3-dimensional transvaginal transducer. All scans were performed by accredited sonographers with 5-25 years (mean: 14 years) experience. Crown-rump length (CRL), MSD, YSD, FHR, TT, TV and bilateral UAPI were measured (Figure 3.1).

The gestational sac was measured in three dimensions (longest diameter (A), perpendicular to this diameter (B) and the widest cross-section (C)) with the MSD calculated using the formula: (A+B+C)/3 (47). The yolk sac was imaged in its longest plane and its diameter measured from the mid-section of one wall to the mid-section of the opposing wall (49). The CRL was measured as the longest length of the embryo (11) and was measured three times with the mean measurement recorded for the purposes of the study. Fetal heart movement was demonstrated using M-Mode and the heart rate measured across two cardiac cycles (227).

Figure 3.1 *(a-h) Composite image showing first trimester ultrasound measurements. (a) mean sac diameter, (b) yolk sac diameter, (c) crown rump length, (d) fetal heart rate, (e) trophoblast volume (larger), (f) trophoblast volume (smaller), (g) trophoblast thickness, (h) uterine artery Doppler with calculation of pulsatility index.*



The trophoblast is demonstrated on ultrasound as the uniformly hyperechoic region surrounding the gestational sac (excluding the decidua). The TT was measured at the cord insertion site (if demonstrable) or adjacent to the yolk sac (42). The volume of the trophoblast was assessed using three-dimensional (3D) mode with the sweep sector centred to include the entire uterus. After the sweep was taken the 3D data was stored for off-line assessment by a single sonographer (TT). Virtual Organ Computer-Aided Analysis (VOCAL) software (3-dimensional Sonoview; GE Healthcare) with 30-degree rotational steps (12 rotations per volume) was used to calculate the volumes of the pregnancy at the outer extent of the trophoblast and the gestational sac (68) taking approximately 3 minutes. The gestational sac volume was subtracted from the total pregnancy volume and used to calculate the trophoblast volume (mL).

Using colour Doppler, each uterine artery was assessed with colour and spectral Doppler with calculation of the pulsatility index (PI) (89). The PI was calculated from the spectral Doppler waveform, using the formula:

$$\frac{\textit{peak systolic velocity - end diastolic velocity)}}{\textit{time averaged velocity.}}$$

The meanUAPI was calculated by adding the left and right PI and dividing by 2.

STATISTICAL ANALYSIS

Maternal and pregnancy characteristics, ultrasound findings and pregnancy outcome were entered into a computer database. Regression lines were fitted for each parameter to determine the expected measurements at a given CRL (as a proxy for gestational age). Each regression model was assessed for the assumptions of linear regression; linearity, normality and homoscedasticity. Models for MSD and YSD were found to satisfy each assumption and the raw residuals were used for further analysis. The most appropriate model for FHR was determined to be a fourth order polynomial regression. Trophoblast thickness, volume and meanUAPI required square root transformation of the residuals before analysis. Z-Scores were calculated for each parameter using the formula:

(observed measurement - expected measurement) / standard deviation of the group.

Z-Scores were compared using a two-sample t-test for independent variables and reported as mean (95%CI). Frequencies were reported as n (%) and compared using Pearson's χ^2 test. A p value of <0.05 was considered statistically significant.

Logistic regression analysis was used to create a prediction model for miscarriage based on the standardised ultrasound measurements recorded. A receiver operator characteristic (ROC) curve was obtained to assess the performance of this model. The diagnostic performance of the model was compared for false positive rates of 5%, 10%, 20% and 30%.

Reporting and presentation follow the principles of the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) initiative (228).

RESULTS

A total of 1036 patients were eligible for inclusion in this study. We excluded 123 cases with unknown pregnancy outcomes at 12 weeks. Of the remaining 913 pregnancies, 863 continued to be viable at 12 weeks gestation (94.3%) whilst 50 women (5.7%) had miscarried. This miscarriage rate is consistent with previous research demonstrating that the number of pregnancies expected to end in miscarriage is significantly lower after the demonstration of fetal heart motion with ultrasound (90, 229). With the exception of maternal age, there was no difference in demographic characteristics between the two groups (Table 3.1).

The CRL measured at the initial ultrasound assessment was significantly greater (p<0.001) in pregnancies that continued beyond 12 weeks (11.9mm (1.7-21.0)) compared to those that subsequently miscarried (9.5mm (2.5-21.0)). These CRL ranges equate to gestational ranges of 7^{+2} weeks (5^{+1} – 8^{+5} weeks) and 7^{+0} weeks (5^{+3} – 8^{+5} weeks) respectively (55). The six ultrasound parameters assessed in this study (MSD, YSD, FHR, meanUAPI, TT and TV) showed a significant correlation with CRL. Regression lines were fitted for each parameter to determine the expected measurements at a given gestational age. In pregnancies that progress past 12 weeks gestational age the fetal heart rate was found to have a quartic relationship with CRL.

Table 3.1. *Maternal characteristics, medical history and current pregnancy characteristics in the two groups of pregnancy outcome; progressive at 12 weeks gestation and miscarriage prior to 12 weeks gestation.*

Maternal Characteristic	Continuing at 12 weeks gestation (n=863)	Miscarried prior to 12 weeks gestation (n=50)	P
Age (years)	31 (16-44)	33 (21-43)	0.002
Weight (kilograms)	72 (36-146)	68 (57-77)	0.494
Height (centimetres)	166 (140-186)	166 (163-174)	0.796
Number of pregnancies	2.5 (1-10)	2.5 (1-6)	0.923
Number of previous miscarriages	0.5 (0-9)	0.65 (0-4)	0.700
Pre-existing medical conditions			0.989
None	722 (83.7)	43 (86.0)	
Hypothyroidism	80 (9.3)	4 (8.0)	
Psychological	13 (1.5)	-	
Asthma	10 (1.2)	2 (4.0)	
Blood clots	4 (0.5)	-	
Epileptic	4 (0.5)	-	
Grave's Disease	2 (0.2)	-	
Hashimoto's thyroiditis	2 (0.2)	-	
Metabolic Syndrome	2 (0.2)	-	
Antiphospholipid Syndrome	1 (0.1)	-	
Factor 5 Leiden	1 (0.1)	-	
Hyperthyroidism	1 (0.1)	-	
SLE/Lupus	1 (0.2)	-	
Multiple	20 (2.3)	1 (2.0)	
Current Medications			0.723
None	705 (81.6)	42 (84.0)	
Thyroxine	74 (8.6)	4 (8.0)	
Anti-depressants	24 (2.8)	1 (2.0)	
Diabetes	14 (1.6)	-	
Asthma	7 (0.8)	1 (2.0)	
Clexane or Aspirin	5 (0.6)	-	
Anti-epileptics	3 (0.4)	-	
Anti-hypertensives	2 (0.2)	1 (2.0)	
Multiple	29 (3.4)	1 (2.0)	
Smoking status			0.347
Non-smoker	848 (98.3)	50 (100.0)	
Smoker	15 (1.7)	-	
Blood pressure			0.152
Normotensive	859 (99.5)	49 (98.0)	
Hypertensive	4 (0.5)	1 (2.0)	
Diabetic Status			0.174
None	735 (85.1)	43 (86.0)	
Type I	2 (0.2)	1 (2.0)	
Type II	6 (0.7)	-	
Menstrual cycle			0.464
28 days	370 (42.9)	22 (44.0)	
< 28 days	103 (11.9)	3 (6.0)	
> 28 days	192 (22.2)	10 (20.0)	
Variable	198 (22.9)	15 (30.0)	
Method of Conception			0.904
Spontaneous	781 (90.5)	44 (88.0)	
Ovulation induction	54 (6.3)	4 (8.0)	
IVF (no ovulation induction)	25 (2.9)	2 (4.0)	
IVF (donor)	2 (0.2)	-	
Indications for ultrasound			0.114
None	266 (30.8)	15 (30.0)	
Dating	418 (48.4)	20 (40.0)	
Viability	132 (15.3)	13 (26.0)	
Previous miscarriage	34 (3.9)	1 (2.0)	
Pain	7 (0.8)	1 (2.0)	
Exclude plurality	4 (0.5)	-	
Pre-NIPS	2 (0.2)	-	

Data are provided as mean (range) or n (%). IVF, in-vitro fertilisation; P.V., per vaginal; NIPS, non-invasive prenatal screening.

All other parameters were found to have a linear relationship with CRL however trophoblast thickness and volume measurements demonstrated increasing variance around the mean as the CRL increased.

The regression formulae for the 863 ongoing pregnancies were used to calculate the expected measurement of each ultrasound parameter as determined from the CRL. Figure 3.2 illustrates the expected median, 5th and 95th centiles for each ultrasound parameter with the observed measurements for pregnancies that miscarried prior to 12 weeks gestation. Z-Scores were significantly different in pregnancies that subsequently miscarried rather than progressed beyond 12 weeks.

The six ultrasound parameters were found to be not linearly related with the logit-outcome and were each categorised into two groups above and below the median. The use of the median as the cut-off had the advantage of dividing each variable into two equal sized groups. The parameters were then analysed as categorical rather than continuous variables within the logistic regression model.

For ease of presentation the mean Z-score of each ultrasound parameter for progressive pregnancies was adjusted to zero and Z-scores for the miscarriage group adjusted accordingly. Comparison of Z-Scores for meanUAPI (0.00 (-0.07-0.07) vs. -0.48 (-0.82- -0.14); p=0.001), TV (0.00 (-0.07-0.07) vs. -0.50 (-1.10-0.10); p=0.002), FHR (0.00 (-0.07-0.07) vs. -0.55 (-1.04- -0.06); p=0.031), MSD (0.00 (-0.07-0.07) vs. -0.52 (-1.05-0.00); p=0.05) demonstrated significant variation between the two groups. (Table 3.2). The statistical significance of these differences varied from their significance when combined in logistic regression analysis, most likely due to interaction between variables. If considered important during validation of the proposed model this could be further assessed with post-hoc analysis.

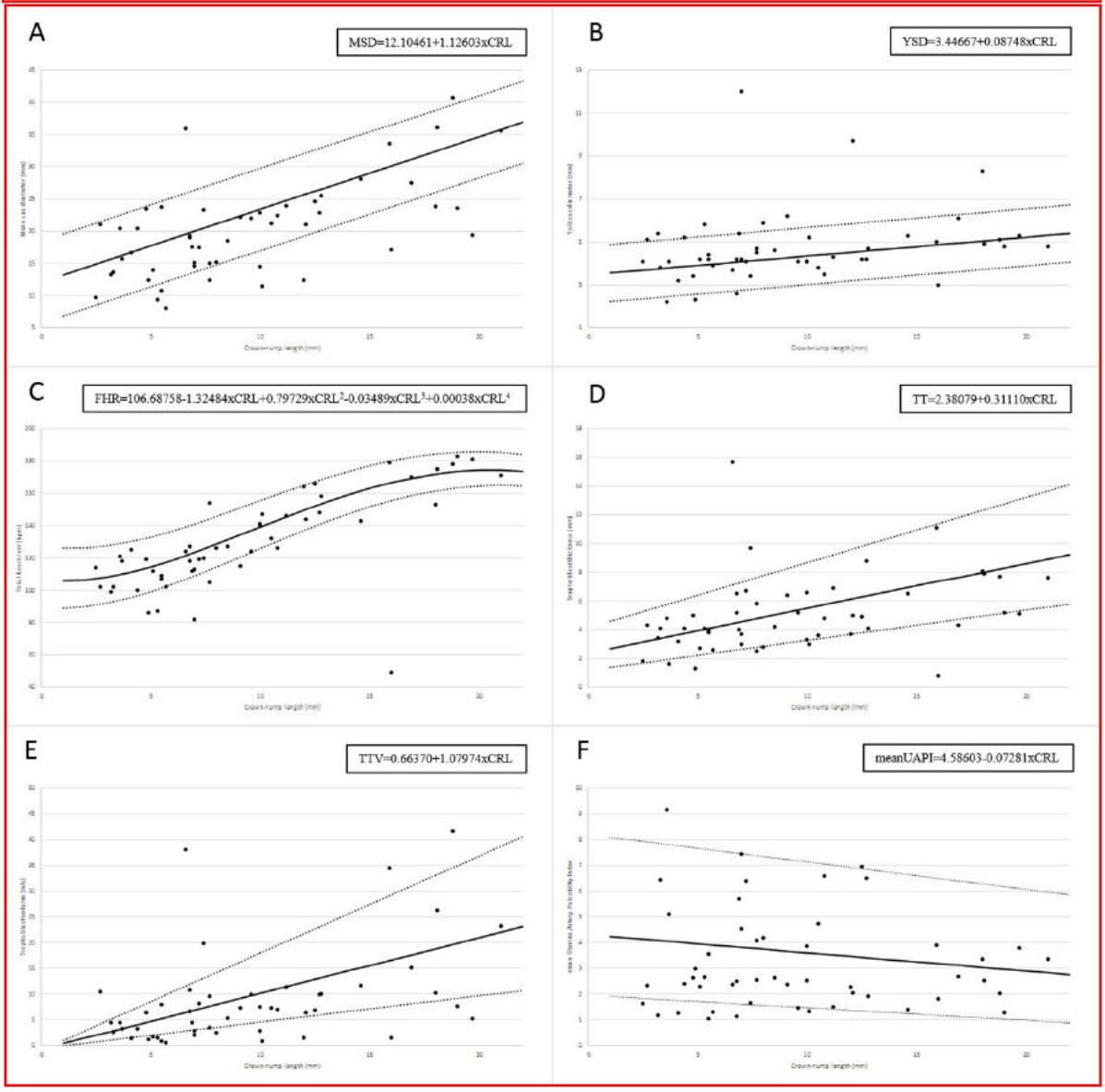
Table 3.3 illustrates the results of the logistic regression analysis. Collinearity was found in the base model between TT (p = 0.06) and TV (p = 0.02). Trophoblast thickness was removed from the model as it appears not to be statistically significant in the initial analysis.

Table 3.2. *Comparison of first trimester ultrasound parameters between pregnancies that were non-progressive prior to 12 weeks gestation and pregnancies that remained progressive at 12 weeks gestation (Z-Score and observed measurement; t-statistic and degrees of freedom).*

Parameter	Continuing at 12 weeks (n=863)	Miscarried prior to 12 weeks (n=50)	P
meanUAPI	0.00 (-0.07-0.07)[†] 3.72 (1.53-7.35)	-0.48 (-0.82- -0.14) 3.23 (1.19-6.94)	0.001 $t_{3.25}$; df_{907}
Trophoblast volume	0.00 (-0.07-0.07)[‡] 12.8mls (3.1-27.6)	-0.50 (-1.10-0.10)[§] 8.8mls (0.9-34.4)	0.002 $t_{3.12}$; df_{886}
FHR	0.00 (-0.07-0.07) 147bpm (113-176)	-0.55 (-1.04- -0.06) 129bpm (86-179)	0.031 $t_{2.23}$; df_{50}
MSD	0.00 (-0.07-0.07) 25.5mm (15.1-36.1)	-0.52 (-1.05-0.00) 20.2mm (9.7-35.9)	0.050 $t_{1.98}$; df_{51}
YSD	0.00 (-0.07-0.07)[¶] 4.5mm (3.1-6.0)	0.71 (-0.04-1.46)[§] 4.7mm (2.6-8.3)	0.065 $t_{-1.89}$; df_{49}
Trophoblast thickness	0.00 (-0.07-0.07)[¶] 6.3mm (3.0-10.5)	-0.35 (-0.78-0.08) 5.0mm (1.6-9.7)	0.113 $t_{-1.61}$; df_{52}

Data are given as mean (95% confidence internal). [†] *n*=859; [‡] *n*=839; [§] *n*=49; [¶] *n*=862 equal variances assumed, meanUAPI, trophoblast volume; equal variances not assumed, FHR, MSD, YSD, trophoblast thickness, MSD, mean sac diameter; meanUAPI, mean uterine artery pulsatility index; YSD, yolk sac diameter; FHR, fetal heart rate; t, t-statistic; df, degrees of freedom.

Figure 3.2. *(a-f) Scatterplots of the expected median (solid line), 5th and 95th centiles (dotted lines) for: (a) mean sac diameter (MSD), (b) yolk sac diameter (YSD), (c) fetal heart rate (FHR), (d) trophoblast thickness (TT), (e) trophoblast volume (TTV), (f) mean uterine artery pulsatility index (meanUAPI);* ● *pregnancies miscarried prior to 12 weeks gestation.*



The final model uses the formula:

$$P_{miscarriage} = \frac{e^{-1.561-(1.260\times MSD\_Z)+(0.584\times YSD\_Z)-(2.330\times FHR\_Z)-(0.612\times TV\_Z)-(0.542\times meanUAPI\_Z)}}{1+e^{-1.561-(1.260\times MSD\_Z)+(0.584\times YSD\_Z)-(2.330\times FHR\_Z)-(0.612\times TV\_Z)-(0.542\times meanUAPI\_Z)}}$$

$P_{miscarriage}$ denotes the probability of miscarriage prior to 12 weeks gestation. Other variables have a value of 0 if below the median and 1 if above the median. This results in an area under the ROC curve (AUC) of 0.81 (95%CI 0.74-0.87); R^2=0.639 (p<0.0001). Yolk sac diameter, TV and meanUAPI demonstrate p values >0.05. Using backwards stepwise elimination, removing meanUAPI then YSD diameter resulted in a model with only significant independent predictors (MSD, FHR and TTV) with an AUC of 0.80 (95%CI 0.74-0.86); R^2=0.636 (p<0.0001), did not significantly change the power of the model. The sensitivity, positive and negative predictive values of the final model are shown for fixed false positive rates (Table 3.4).

Table 3.3. *Logistic regression analysis of the parameters used to predict miscarriage prior to 12 weeks gestation.*

Parameters	β	OR (CI)	*P*
MSD Z-score	-1.260	0.284 (0.144-0.561)	0.0003
YSD Z-score	0.584	1.793 (0.970-3.312)	0.062
FHR Z-score	-2.330	0.097 (0.038-0.250)	<0.0001
TV Z-score	-0.612	0.542 (0.290-1.014)	0.055
meanUAPI Z-score	-0.542	0.582 (0.313-1.081)	0.087
Constant	-1.561		

The data were analysed using binary logistic regression analysis. Model R^2 = 0.639; P < 0.0001
Dependent variable: progressive pregnancy = 0, miscarriage = 1
All independent variables: (Dummy variable), less than median = 0, median and above = 1
OR, Odds ratio; CI, confidence interval; MSD, mean sac diameter; YSD, yolk sac diameter; FHR, fetal heart rate; TV, trophoblast volume; meanUAPI, mean uterine artery pulsatility index.

Table 3.4. *Diagnostic performance of the logistic regression model.*

FPR	Sensitivity	PPV	NPV	LR+	LR-
5%	10% (3-22)	0.6%	45.8%	0.10	20.4
10%	46% (32-61)	2.9%	75.5%	0.51	5.6
20%	60% (45-74)	4.0%	88.0%	0.72	2.36
30%	76% (62-87)	6.3%	96.1%	1.16	0.70

Data for sensitivity are given as mean (95% confidence internal).
FPR, False positive rate; PPV, positive predictive value; NPV, negative predictive value; LR+, positive likelihood ratio; LR-, positive likelihood ratio

DISCUSSION

The main findings were that meanUAPI, TV and FHR were all significantly reduced in pregnancies that were viable at the time of the scan then subsequently miscarried prior to 12 weeks gestation. In addition, including MSD with these parameters improves the efficacy of the proposed miscarriage prediction model. These findings are important because with appropriate equipment and operator experience each parameter is readily measurable with ultrasound in the first trimester and potentially of value in developing an algorithm aiming to improve the prediction of miscarriage prior to 12 weeks gestation.

A reduction in early first trimester TV in pregnancies that miscarry compared to pregnancies that resulted in a live birth has been reported by Reus et al. (2013). This study used serial measurements from a sample of 112 pregnancies with normal outcomes compared with measurements from 56 pregnancies that miscarried. No definition of miscarriage was provided and it is unclear if the miscarriages occurred prior 12 weeks, as in this study, or later in pregnancy (43). The inclusion of "empty sac" miscarriages in their data may have also overestimated the significance of their findings. In contrast, limiting assessment to viable pregnancies likely increases the clinical validity and usefulness of these results. Nardozza et al. (2009) presented normative values for

placental volume at 7-10^{+6} weeks of pregnancy based on a small series of 70 pregnancies (67). This data did not include the entire trophoblast surrounding the pregnancy relying on subjective assessment of the placental boundaries. This study provides a more reliable and reproducible volume estimation by inclusion of the entire trophoblast.

Uterine artery Doppler waveforms change dramatically through pregnancy. Early in the first trimester researchers have shown that the spiral arteries are occluded by invading trophoblast to restrict perfusion of the developing pregnancy (80) with many concluding that this is necessary to protect the developing fetus from very high level of oxygen at this stage (82). A study investigating the degree of uterine perfusion using ultrasound to assess intervillous blood flow (IBF) demonstrated increased IBF with advancing gestational age in the first trimester however flow was found significantly earlier in pregnancies that had miscarried (116). No studies were identified investigating the use of meanUAPI early in the first trimester or its relationship with miscarriage prior to 12 weeks gestation. Since a decrease in meanUAPI is reflective of an increase in uterine blood flow, this is consistent with these results that pregnancies that fail before 12 weeks gestation demonstrate a decreased mean UAPI compared to those that progress.

The reduction in MSD has been reported previously. Papaioannou et al. (2011) reported a retrospective series of 5427 cases (729 with miscarriage and 4698 with normal outcomes) assessed by ultrasound at 6-10 weeks gestation. They reported that the risk of miscarriage was inversely related to gestational sac diameter (odds ratio 0.84) (96).

A low FHR has previously been associated with an increased risk of first trimester miscarriage; one retrospective study including 729 pregnancies reported that the risk of miscarriage was inversely related to FHR (96). A heart rate of below the 5th centile for gestational age was demonstrated in approximately 24% of pregnancies that subsequently miscarried compared to 5% of pregnancies with a normal outcome.

In this study we demonstrated that the combination of these ultrasound parameters is of good predictive value for miscarriage with an AUC of 0.81. At a fixed 10% screen positive rate, this would identify 76% of pregnancies destined to

miscarry with positive and negative predictive values of 6.3% and 96.1% respectively. This information may be useful in counselling and guiding the short-term management of these pregnancies; women with an increased risk of miscarriage could be offered a repeat scan to assess progression of their pregnancy before the routine 11-13^{+6} week scan or prior to incurring the cost of non-invasive prenatal testing. In addition, the high negative predictive value of the test would help alleviate patient and practitioner concerns particularly in cases of recurrent miscarriage.

Previous models for the prediction of miscarriage have been reported however most have focussed on pregnancies complicated by vaginal bleeding (96, 117, 230). More similar to this model a smaller prospective study used a combination of gestational sac volume, gestational sac diameter, FHR and YSD resulting in a detection rate of 78% (231).

There were no studies identified that used TV, meanUAPI or the combination of these parameters such as in this proposed model. Although the sensitivity and specificity of this model are lower than reports that included pregnancies presenting with PV. bleeding, confidence intervals remain large as the number of miscarriage cases in this cohort was relatively small.

Strengths of this study include the use of standardised measurement methods for the ultrasound parameters of interest. The techniques for measurement of the MSD, CRL and FHR have been widely documented (47, 96, 227). The measurement methods for TT and TV, while more recently established, have been standardised and verified by previous research groups (42, 74, 232). The method used for the documentation and measurement of the UAPI is in common usage with a transabdominal approach (89) with the methodology readily extrapolated to the transvaginal approach used in this study (87). Another strength of the study is the restriction of recruitment to pregnancies with a detectable fetal heartbeat and absence of PV. bleeding, which improves the clinical focus of the results for low-risk pregnancies. Similarly, the rate of miscarriage after 12 weeks also reduces significantly, so this outcome measure is also more relevant to clinical practice.

A limitation of this study is that despite recruitment of a large cohort, the absolute numbers of cases that miscarried

by 12 weeks was small. This limited the number of variables that could be assessed in regression analyses. We did find that CRL measured at the initial ultrasound assessment was significantly greater ($p<0.001$) in pregnancies that continued beyond 12 weeks compared to those that subsequently miscarried. This equates to a gestational age of 7^{+2} weeks and 7^{+0} weeks respectively (55). Despite this difference being statistically significant, in the clinical setting the difference equates to a difference of only 2 days gestational age. It is possible that this result is a feature of the sample population, however early fetal growth restriction in the subsequent miscarriage population cannot be excluded. Further research addressing this is needed.

Measurement of TV is restricted to services with dedicated 3D transvaginal transducers and access to VOCAL measurement software. This may limit the translatability of this research into clinical practice. In this study we were interested in assessing if more novel ultrasound parameters in the first trimester, in particular, meanUAPI, TT and TV were able to predict miscarriage prior to 12 weeks gestation. Inclusion of TT in the model may appear practical as it is more easily measured in the clinical environment however univariate analysis demonstrated it was not clinically significant and it was removed from the presented model.

Further research is needed to validate this model as well as to determine whether these first trimester parameters are effective in predicting other, late, adverse pregnancy outcomes, such as pre-eclampsia or intrauterine growth restriction.

CONCLUSION

This study is the first to show a that a reduction in meanUAPI in early pregnancy is a significant independent predictor of subsequent miscarriage. In addition, we have shown that a combination of meanUAPI TV, MSD, YSD and FHR is of good predictive value for miscarriage and may be useful in counselling and guiding the short-term management of early pregnancies.

Chapter 4. Uterine artery pulsatility index prior to 11 weeks gestation

Addressing the second aim of this work, this chapter discusses the value of measuring uterine artery PI prior to 11 weeks gestation in the prediction of SGA and MHD.

This chapter has been published in a different format in a peer reviewed journal.

Citation:

Taylor TJ, Quinton AE, de Vries BS, Hyett JA. Uterine Artery Pulsatility Index Assessment at <11 Weeks Gestation: A Prospective Study. Fetal Diagn Ther. 2020;47(2):129-137.

Contributions:

***Tracey Hanchard**: research planning, data collection, statistical analysis and drafting of the manuscript*

***Associate Professor Ann Quinton:** research planning and review of the manuscript*

***Dr Bradley de Vries**: statistical advice and review of the manuscript*

***Clinical Professor Jonathon Hyett:** research planning and review of the manuscript.*

Relevance:

An increased meanUAPI at 11-13^{+6} weeks gestation is associated with an increased risk of developing SGA and/or MHD later in pregnancy. The value of meanUAPI in the prediction of SGA and/or MHD measured <11 weeks gestation has not been investigated.

Chapter 4. Uterine artery pulsatility index prior to 11 weeks gestation

INTRODUCTION

Placental dysfunction has significant implications for both mother and fetus. Manifestations of chronic placental dysfunction include a small fetus, maternal hypertensive disorders (MHD), including pre-eclampsia (PET) and pregnancy complications such as placental abruption, preterm labour and delivery (16). Being small-for-gestational age (SGA) is the first evidence of a growth restricted fetus and is associated with an increase in morbidity and mortality in both the short and long term. A large proportion of fetuses diagnosed with growth restriction are born early and, as such, are susceptible to the risks of prematurity (31). In addition, they are also vulnerable to a higher incidence of a myriad of perinatal conditions including oligohydramnios, caesarean delivery, low Apgar scores, acidosis, polycythemia, hypoglycemia, hypothermia, apnoea, sepsis, seizures, stillbirth and neonatal death(32). Approximately 50% of stillbirths are associated with undiagnosed intrauterine growth restriction (IUGR) (33). Along with these perinatal complications, individuals who are born growth restricted are at an increased risk later in life of developing osteoporosis (28), obesity and some cancers (34), stroke (28, 35), diabetes (28, 34, 35), hypertension and heart disease (28, 32, 34, 35).

In addition to an increase in fetal morbidity and mortality, there is also an increased risk of maternal complications. Short-term maternal complications of PET include placental abruption, progression to eclampsia requiring immediate delivery, and the development of life-threatening HELLP Syndrome (haemolysis, elevated liver enzymes and a low platelet count) (36). Further, there is an increased risk that subsequent pregnancies will be affected and, in the long term, that the mother will develop cardiovascular disease and metabolic disorders later in life if they have a history of PET or delivering a low birthweight infant (29, 37, 38).

Chapter 4: Uterine artery pulsatility index prior to 11 weeks gestation

Poor placentation and transformation of the spiral arteries within the myometrium in the first trimester of pregnancy, resulting in decreased perfusion of the placenta later in pregnancy, has been linked with the development of SGA and MHD (9). It is well-established that uterine artery blood flow changes throughout pregnancy and is a reflection of the downstream perfusion of the placenta (44). There have been decades of research into the ability of uterine artery Doppler waveforms in the first and second trimesters to predict the development of PET, low birthweight infants and other placental related disorders in the third trimester. This work originally focussed on the assessment of uterine artery Doppler waveforms after 20 weeks gestation (233) and it is well-established that the waveform appearances (168) and various Doppler indices (234) are able to predict the likelihood of development of placental-related disease later in pregnancy (84).

More recently, the application of uterine artery Doppler has been investigated at 11-13^{+6} weeks gestational age (78). Uterine artery Doppler changes are extensively described at this stage with an increase in mean uterine artery pulsatility index (meanUAPI) reported in pregnancies that subsequently develop SGA and/or PET (78, 85). The test can be combined with aneuploidy screening to be cost-effective and, with the instigation of low-dose aspirin therapy, has the potential to significantly improve pregnancy outcome in those screened 'high-risk' (178). The value of a 11-13^{+6} week ultrasound has been questioned following the introduction of non-invasive prenatal aneuploidy screening (NIPS) [16]. Different scenarios are being considered including the routine use of ultrasound to confirm location, plurality and viability of a pregnancy *prior* to NIPS at 10-11 weeks. As aspirin prophylaxis against SGA and PET becomes significantly less effective after 16 weeks (3), the loss of risk assessment at 11-13^{+6} weeks has the potential to impact the success of prediction and prevention.

Spiral artery transformation begins soon after fertilisation and results in changes to uterine artery blood flow as early as 8 weeks gestation (44). Uterine artery Doppler assessment <11 weeks may therefore be of value for prediction of SGA and/or PET. There has been little investigation of uterine artery Doppler indices <11 weeks, with no large

prospective studies identified. We hypothesised that the meanUAPI <11 weeks gestation will be increased in pregnancies complicated by an adverse outcome; defined as SGA or MHD. There were three aims of this study. The first was to establish a population specific reference range for meanUAPI <11 weeks gestation. The second was to use this range to determine if an abnormal meanUAPI <11 weeks was associated with adverse pregnancy outcome, and the third was to assess changes in meanUAPI between <11 weeks and 11-13^{+6} weeks gestation.

MATERIALS AND METHODS

This was a prospective cohort study designed to assess the predictive value of early ultrasound measurements in the assessment of hypertensive disorders, SGA and other adverse pregnancy outcomes. Consecutive patients that attended for an early (<11 weeks) ultrasound scan at a single private obstetric ultrasound practice were enrolled in the study between February 2016 and August 2018. Eligibility criteria included consent for a transvaginal scan, a single viable intrauterine pregnancy with a crown rump length (CRL) measuring <45mm and likely availability of pregnancy outcome data.

All participants completed a questionnaire detailing their clinical and obstetric history at the time of the ultrasound assessment. Transvaginal ultrasound examination was conducted with a GEVolusonE8 ultrasound machine (GEHealthcare, Milwaukee, WI) equipped with a 5-to 9-MHz 3-dimensional transvaginal transducer. In addition to routine measurements, the uterine artery pulsatility index was measured bilaterally.

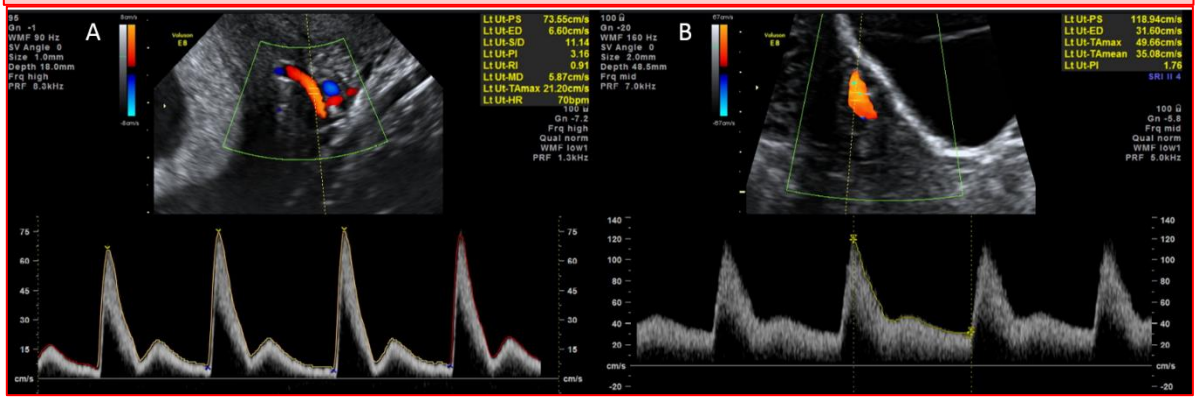
Standard first trimester ultrasound images and measurements were recorded (cervical length, gestational sac size, crown-rump length and fetal heart rate). Then, using colour Doppler, each uterine artery (UA) was identified laterally at the level of the internal os. Colour Doppler was used to locate the ascending branch of the vessel. Spectral Doppler was then used with a sample gate size of 2mm and insonation angle of <30 degrees to record a representative waveform of the flow within this vessel with subsequent measurement of the pulsatility index (PI) (87) (Figure 4.1a). The PI was calculated by the ultrasound machine, from the spectral Doppler waveform, using the formula:

$$\frac{\textit{peak systolic velocity - end diastolic velocity}}{\textit{time averaged velocity.}}$$

The meanUAPI was calculated by adding the left and right PI and dividing by 2.

For participants who returned for First Trimester Screening at 11-13^{+6} weeks gestation, the uterine artery waveforms were recorded using a transabdominal approach (89) (Figure 4.1b) and the meanUAPI calculated by the same method. An abnormal meanUAPI was defined as greater than the 95th percentile (235).

Figure 4.1. *Normal uterine artery Doppler waveform*
a) < 11 weeks gestation (b) 11-13^{+6} weeks gestation. CRL, crown-rump length.



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Maternal and pregnancy characteristics, ultrasound findings and pregnancy outcome were entered into a computer database. Pregnancy outcome was retrieved from participant medical records at the centre of delivery. Adverse pregnancy outcome was defined as the development of hypertensive disorders: gestational hypertension (GH) or PET as defined by the International Society for the Study of Hypertension in Pregnancy (236) and/or SGA defined as a birth weight <10th percentile for local standards (149).

SAMPLE SIZE ESTIMATION

It was estimated that approximately 680 participants would be required to demonstrate an effect size (difference in meanUAPI between pregnancies with a normal and adverse outcome) of 10% with a power of 80%. To compensate for a rate of miscarriage prior to 12 weeks gestation and lost to follow-up of 10%, we aimed to

recruit a total of 750 participants prior to 11 weeks gestation.

AIM 1: Pregnancies with an adverse outcome were excluded from the regression analysis to determine the expected population specific meanUAPI 5th, 50th and 95th centiles at a given CRL (as a proxy for gestational age) (235) for measurements taken <11 weeks using methods described by Royston (1991)(235). This process was repeated for measurements taken between 11-13^{+6} weeks gestation to allow comparisons addressing aim 3.

AIM 2: Based on the regression formula established for meanUAPI measured at each time point square root transformation of the residuals was required before further analysis. Z-scores were then calculated for meanUAPI using the formula:

(observed measurement - expected measurement) / standard deviation of the group.

Z-Scores were compared between outcome groups using a two-sample t-test for independent variables and reported as mean (95%CI).

AIM 3: Changes in the meanUAPI between gestational age time points were assessed by two-way mixed ANOVA; the two time points were considered as the 'within-subject factor', adverse vs. normal outcome as the 'between-subjects' factor and the meanUAPI as the dependent variable of interest.

All results are reported as mean(95%CI). Frequencies were reported as n (%) and compared using Pearson's χ^2 test. A p value of <0.05 was considered statistically significant. Reporting and presentation follow the principles of the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) initiative (228).

RESULTS

A total of 763 patients were eligible for inclusion in this study. We excluded 54 cases that miscarried prior to 12 weeks gestation, one case that resulted in a neonatal death, four cases treated with aspirin or Clexane, and two cases treated with anti-hypertensive medication prior to enrolment (Figure 4.2). 702 cases were available for analysis of meanUAPI at <11weeks. With the exception of maternal height and number of pregnancies, there was no significant difference in demographic characteristics

between the two groups of pregnancy outcome (Table 4.1). 662 women (94.3%) returned to our centre for First Trimester Screening at 11-13^{+6} weeks. Of these, 601 had meanUAPI data recorded at this time.

Figure 4.2. *Flowchart illustrating study inclusion criteria, cases excluded from analysis and pregnancy outcome. CRL, crown-rump length, ET, essential hypertension, GH, gestational hypertension, PET, pre-eclampsia, BW, birthweight.*

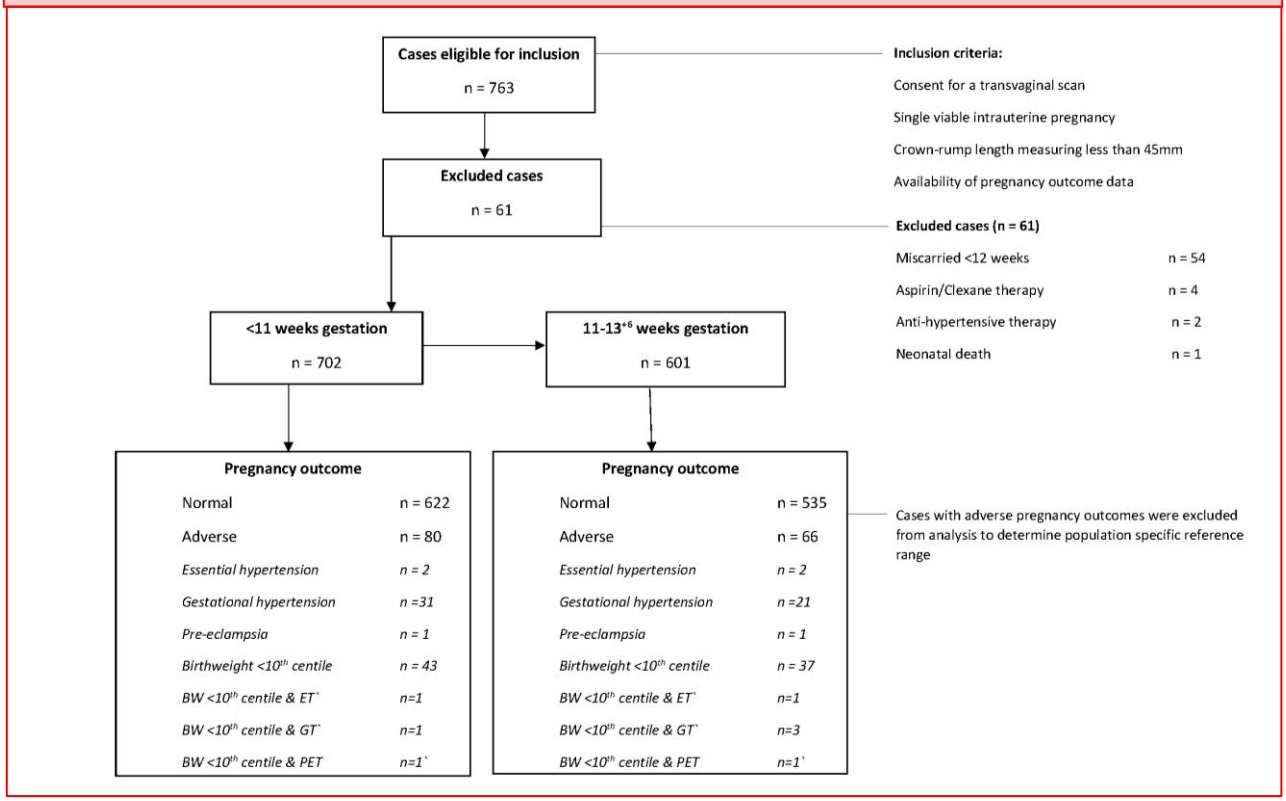
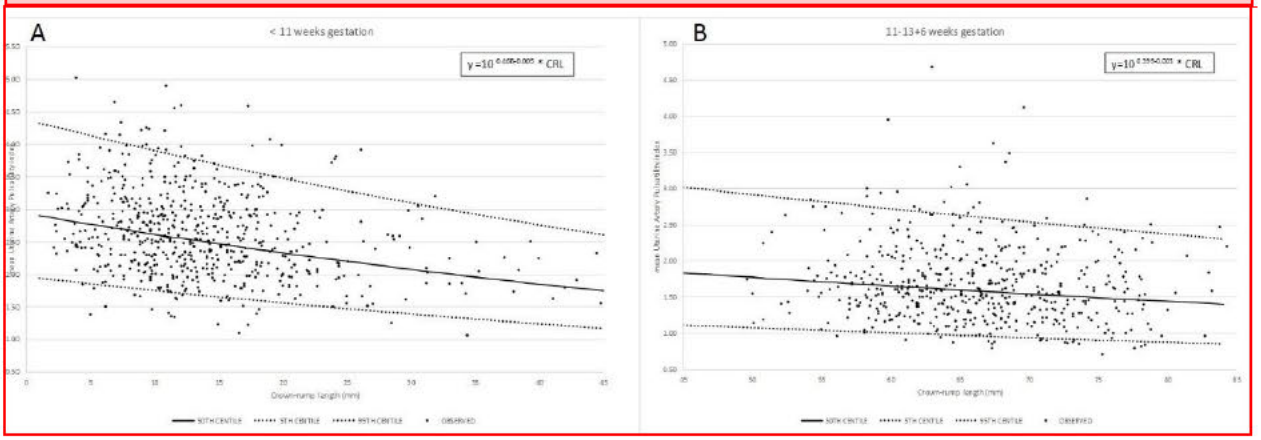


Table 4.1. *Maternal characteristics, medical history and current pregnancy characteristics in the two groups of pregnancy outcome (n=702); normal and adverse.*

Maternal Characteristic	Normal outcome (n=622)	Adverse outcome (n=80)	P
Age (years)	32 (16-44)	30 (19-42)	0.707
Weight (kilograms)	72 (36-142)	75 (48-136)	0.192
Height (centimetres)	166 (140-186)	164 (145-189)	0.044
Number of pregnancies	2.6 (1-10)	1.9 (1-5)	0.026
Pre-existing medical conditions			0.070
None	529 (85.0)	70 (87.5)	
Hypothyroidism	50 (8.0)	7 (8.6)	
Psychological	9 (1.5)	-	
Asthma	11 (1.8)	-	
Epileptic	4 (<1.0)	-	
Grave's Disease	1 (<1.0)	-	
Hashimoto's thyroiditis	-	1(1.3)	
Metabolic Syndrome	-	1(1.3)	
Factor 5 Leiden	1 (<1.0)	-	
Antiphospholipid Syndrome	1 (<1.0)	-	
Blood clots	3 (<1.0)	-	
SLE/Lupus	2 (<1.0)	-	
Multiple	11 (1.8)	1 (1.3)	
Current Medications			0.796
None	509 (81.8)	68 (85.0)	
Thyroxine	46 (7.4)	7 (8.8)	
Anti-depressants	20 (3.2)	-	
Diabetes	16 (2.6)	1 (1.3)	
Asthma	9 (1.4)	1 (1.3)	
Anti-epileptics	3 (<1.0)	-	
Multiple	19 (3.1)	3 (3.8)	
Smoking status			0.349
Non-smoker	609 (97.9)	77 (96.3)	
Smoker	13 (2.1)	3 (3.8)	
Blood pressure			0.534
Normotensive	619 (99.5)	80 (100.0)	
Hypertensive	3 (<1.0)	-	
Diabetic Status			0.681
None	527 (84.7)	66 (82.5)	
Type I	3 (<1.0)	1 (1.3)	
Type II	4 (<1.0)	-	
Gestational	88 (14.1)	13 (16.3)	
Menstrual cycle			0.517
28 days	268 (43.1)	40 (50.0)	
< 28 days	76 (12.2)	6 (7.5)	
> 28 days	140 (22.5)	18 (22.5)	
Variable	138 (22.2)	16 (20.0)	
Method of Conception			0.057
Spontaneous	569 (91.5)	66 (82.5)	
Ovulation induction	37 (5.9)	10 (12.5)	
IVF (no ovulation induction)	14 (2.3)	4 (5.0)	
IVF (donor)	2 (<1.0)	-	
Indication for ultrasound			0.114
None	206 (33.1)	27 (33.8)	
Dating	288 (46.3)	33 (41.3)	
Viability / P.V. bleeding	93 (15.0)	15 (18.8)	
Previous miscarriage	30 (4.8)	2 (2.5)	
Pain	2 (<1.0)	1 (1.3)	
Exclude plurality	2 (<1.0)	1 (1.3)	
Pre-NIPS	1 (<1.0)	-	
Uterine anomaly	-	1 (1.3)	

Data are provided as mean (range) or n (%). IVF, in-vitro fertilisation; P.V., per vaginal; NIPS, non-invasive prenatal screening.

Figure 4.3. *Scatterplots of the expected median (solid line), 5th and 95th centiles (dotted lines) for uterine artery pulsatility index (A) < 11 weeks gestation, (B) 11-13+6 weeks gestation observed values* • .



AIM 1: Development of population specific reference ranges

Eighty pregnancies with an adverse outcome were excluded from analysis during development of a population specific reference range for meanUAPI at <11 weeks gestation. These exclusions included 3 cases of essential hypertension, 32 cases of GH, 2 cases of PET, and 43 cases with an infant birthweight <10th centile. Sixty-six of these women were also excluded from development of the normal range for 11-13+6 weeks gestation: 3 cases of essential hypertension, 24 cases of GH, 2 cases of PET and 37 cases with an infant birthweight <10th centile. These exclusions left 622 and 535 cases respectively available for development of normal ranges.

The meanUAPI showed a significant correlation with CRL for both gestational age time points. Initial analysis of meanUAPI measurements demonstrated a non-normal distribution of the data for both time points (Shapiro-Wilk test < 11 week cohort: p-value: <0.0001 and 11-13+6 weeks cohort: p-value: <0.0001). Logarithmic ($\log_{10}$) transformation of meanUAPI values resulted in a normal distribution for both time points (Shapiro-Wilk test <11 weeks cohort: p-value: 0.155 and 11-13+6 weeks cohort: p-value: 0.327. Various regression models using the transformed values were fitted to

determine the expected meanUAPI at a given CRL (as a proxy for gestational age). Each regression model was assessed for the assumptions of linear regression; linearity, normality and homoscedasticity with a simple linear regression model providing the best fit.

These models was internally validated using bootstrapping of 1000 samples (237). The regression model used to calculate the expected meanUAPI for pregnancies <11 weeks gestation has a constant of 0.468 (p <0.0001). Internal validation by bootstrapping indicates likely significance in the actual population with a narrow 95%CI for the constant (0.468) of 0.452-0.483 (p=0.001). The regression model used to calculate the expected meanUAPI for pregnancies 11 - 13^{+6} weeks gestation has a constant of 0.399 (p <0.0001). Internal validation by bootstrapping indicates likely significance in the actual population with a narrow 95%CI for the constant (0.399) of 0.289-0.501 (p=0.001).

Table 4.2 illustrates the expected 5^{th}, 50^{th} and 95^{th} centiles using this model. Figure 4.3 graphically illustrates these centiles and the observed measurements from the data.

AIM 2: Assessment of meanUAPI by pregnancy outcome at the <11 weeks gestation

For ease of presentation the mean Z-score for pregnancies with a normal outcome was adjusted to zero and Z-scores for the adverse outcome group adjusted accordingly. Comparison of Z-Scores for meanUAPI measured <11 weeks gestation was not significantly different between outcome groups (normal: 0.00 (-0.08-0.07) vs. adverse: 0.02 (-0.22- 0.27); p=0.807). In contrast, there was a significant difference between outcome groups of Z-Scores for meanUAPI measured at 11-13^{+6} weeks gestation (normal: 0.00 (-0.09-0.07) vs. adverse: 0.27 (-0.03-0.56); p=0.040) (Table 4.3).

AIM 3: Comparison of meanUAPI at two-time points (<11 weeks and 11-13+6 weeks) in normal and adverse outcome groups

Using the $\log_{10}$ transformed values for meanUAPI for each gestational age time point all statistical assumptions were satisfied. Outliers identified by boxplots were considered accurate and within plausible range. The data were normally distributed, as assessed by Shapiro-Wilk's test for normality ($p>0.05$). There was homogeneity of covariances ($p>0.05$) and variances ($p>0.05$) as assessed by Box's M test and Levene's test of homogeneity of variances, respectively. Results demonstrated a statistically significant decrease in the meanUAPI between <11 weeks gestation and 11-13^{+6} weeks gestation independent of pregnancy outcome ($F_{(1,599)}=364.861$; $p<0.0001$; partial $n^2=0.379$). While this decrease was smaller in pregnancies that resulted in an adverse rather than a normal outcome; mean (95%CI): 0.78 (0.75-0.81) vs. 0.91 (0.90-0.92) the difference was not statistically significant ($F_{(1,599)} = 3.764$; $p=0.053$) (Figure 4.4).

Table 4.2. *Expected mean uterine artery pulsatility index (5th, 50th and 95th centile) based on crown-rump length.*

	meanUAPI centile				meanUAPI centile		
CRL	5th	50th	95th	CRL	5th	50th	95th
1	1.95	2.90	4.33	43	1.20	1.79	2.67
2	1.93	2.87	4.28	44	1.19	1.77	2.64
3	1.90	2.84	4.23	45	1.12	1.84	3.02
4	1.88	2.81	4.18	46	1.11	1.82	3.00
5	1.86	2.77	4.13	47	1.10	1.81	2.98
6	1.84	2.74	4.09	48	1.09	1.80	2.96
7	1.82	2.71	4.04	49	1.09	1.79	2.94
8	1.80	2.68	3.99	50	1.08	1.77	2.92
9	1.78	2.65	3.95	51	1.07	1.76	2.90
10	1.76	2.62	3.90	52	1.06	1.75	2.88
11	1.74	2.59	3.86	53	1.06	1.74	2.86
12	1.72	2.56	3.81	54	1.05	1.73	2.84
13	1.70	2.53	3.77	55	1.04	1.71	2.82
14	1.68	2.50	3.73	56	1.03	1.70	2.80
15	1.66	2.47	3.68	57	1.03	1.69	2.78
16	1.64	2.44	3.64	58	1.02	1.68	2.76
17	1.62	2.42	3.60	59	1.01	1.67	2.74
18	1.60	2.39	3.56	60	1.01	1.66	2.73
19	1.58	2.36	3.52	61	1.00	1.64	2.71
20	1.57	2.33	3.48	62	0.99	1.63	2.69
21	1.55	2.31	3.44	63	0.99	1.62	2.67
22	1.53	2.28	3.40	64	0.98	1.61	2.65
23	1.51	2.25	3.36	65	0.97	1.60	2.63
24	1.49	2.23	3.32	66	0.97	1.59	2.61
25	1.48	2.20	3.28	67	0.96	1.58	2.60
26	1.46	2.18	3.25	68	0.95	1.57	2.58
27	1.44	2.15	3.21	69	0.95	1.56	2.56
28	1.43	2.13	3.17	70	0.94	1.55	2.54
29	1.41	2.10	3.14	71	0.93	1.53	2.53
30	1.39	2.08	3.10	72	0.93	1.52	2.51
31	1.38	2.06	3.06	73	0.92	1.51	2.49
32	1.36	2.03	3.03	74	0.91	1.50	2.47
33	1.35	2.01	3.00	75	0.91	1.49	2.46
34	1.33	1.99	2.96	76	0.90	1.48	2.44
35	1.32	1.96	2.93	77	0.89	1.47	2.42
36	1.30	1.94	2.89	78	0.89	1.46	2.41
37	1.29	1.92	2.86	79	0.88	1.45	2.39
38	1.27	1.90	2.83	80	0.88	1.44	2.37
39	1.26	1.87	2.80	81	0.87	1.43	2.36
40	1.24	1.85	2.76	82	0.86	1.42	2.34
41	1.23	1.83	2.73	83	0.86	1.41	2.32
42	1.21	1.81	2.70	84	0.85	1.40	2.31

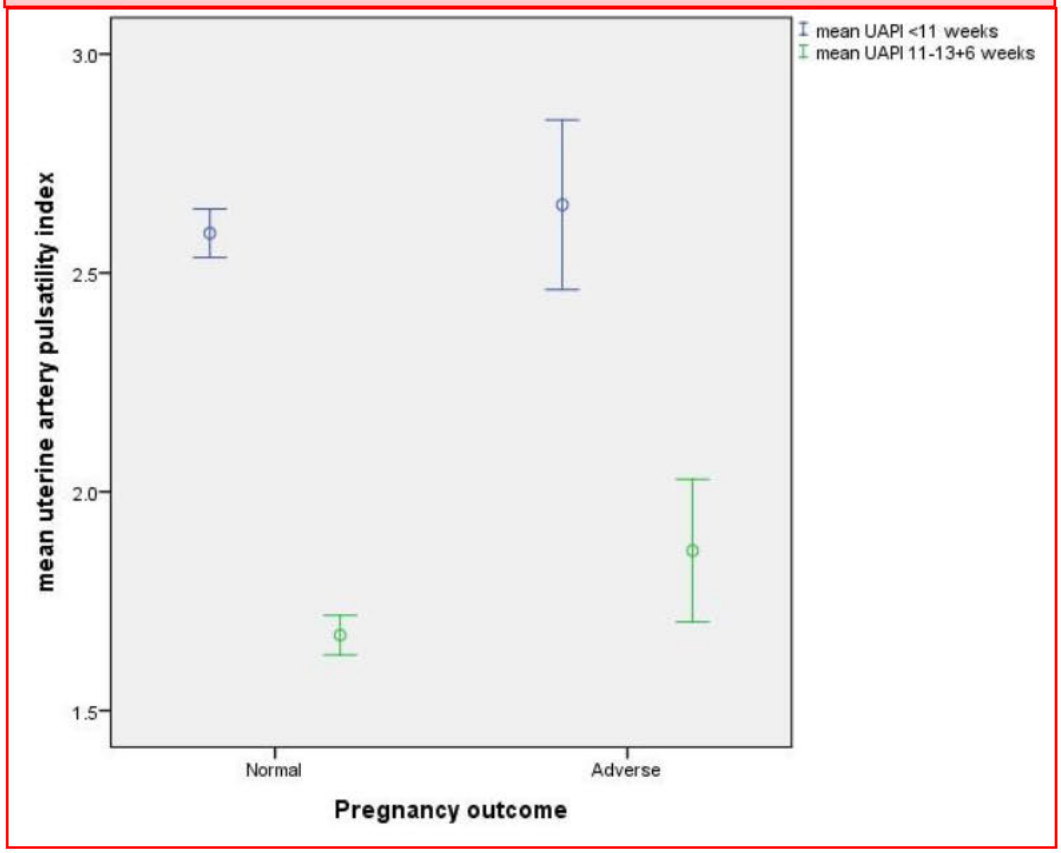
CRL crown-rump length, meanUAPI mean uterine pulsatility index, mm millimetres

Table 4.3. *Comparison of meanUAPI measured <11 weeks and 11-13^{+6} weeks gestation between pregnancies that resulted in a normal outcome and those with an adverse (Z-Score and observed measurement).*

Gestation	Normal outcome	Adverse outcome	P
< 11 weeks	0.00 (-0.08-0.07)*	0.02 (-0.22-0.27)**	0.807
	2.62 (2.57-2.67)	2.67 (2.50-2.84)	$t_{0.24}$; df_{700}
11-13^{+6} weeks	0.00 (-0.09-0.07)***	0.27 (-0.03-0.56)****	0.040
	1.67 (1.63-1.72)	1.87 (1.70-2.03)	$t_{2.11}$; df_{599}

Data are given as mean (95% confidence internal); equal variances assumed. meanUAPI, mean uterine artery pulsatility index; t, t-statistic; df, degrees of freedom. * n=622; ** n=80; *** n= 535; **** n=66

Figure 4.4. *Error plots demonstrating changes in meanUAPI over time points for normal and adverse pregnancy outcomes.*



DISCUSSION

This prospective cohort study has enabled the development of population-specific reference ranges for meanUAPI measured <11 weeks gestation. The data show that median of meanUAPI decreases with advancing gestation from 2.90 at 5^{+3} weeks to 1.75 at 10^{+6} weeks gestation. To facilitate analysis a reference range was calculated for 11-13^{+6} weeks based on data from participants at this gestational age. There was a similar negative relationship between meanUAPI and gestation across this period. This confirms previous studies in the 11-13^{+6} week gestational window (78, 84, 168).

MeanUAPI measured <11 weeks gestation was not significantly different in pregnancies that resulted in an adverse outcome compared to those with a normal outcome. In comparison, there was a significant difference in meanUAPI measured between 11-13^{+6} weeks gestation in the same cohort. The process of placentation normally involves 'transformation' from a high resistance/low flow system to a low resistance/ high flow system; a process which is normally complete by 16 weeks (17). After 11 weeks gestation, failure to develop this low resistance circulation is recognised as a key feature of placental insufficiency (83). It has been suggested that failure in implantation is of fundamental importance in the development of PET and IUGR - leading to placental hypoxia and the release of vasoactive angiogenic markers that affect the maternal circulation as well as the placenta (77, 78). As there is evidence suggesting that spiral artery transformation begins as early as 8 weeks gestation (44), we had anticipated that a failure in this process, associated with placental insufficiency, would be detectable earlier in the first trimester. We were, however, unable to recognise such a difference (reflected in meanUAPI) in this dataset. This marker does not, therefore, appear to be useful < 11-13^{+6} weeks gestation and if meanUAPI is to be included in the predictive algorithm used, traditional SGA/PET screening should continue to be focused at this time point.

This data indicates a significant decrease in meanUAPI measured from <11 weeks gestation compared to 11-13^{+6} weeks gestation in all pregnancies regardless of outcome. The degree of change was smaller in pregnancies that resulted in an adverse outcome, but this was not

statistically significant. Although we have not identified previous studies comparing meanUAPI between these two early time points, there are reports comparing repeated meanUAPI measures at later gestations. In a case-control study (control and IUGR pregnancies) comparing longitudinal changes (20 to 28/34 weeks gestation) in meanUAPI, Contro et al. (2014) demonstrated a decline in the meanUAPI in all pregnancies over time although the decrease was smaller in pregnancies affected by IUGR (238). Prefumo et al. (2004) defined the presence of diastolic notching in the uterine artery waveform as an indication of a high resistance/low flow system. These authors showed that pregnancies that resulted in a <10th centile birthweight infant were less likely to convert to a low resistance/high flow system by 18-23 weeks gestation than those that resulted in an appropriate-for-gestational-age infant (239). Further investigation of meanUAPI changes over time beginning <11 weeks gestation is needed to assess the potential benefit of including this marker in any algorithm to predict PET.

The strengths of this study included the use of standardised methodology for the measurement of meanUAPI that is commonly performed via a transabdominal approach at 11-13^{+6} weeks gestation (89, 164). However, the methodology was readily extrapolated to the transvaginal approach used in this study. The two normal ranges we developed therefore used different ultrasound approaches and this in part explains why the normal ranges do not completely overlap at 11 weeks. A further strength of the study is that these population-specific references ranges are based on a large sample of pregnancies with normal outcomes.

A limitation of this study may be the comparatively small number of pregnancies affected by PET (240). This may be due to the similarly small incidence of PET risk factors including previous PET (4.1%), chronic hypertension (<1%), pre-existing diabetes (1.2%), autoimmune disorders (<1% and pre-existing renal disease (<1.0%) (241). In addition, comparison of mean gestational age at delivery in this cohort demonstrated that PIH-affected pregnancies delivered significantly earlier than normotensive pregnancies (37^{+5} weeks' vs. 39^{+1} weeks' respectively; p=0.036). The low prevalence of PET may be due to the earlier delivery of PIH-

affected pregnancies compared to normotensive pregnancies, preventing the progression to PET in this sub-group. We feel the large number of pregnancies affected by maternal hypertension and/or resulting in a low birthweight infant (11.4%) is significant enough to establish the reliability of these results and encourage further research with a larger cohort of PET-affected pregnancies.

A further limitation of this study may be the unequal distribution of cases across gestational age weeks prior to 11 weeks gestation with the majority of cases being assessed between 6 and 8 weeks (CRL: 5-16mm) (55). This unequal distribution may influence the reliability of the regression model used to calculate the reference ranges for meanUAPI with values estimated outside these gestational weeks possibly less accurate. The clinical relevance of the results should not be underestimated by this as the majority of routine scans are performed during this 6-8 week window and further research at gestational ages outside this range is suggested.

CONCLUSION

By establishing a population specific reference range for meanUAPI <11 weeks gestation, this study has demonstrated that this marker does not appear useful in the prediction adverse pregnancy outcomes at these early gestational ages. This supports the argument for the prediction of PET and SGA risk at 11-13^{+6} weeks gestation if meanUAPI is to be included in the predictive algorithm applied. Further investigation of meanUAPI changes over time beginning prior to 11 weeks gestation is needed to assess the potential benefit of including this marker in any longitudinal algorithm to predict adverse pregnancy outcomes.

Chapter 5. Combining early first trimester ultrasound features, maternal characteristics and serum biochemistry to predict small-for gestational age neonates

Addressing the third aim of this research this chapter discusses the predictive value of conventional and novel ultrasound measures prior to 11 weeks in the prediction of SGA neonates.

This chapter has been submitted in a different format for publication and is currently under peer review.

Citation:

Hanchard TJ, de Vries BS, Quinton AE, Sinosich M, Hyett JA. Combining early (<11 weeks' gestation) ultrasound features and maternal factors to predict small-for-gestational age neonates.

Contributions:

Tracey Hanchard*: research planning, data collection, statistical analysis and drafting of the manuscript*

Dr Bradley de Vries*: statistical advice and review of the manuscript*

Associate Professor Ann Quinton: *research planning, statistical advice and review of the manuscript*

Dr Michael Sinosich: *statistical advice and review of manuscript*

Clinical Professor Jonathon Hyett: *research planning, statistical advice and review of the manuscript*

Relevance:

Conventional and novel ultrasound measures prior to 11 weeks gestation may be associated with the development of a small-for-gestational neonate. In conjunction with maternal characteristics and serum biochemistry these measures may facilitate the prediction of disease earlier in pregnancy than is currently possible.

Chapter 5. Combining early first trimester ultrasound features, maternal characteristics and serum biochemistry for the prediction of small-for-gestational age neonates

INTRODUCTION

One of the main aims of modern obstetric practice is to optimise fetal and maternal outcomes. One approach involves the early prediction of complications that manifest later in pregnancy in order to guide pregnancy management and influence outcome. Disorders of placentation with their origins early in the first trimester of pregnancy (9), may cause intrauterine growth restriction (IUGR) or the delivery of infants that are small-for-gestational age (SGA) and can have a significant impact on both the mother and the fetus.

A fetus with an estimated weight of <10th centile is considered SGA and is considered at risk for IUGR (30) with an increased risk of numerous adverse short- and long-term outcomes. In the short-term these include prematurity (31), caesarean delivery, oligohydramnios, low Apgar scores, hypothermia, polycythemia, acidosis, hypoglycemia, apnoea, sepsis, seizures, stillbirth and death (32). In the long-term babies born growth restricted are at an increased risk of developing osteoporosis (28), stroke (28, 35), diabetes (28, 34, 35), obesity (34), some cancers (34), hypertension and heart disease later in life (28, 32, 34, 35). From a maternal perspective, placental insufficiency is associated with placental abruption, severe pre-eclampsia, HELLP Syndrome and eclampsia (36). In addition to these life-threatening complications, there is increased risk that subsequent pregnancies will also be affected and that the mother will develop metabolic disorders and cardiovascular disease later in life (29, 37, 38).

Placentation begins as soon as the conceptus implants into the endometrium (10). Proliferation of the surrounding trophoblast initiates transformation of the spiral arteries within the myometrium (12). Incomplete transformation of the spiral arteries into compliant, low-resistance channels may result in placental hypoxia and a cascade of events

that result in adverse pregnancy outcomes (9). It is already possible to predict SGA/IUGR using models implemented at 11-13^{+6} weeks' gestation that include maternal characteristics and markers of placental function, such as, the uterine artery Doppler and serum markers placental growth factor (PlGF) and pregnancy – associated plasma protein-A (PAPP-A) (219). Early prediction can guide pregnancy management, influence outcome and coupled with prophylactic intervention prior to 16 weeks gestation may modify progression of disease (3). It is possible that if prediction of SGA and instigation of therapy occurred at an earlier gestation it would be more effective than the current approaches.

This study aimed to determine whether maternal characteristics, ultrasound and biochemical markers can be combined early in the first trimester for effective prediction of SGA; defined as a birthweight (BW) < 10th centile for gestational age at delivery.

MATERIAL AND METHODS

This was a prospective cohort study designed to assess the value of early ultrasound measurements in the prediction of SGA. Consecutive patients that attended for an early (<11 weeks) ultrasound scan at a private obstetric ultrasound practice were enrolled in the study between February 2016 and August 2018.

Eligibility criteria included consent for a transvaginal scan, the presence of a single intrauterine pregnancy, an embryo with a crown rump length (CRL) ≤45mm with demonstration of a heartbeat, capture of additional ultrasound measurements and availability of pregnancy outcome data. All participants provided written informed consent. Fetal ultrasound exposure was as low as reasonably achievable by minimising mechanical and thermal indices and output power.

All participants completed a questionnaire detailing their obstetric and clinical history upon enrolment in the study. Transvaginal ultrasound examination was conducted with a GEVolusonE8 ultrasound machine (GEHealthcare, Milwaukee, WI) using a 5-9MHz transducer with 3-dimensional capabilities. All scans were performed by accredited sonographers (average 14 years' experience). Trophoblast thickness (TT), trophoblast volume (TV), bilateral

uterine artery pulsatility index (UAPI), mean sac diameter (MSD), yolk sac diameter (YSD), CRL, and fetal heart rate (FHR) were measured (Figure 1).

The measurement techniques for MSD (47), YSD (49), FHR (227) and CRL (11) are well-documented by previous authors. The trophoblast is demonstrated on ultrasound as the uniformly hyperechoic region surrounding the gestational sac (excluding the decidua). The techniques used to measure TT and TV have been described in Chapters 1 and 3.

Each uterine artery (UA) was identified with colour Doppler and spectral Doppler used to calculate the pulsatility index (PI) (89) using the formula:

$$\frac{\textit{peak systolic velocity - end diastolic velocity}}{\textit{time averaged velocity.}}$$

The meanUAPI was calculated by adding the left and right PI then dividing by 2.

For participants subsequently returning for First Trimester Screening maternal blood was collected at 10-14 weeks' gestation. After clotting, serum was aspirated and stored frozen (-18^{o}C) until required for assay. Biochemical markers; free-beta subunit of human chorionic gonadotropin (FBhCG), PAPP-A, alpha fetoprotein (αFP) and PlGF were quantified on DELFIA® Xpress random access immunoanalyzer (Wallac Oy, PerkinElmer) and reported as MoMs.

STATISTICAL ANALYSIS

Ultrasound findings, maternal characteristics, biochemistry results and details of pregnancy outcome were entered into a computer database. Based on pregnancies with a normal outcome (BW ≥10th centile and absence of maternal hypertension at delivery) regression lines were fitted for each ultrasound parameter to determine the expected measurements at a given CRL (as a proxy for gestational age) with each regression model assessed for the assumptions of linear regression; linearity, normality and homoscedasticity. Models for mean UAPI, TT, YSD and MSD satisfied each assumption and the raw residuals were used for further analysis. The most appropriate model for FHR was found to be a fourth order polynomial regression. Trophoblast volume required square root transformation before analysis. For consistency with maternal serum results MoMs were calculated for each parameter using the formula:

Observed measurement

regression formula for expected median measurement.

Multiples of the median were compared using a two-sample t-test for independent variables and reported as means (95%CI). Frequencies were reported as n (%) and compared using Pearson's χ^2 test. A p value of <0.05 was considered statistically significant.

Logistic regression analysis was used to create a prediction model for SGA based on ultrasound measurements, maternal characteristics and biochemistry. A receiver operator characteristic (ROC) curve was obtained to assess the performance of the model. Reporting and presentation follow the principles of the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) initiative (228).

RESULTS

A total of 1,245 patients were eligible for inclusion in this study. We excluded a total of 104 cases (one participant withdrew consent for the study, two neonatal deaths, three miscarried after 12 weeks gestation, three chose termination of pregnancy, eight with essential hypertension, 10 taking aspirin, clexane or anti-hypertensive medication, 10 fetuses that later demonstrated morphological anomalies, six with proven aneuploidy and 61 pregnancies miscarried prior to 12 weeks gestation (Figure 5.1). Of the remaining 1,141 pregnancies, 1,068 had a BW ≥10^{th} centile (93.6%) and 73 had a BW <10^{th} centile (6.4%).

There was no difference in demographic characteristics between the two groups except for indications for ultrasound, method of conception and maternal weight and height (Table 5.1). CRL at the initial ultrasound assessment was similar (p=0.493) in pregnancies that had a BW ≥10^{th} centile (13.7mm (range 1.7-44.8)) compared to those with a BW <10^{th} centile (13.2mm (range 2.4-40.0)). These CRLs equate to gestational ages of 7^{+5} weeks (5^{+1} –11^{+1} weeks) and 7^{+4} weeks (5^{+3} – 10^{+5} weeks) respectively (55).

The regression formulae for the expected measurement of each ultrasound parameter as determined from the CRL were calculated using 1,019 pregnancies with a normal outcome. The six ultrasound parameters assessed in this study (MSD, YSD, FHR, meanUAPI, TT and TV) showed a significant correlation with CRL. In pregnancies that had a normal

outcome all parameters, with the exception of FHR were found to have a linear relationship with CRL. Trophoblast volume and TT measurements demonstrated increasing variance around the mean as the CRL increased. The FHR was found to have a quartic relationship with CRL. Figure 5.2 illustrates the expected median, 5th and 95th centiles for each parameter with the observed measurements for pregnancies that ultimately delivered an SGA fetus.

Figure 5.1. Flowchart illustrating study inclusion criteria, cases excluded from analysis and pregnancy outcome. CRL, crown-rump length, ET, essential hypertension, GH, gestational hypertension, PET, pre-eclampsia, BW, birthweight.

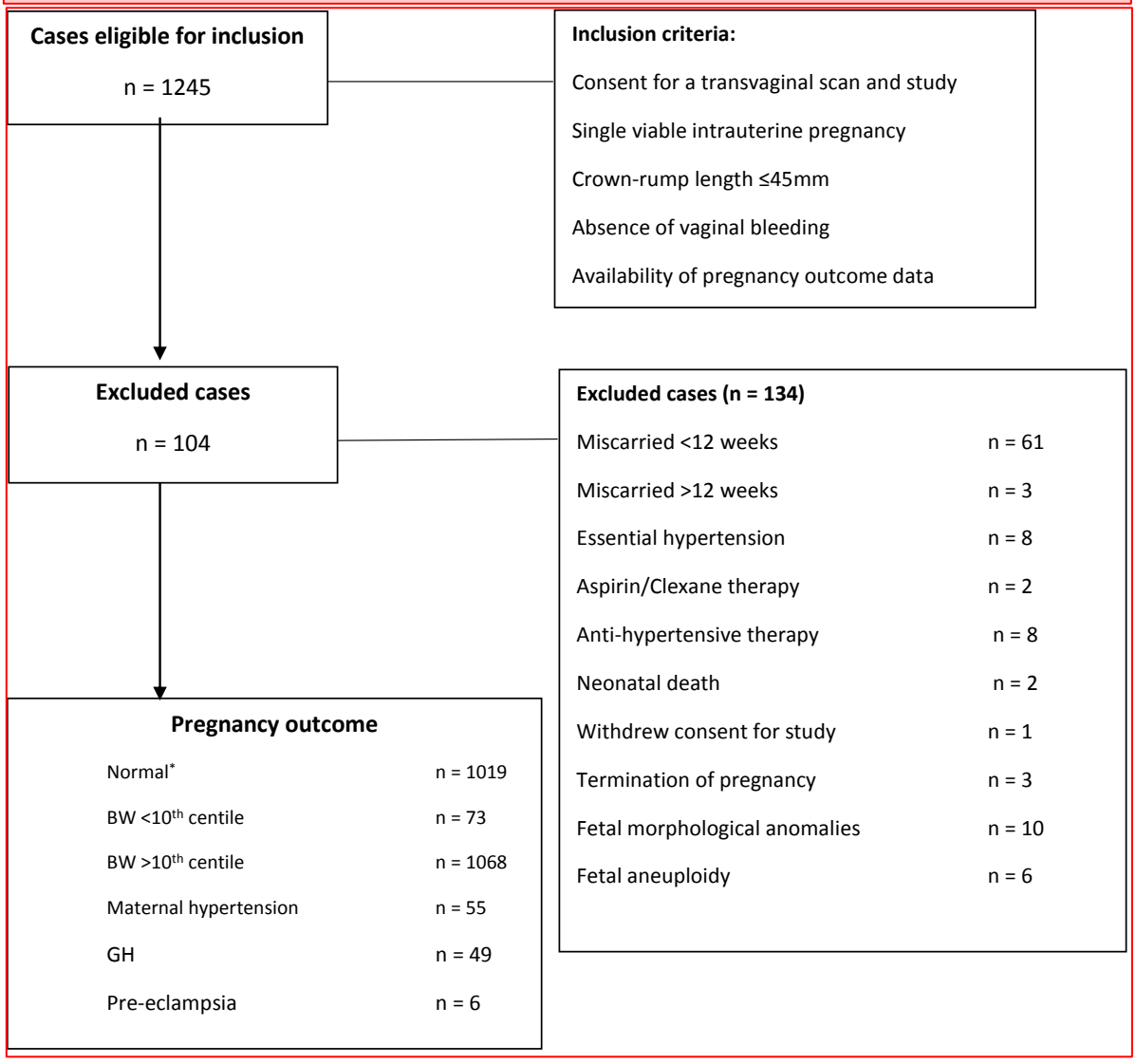


Table 5.1. *Maternal characteristics, medical history and current pregnancy characteristics in the two groups of pregnancy outcome (n=1141); birthweight ≥10th centile and birthweight <10th centile.*

Maternal Characteristic	BW ≥10th centile (n=1068)	BW <10th centile (n=73)	P
Age (years)	31 (16-44)	31 (19-40)	0.389
Weight (kilograms)	72 (36-146)	69 (48-98)	0.035
Height (centimetres)	166 (140-186)	163 (145-189)	0.009
Number of pregnancies	2.5 (1-10)	2.1 (1-6)	0.374
Pre-existing medical conditions			0.513
None	898 (84.1)	61 (83.6)	
Hypothyroidism	86 (8.1)	9 (12.3)	
Psychological	23 (2.2)	-	
Asthma	22 (2.1)	-	
Epileptic	6 (<1.0)	-	
Grave's Disease	2 (<1.0)	-	
Hashimoto's thyroiditis	2 (<1.0)	1 (1.4)	
Metabolic Syndrome	2(<1.0)	-	
Factor 5 Leiden	1 (<1.0)	-	
Antiphospholipid Syndrome	1 (<1.0)	-	
Blood clots	6 (<1.0)	-	
SLE/Lupus	2 (<1.0)	-	
Multiple	17 (1.6)	2 (2.7)	
Current Medications			0.243
None	897 (84.0)	59 (80.8)	
Thyroxine	83 (7.8)	8 (11.0)	
Anti-depressants	32 (3.0)	-	
Diabetes	13 (1.2)	-	
Asthma	14 (1.3)	1 (1.4)	
Anti-epileptics	3 (<1.0)	-	
Multiple	26 (2.4)	5 (6.9)	
Smoking status			0.270
Non-smoker	1047 (8.0)	70 (95.9)	
Smoker	21 (2.0)	3 (4.1)	
Diabetic Status			0.651
None	966 (90.5)	69 (94.5)	
Type I	5 (<1.0)	-	
Type II	6 (<1.0)	-	
Gestational	91 (8.5)	4 (5.5)	
Menstrual cycle			0.843
28 days	479 (44.9)	36 (49.3)	
<28 days	117 (11.0)	7 (9.6)	
>28 days	212 (19.9)	12 (16.4)	
Variable	2 (24.3)	18 (24.7)	
Method of Conception			0.004
Spontaneous	982 (92.0)	59 (80.8)	
Ovulation induction	60 (5.6)	8 (11.0)	
IVF (no ovulation induction)	24 (2.3)	6 (8.2)	
IVF (donor)	2 (<1.0)	-	
Indication for ultrasound			<0.0001
None	315 (29.5)	323 (31.5)	
Dating	543 (50.8)	30 (41.1)	
Viability	148 (13.9)	17 (23.3)	
Previous miscarriage	46 (4.3)	-	
Pain	10 (<1.0)	-	
Exclude plurality	3 (<1.0)	1 (1.4)	
Pre-NIPS	3 (<1.0)	-	
Uterine anomaly	-	2 (2.8)	

Data are provided as mean (range) or n (%). IVF, in-vitro fertilisation; P.V., per vaginal; NIPS, non-invasive prenatal screening.

Multiples of the median were calculated for each ultrasound parameter based on the formula:

$$\frac{\textit{Observed measurement}}{\textit{expected median for CRL.}}$$

Relative to pregnancies with normal outcomes, mean MoM values for TV (p <0.0001), MSD (p=0.007) and TT (p=0.017) were significantly decreased in pregnancies delivered of SGA infants (Table 5.2). Similarly, trophoblast-derived markers, PAPP-A (p=0.007) and PlGF (p=0.008), were both significantly decreased in pregnancies delivered of SGA babies. Free-beta subunit of human chorionic gonadotropin (p = 0.981) and AFP (p = 0.105) levels were undistinguishable from control population.

To identify markers with potential clinical efficacy, we applied logistic regression analyses. Due to lack of statistical significance, markers / parameters excluded from further modelling included maternal weight (p=0.229), TT (p=0.683), MSD (p=0.509), mean UAPI (p=0.322), PAPP-A (p=0.442) and AFP (p=0.680). In contrast, TV MoM (p=0.010), maternal height (p=0.02) and PlGF MoM (p=0.003) were incorporated into the final logistic model (Table 5.3). Parameters not linearly related to the logit outcome (TV MoM and PlGF MoM) were categorised into two groups above and below the median and included in analysis as categorical variables. Parameters linearly related to the logit outcome (maternal height) were included in the model as continuous variables.

The diagnostic performance of this three-marker algorithm is shown as a ROC curve (Figure 5.2) and summarised in Table 5.4. With area under curve (AUC) of 0.70 (95%CI:0.63-0.76), for a FPR of 5%, the PPV was 18.5% whilst NPV = 94.8%. Since the NPV remained consistent at 94.8 – 96.4%, this indicates that a low risk assessment is likely to be correct in about 95% of cases.

Figure 5.2. *(a-f) Scatterplots of the expected median (solid line), 5th and 95th centiles (dotted lines) for: (a) mean sac diameter (MSD), (b) yolk sac diameter (YSD), (c) fetal heart rate (FHR), (d) trophoblast thickness (TT), (e) trophoblast volume (TV), (f) mean uterine artery pulsatility index (meanUAPI); ● pregnancies that resulted in a small-for-gestational age neonate.*

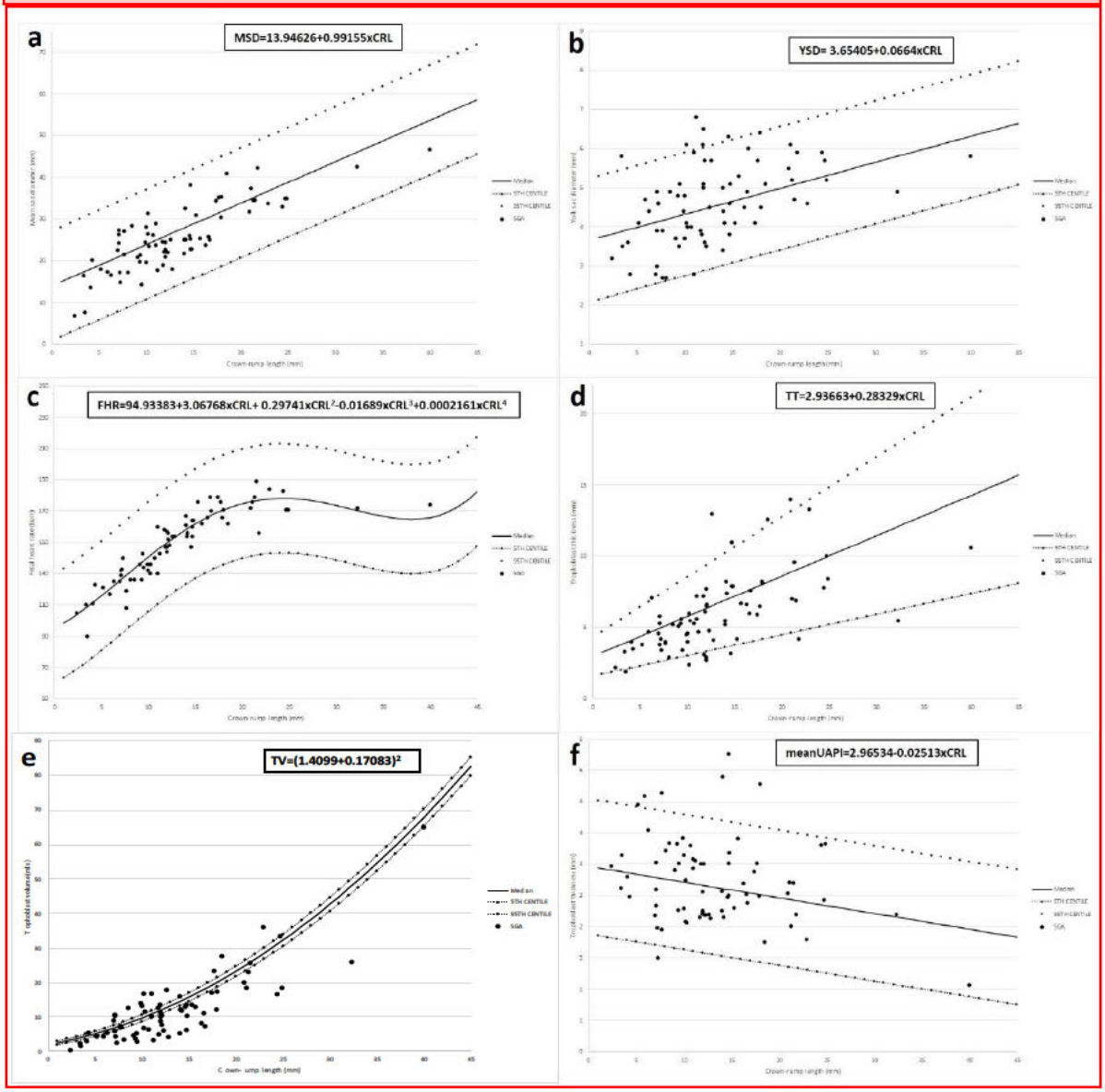


Table 5.2. *Comparison of first trimester ultrasound parameters between pregnancies that resulted in birthweight ≥10th centile vs. birthweight <10th centile (MoM and observed measurement; t-statistic and degrees of freedom).*

Parameter	BW ≥10th centile (n=1068)	BW <10th centile (n=73)	P
Trophoblast volume	1.00 (0.98-1.03)‡ 15.8mls (0.5-105.8)	0.81 (0.73-0.89)§ 12.1mls (0.4-65.0)	<0.0001 $T_{3.99}$; df_{1103}
MSD	1.00 (0.97-1.00) 27.5mm (4.6-58.4)	0.94 (0.90-0.99) 25.6mm (6.8-46.7)	0.007 $t_{2.69}$; df_{1139}
PAPP-A	1.47 (1.41-1.52)*	1.24 (1.09-1.39)#	0.007 $t_{2.78}$; df_{85}
PIGF	1.09 (1.06-1.12)^	0.94 (0.85-1.03)€	0.008 $t_{2.65}$; df_{884}
Trophoblast thickness	1.00 (0.99-1.02)¶ 6.8mm (0.4-24.1)	0.92 (0.85-1.00) 6.1mm (1.9-14.0)	0.017 $t_{2.40}$; df_{1137}
αFP	1.22 (1.18-1.25)ĭ	1.37 (1.19-1.55)˜	0.105 $t_{-1.65}$; df_{65}
YSD	1.00 (0.99-1.01)¶ 4.6mm (2.4-7.8)	1.02 (0.98-1.07) 4.6mm (2.7-6.8)	0.303 $t_{-1.03}$; df_{1137}
FHR	1.00 (1.00-1.00) 150bpm (88-191)	0.99 (0.98-1.00) 148bpm (90-189)	0.500 $t_{0.60}$; df_{1139}
meanUAPI	1.00 (0.98-1.01)† 2.62 (1.00-5.00)	1.03 (0.97-1.09) 2.72 (1.07-4.76)	0.604 $t_{-0.52}$; df_{1133}
βHcG	1.14 (1.10-1.19)*	1.15 (0.98-1.31)#	0.981 $t_{-0.02}$; df_{1028}

Data are given as mean (95% confidence internal), or mean (range), † *n*=1062; ‡ *n*=1035; § *n*=70; ¶ *n*=1066; * n=962; # n=68; ^ n=828; € n=58, ĭ n=909; ˜ n=61

equal variances assumed, FHR, MSD, YSD, trophoblast thickness, meanUAPI, trophoblast volume, βHcG, PIGF;

equal variances not assumed, PAPP-A, αFP;

MSD, mean sac diameter; meanUAPI, mean uterine artery pulsatility index; YSD, yolk sac diameter; FHR, fetal heart rate; βHcG, beta human chorionic gonadotropin; PAPP-A, pregnancy – associated plasma protein A; PIGF, placental growth factor; αFP, alpha fetoprotein; t, t-statistic; df, degrees of freedom.

Table 5.3. *Logistic regression analysis of the parameters used to predict birthweight <10th centile.*

Parameter	β	OR (CI)	P
TV MoM	-0.7614	0.467 (0.261-0.835)	0.010
Maternal height	-0.0642	0.938 (0.901-0.976)	0.002
PIGF MoM	-1.5445	0.213 (0.076-0.597)	0.003
Constant	10.555		

The data were analysed using binary logistic regression analysis. Model R^2 = 0.027; p<0.0001

Dependent variable: BW≥10th centile = 0, BW<10th centile = 1

Independent variables: TV MoM and PIGF MoM (Dummy variables; below the median = 0 and above = 1)

OR, Odds ratio; CI, confidence interval; BW, birthweight; TTV, trophoblast volume; MoM, multiple of the median; PIGF, placental growth factor.

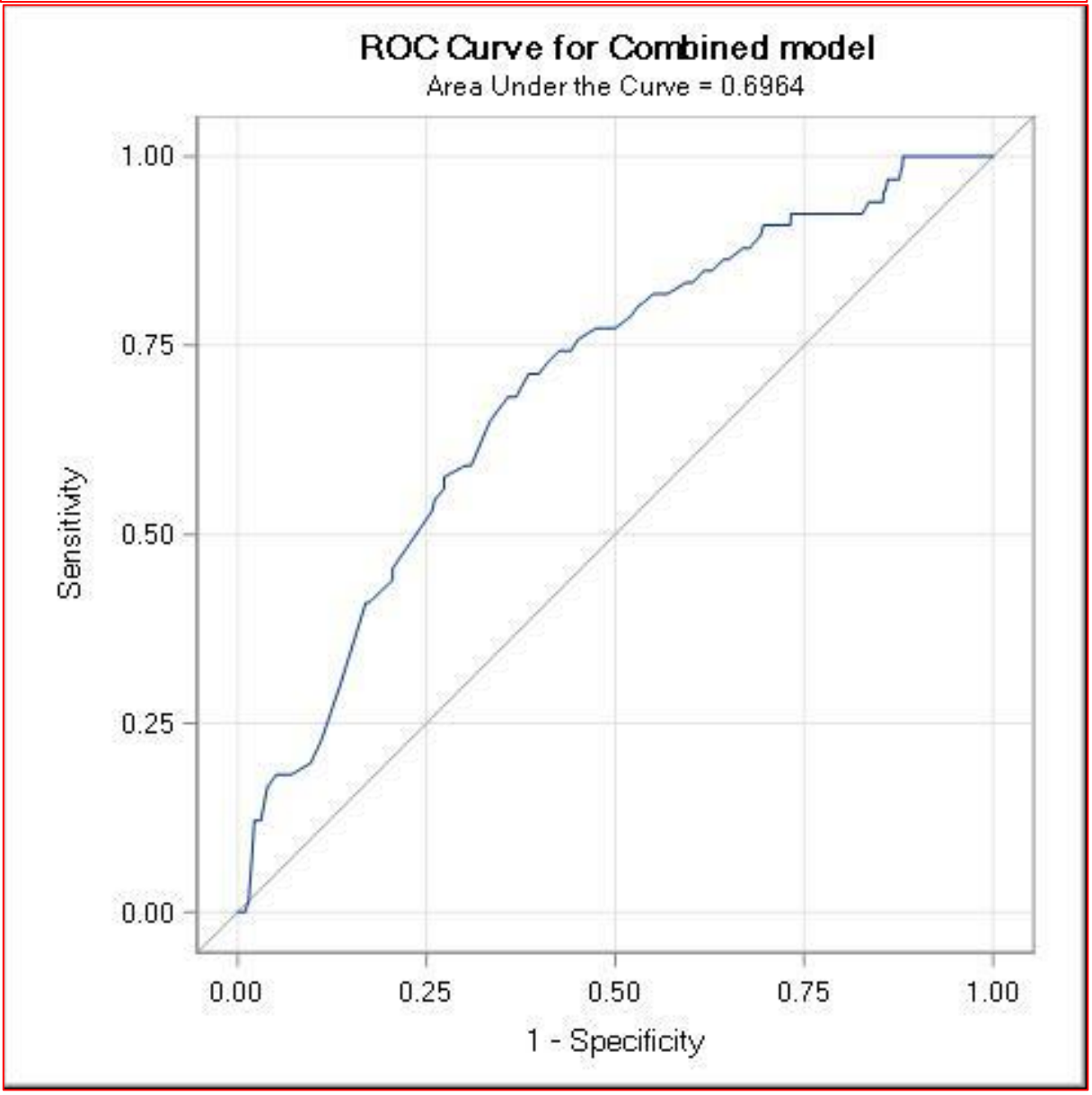
Table 5.4. *Diagnostic performance of the logistic regression model.*

FPR	Sensitivity	PPV	NPV	LR+	LR-
5%	18% (9-27)	18.5%	94.8%	3.6	0.9
10%	20% (10-29)	11.5%	94.7%	2.0	0.9
30%	59% (47-71)	11.1%	96.4%	2.0	0.6

Data for sensitivity are given as mean (95% confidence internal).

FPR, False positive rate; PPV, positive predictive value; NPV, negative predictive value; LR+, positive likelihood ratio; LR-, positive likelihood ratio

***Figure 5.3**. Area under the curve for combined prediction model for birthweight <10th centile.*



DISCUSSION

In this study, multivariate analysis found that TV, maternal height and PIGF levels were all significantly less in pregnancies that resulted in an infant BW of <10th centile for gestational age. These findings are important as this is the first study to demonstrate that TV is potentially of value in conjunction with maternal and placental factors in developing an algorithm aiming to improve the early prediction of this adverse pregnancy outcome.

Despite the placenta beginning to develop during the 8th week of pregnancy the boundaries of the placenta are difficult to delineate with ultrasound prior to 10 weeks' gestation (74) and the majority of studies have focussed on assessment of placental volume (PV) after 11 weeks' for the prediction of SGA (68, 69, 112, 162, 163). The inclusion of the entire trophoblast and calculation of TV in this study is less subjective and a more reliable and reproducible method than PV estimation alone.

Comparison between studies addressing the association between PV and SGA is difficult due to differences in the definition for SGA (<5th or <10th centile) (68, 112, 162). Compared to pregnancies with normal outcomes, the PV has been reported as significantly lower in pregnancies which subsequently develop SGA <5th centile (median PV MoM 0.88 vs. 1.00 (0.74-1.08); p<0.0001) (68) and SGA <10th centile (median PV MoM 0.79 vs. 1.00 (0.62-1.00; p<0.001) (69). In contrast, other studies have demonstrated no significant differences in PV between these groups (112, 163). These differences are possibly accounted for by smaller sample sizes and differences in reporting values for PV; MoM's in the groups reporting significant results and mm^3 in groups reporting non-significant findings.

No large prospective studies including measurements of trophoblast or placental volume have been identified investigating the prediction of SGA prior to 11 weeks' gestation. In this study, we demonstrated a significant association between TV and SGA/BW <10th centile (p <0.0001). In combination with other significant variables; PIGF and maternal height, the proposed prediction model demonstrates moderate efficacy with an AUC of 0.70 (95%CI:0.63-0.76).

Placental growth factor promotes angiogenesis and supports endothelial function (192) supporting effective trophoblastic proliferation and early placentation (242). These results indicate a decreased PIGF level in the first trimester is associated with an increased risk of SGA later in pregnancy and are consistent with previous studies (180, 185, 188). Also consistent with these results, it is well-documented lower maternal height (243) also increases the risk of developing SGA in the third trimester.

The Fetal Medicine Foundation provide a prediction model specific to SGA that can be used at 11-13^{+6} days gestation with a 73% detection rate for SGA fetuses (<5th centile) requiring delivery before 37 weeks' gestation and 46% for those delivering at term (219). Improvement in this detection rate was found in the SGA prediction model (BW <3rd centile) designed by Crovetto et al. 2016 using a combination of maternal factors, meanUAPI, PIGF and soluble fms-like tyrosine kinase-1 implemented at 11-13^{+6} days gestation with a reported detection rate of 86% for early IUGR (AUC 0.93 (95% CI: 0.87-0.98)) and 66% for late IUGR (AUC: 0.76 (95% CI: 0.73-0.80)) with a 10% FPR) (5). The small amount of SGA/IUGR cases in this cohort precludes stratification into early and late cases however the results overall are reasonably comparable to Crovetto et al.'s model. Overall, for the prediction of SGA the results demonstrate an excellent negative predictive value (94.7%) for a FPR of 10%.

Despite the moderate discriminant value of the final model the finding of a significant association between TV and SGA/BW <10th centile is new knowledge and deserving of further investigation. These results suggest that the addition of TV prior to 11 weeks gestation may improve the efficacy of current prediction models for SGA. Further research is needed to address this question.

A strength of this study is the restriction of recruitment of viable intrauterine pregnancies with an absence of maternal hypertension at recruitment and the use of a standardised methodology for the measurement of TV with ultrasound (74, 232). This improves the clinical focus of the results for pregnancies at a lower *a-priori* risk of SGA.

A limitation of this study is that despite recruitment of a large cohort, the absolute numbers of cases that were SGA were smaller than expected. With a cut-off for SGA of <10th centile it would be expected that approximately 10% of cases would result in an SGA infant. With a total number of participants on 1141 we would have expected approximately 110 SGA fetuses however there were only 73 within this cohort (6.4%). This may be due to the small differences in population used in the algorithm to calculate the expected the 10th centile (149) including

variations in ethnicity that were not addressed.

Limited clinical access to VOCAL measurement software and dedicated 3D transvaginal transducers may limit the translatability of this research into general clinical practice. Inclusion of trophoblast thickness may appear more practical as it is more readily measured however univariate analysis demonstrated it was not clinically significant (p=0.683) and it was removed from the presented model.

Despite the good predictive value of the proposed model the R^2 value of 0.027 suggests that <3% of SGA can be attributed to the parameters in the model (maternal height, TV and PIGF). Further sub-analysis found maternal height and TV individually each have a R^2 value of 0.027, whereas slightly more predictive value was found using PIGF alone (R^2 =0.011).

A further limiting factor is the collection of maternal serum at 10-14 weeks gestation. It is possible that future studies may find biochemical markers to be useful at earlier gestational ages, leading to the potential for earlier prediction models and interventions. Alternatively, the inclusion of early pregnancy parameters may improve the efficacy of current prediction models implemented at 11-13^{+6} weeks' gestation.

Further research is needed to validate the model that is presented and to further investigate the value of TV prior to 11 weeks' gestation in the prediction of SGA in different populations of pregnant women.

CONCLUSION

This study is the first to show a significant reduction in TV in early pregnancy in those destined to result in an SGA fetus. In addition, we have shown that maternal PIGF levels and maternal height are also significantly less in pregnancies that subsequently develop SGA. More research is needed to identify whether this or other combinations of early pregnancy parameters are of good predictive value for SGA at an earlier gestation than is currently possible.

Chapter 6. Combining early first trimester ultrasound features maternal characteristics and serum biochemistry for the prediction of maternal hypertensive disorders

Addressing the fourth and final aim of this research this chapter discusses the predictive value of conventional and novel ultrasound measures prior to 11 weeks in the prediction of MHD.

This chapter has been submitted in a different format for publication and is currently under peer review.

Citation:

Hanchard TJ, de Vries BS, Quinton AE, Sinosich M, Hyett JA. Ultrasound features prior to 11 weeks' gestation and first-trimester maternal factors in prediction of hypertensive disorders of pregnancy. Ultrasound Obstet Gynecol. 2020; 55(5):629-636.

Contributions:

Tracey Hanchard*: research planning, data collection, statistical analysis and drafting of the manuscript*

Dr Bradley de Vries*: statistical advice and review of the manuscript*

Associate Professor Ann Quinton: *research planning, statistical advice and review of the manuscript*

Dr Michael Sinosich: *statistical advice and review of manuscript*

Clinical Professor Jonathon Hyett: *research planning, statistical advice and review of the manuscript.*

Relevance:

Conventional and novel ultrasound measures prior to 11 weeks gestation may be associated with the development of maternal hypertensive disorders. In conjunction with maternal characteristics and serum biochemistry these measures may facilitate the prediction of disease earlier in pregnancy than is currently possible.

Chapter 6. Combining early first trimester ultrasound features, maternal factors and serum biochemistry for the prediction of maternal hypertensive disorders

INTRODUCTION

Maternal hypertensive disorders that include gestational hypertension (GH) and pre-eclampsia (PET) are disorders of deep placentation originating early in the first trimester of pregnancy (9). Within Australia, GH and PET are estimated to occur in approximately 4.3% and 3-3.3% of pregnancies respectively (240, 244). Early prediction of MHD enables prophylactic intervention modifying progression of disease and improving pregnancy outcomes for both mother and fetus (245).

Gestational hypertension, particularly with progression to PET, confers significant risk to both mother and fetus. Maternal complications include renal insufficiency (serum or plasma creatinine >90µmol/L), liver involvement (elevated transminases with or without right epigastric or right upper quadrant pain), neurological complications (altered mental status, stroke, blindness, hyperreflexia, severe headache) and haematological complications (165). In a GH-complicated pregnancy the fetus is considered at risk for intrauterine growth restriction (IUGR) (30) with an increased risk of numerous adverse short- and long-term outcomes for both fetus (28, 31, 32, 34, 35) and mother (29, 36-38).

At the beginning of pregnancy, the fertilised ovum is surrounded by trophoblast as it implants into the endometrium (10). The trophoblast initiates transformation of the spiral arteries within the myometrium (12) with inadequate transformation resulting in varying degrees of placental hypoxia and an increased risk of the woman developing hypertension later in pregnancy (9). Current prediction models for MHD are currently implemented at 11-13^{+6} weeks' gestation (246). Intervention in pregnancies considered to be at an increased risk, may reduce rates of severe early onset PET by 80-90% but are less effective if given after 16 weeks' gestation

(245). It is possible that if prediction and prevention occurred at an earlier gestation it may be more effective than the current regimen.

In this study, we aimed to determine whether ultrasound measures prior to 11 weeks' gestation can be combined with maternal characteristics and biochemical markers for effective prediction of MHD.

MATERIAL AND METHODS

This was a prospective cohort study designed to assess the value of early ultrasound measurements in the prediction of MHD. The same participant cohort presented in Chapter 5 was used for this study. Data collection methods and measurement methodology for ultrasound parameters and maternal serum have been previously described (Chapter 5). Gestational hypertension is defined as the new onset of hypertension (>140 mmHg systolic or >90 mmHg diastolic) after 20 weeks gestation (165). Pre-eclampsia is defined as the new onset of hypertension (>140 mmHg systolic or >90 mmHg diastolic) after 20 weeks gestation in conjunction with one or more complications (165).

STATISTICAL ANALYSIS

Statistical analyses were similar to those presented in Chapter 5. Maternal characteristics, ultrasound findings, maternal biochemistry results and pregnancy outcome were entered into a computer database. Regression techniques applied to determine the expected value for each ultrasound parameter measurement at a given CRL and MoM calculated as previously described. Non-parametric analysis was used to compare CRL between groups and reported as mean range). Parameters were compared using a two-sample t-test for independent variables; reported as mean (95%CI). Frequencies were reported as n (%) and compared using Pearson's χ^2 test. A p value of <0.05 was considered statistically significant.

Logistic regression analysis was used to create a prediction model for MHD based on maternal characteristics, standardised ultrasound measurements and maternal biochemistry. A receiver operator characteristic (ROC) curve was obtained to assess the performance of this model. Reporting and presentation follow the principles of the STROBE initiative (228).

RESULTS

A total of 1,245 women were eligible for inclusion in this study. We excluded a total of 104 cases (Figure 5.1). Of the remaining 1,141 pregnancies, 1086 women were normotensive at delivery (95.2%) and 55 developed GH (4.8%). Of the 55 pregnancies affected by hypertension, six developed PET (0.5% overall). Due to the small number of PET-affected pregnancies, GH and PET-affected pregnancies were combined for analysis.

With the exception of maternal weight and height there was no difference in demographic characteristics between normotensive and hypertensive pregnancies (Table 6.1). There was no significant difference in the CRL measured at the initial ultrasound assessment between these two groups; 13.7mm (range 1.7-44.8) in the normotensive groups compared to 14.1mm (range 4.8-36.6) in the hypertensive group (p=0.876). These CRL ranges equate to gestational ages of 7^{+5} weeks (5^{+1} –11^{+1} weeks) and 7^{+5} weeks (6^{+0} – 11^{+1} weeks) respectively (55). The regression formulae for the expected measurement of each ultrasound parameter as determined from the CRL were calculated using 1,019 pregnancies with a normal outcome (see Chapter 5). The six ultrasound parameters assessed in this study each showed a significant correlation with CRL with each relationship described in Chapter 5.

Figure 6.1 illustrates the expected median, 5^{th} and 95^{th} centiles for each ultrasound parameter with the observed measurements for pregnancies that were ultimately complicated by MHD. Multiples of the median were calculated as described in Chapter 5 with comparison of MoM for FHR (1.00 (95%CI: 1.00-1.00) vs. 0.98 (95%CI :0.96-1.00); p=0.051) and TV (1.00 (95%CI: 0.98-1.02) vs. 0.85 (95%CI: 0.76-0.94); p=0.006) significantly lower for women who subsequently developed MHD compared to those with a normal outcome. Maternal biochemistry results showed that PAPP-A (1.46 (95%CI: 1.41-1.52) vs. 1.27 (95%CI: 1.10-1.47); p=0.031) and PIGF (1.09 (95%CI: 1.06-1.12) vs. 0.95 (95%CI: 0.84-1.06); p=0.004) were both significantly lower in women who developed MHD. Maternal weight was higher in GH affected pregnancies: mean (range): 82kg (51-136) vs. 72kg (36-146); p=<0.0001 (Table 6.2).

Table 6.1. *Maternal characteristics, medical history and current pregnancy characteristics in the two groups of pregnancy outcome (n=1141); normotensive (n=1086) and hypertensive (n=55).*

Maternal Characteristic	Normotensive (n=1086)	Hypertensive (n=55)	P
Age (years)	31 (16-44)	30 (21-42)	0.057
Weight (kilograms)	72 (36-146)	81 (51-129)	<0.0001
Height (centimetres)	166 (140-189)	164 (145-180)	0.010
Number of pregnancies	2.5 (1-10)	1.9 (1-5)	0.332
Pre-existing medical conditions			0.149
None	916 (84.3)	44 (80.0)	
Hypothyroidism	88 (8.1)	7 (12.7)	
Psychological	20 (1.8)	3 (5.5)	
Asthma	22 (2.0)	-	
Epileptic	6 (<1.0)	-	
Grave's Disease	2 (<1.0)	-	
Hashimoto's thyroiditis	3 (<1.0)	-	
Metabolic Syndrome	1 (<1.0)	1 (1.8)	
Factor 5 Leiden	1 (<1.0)	-	
Antiphospholipid Syndrome	1 (<1.0)	-	
Blood clots	6 (<1.0)	-	
SLE/Lupus	2 (<1.0)	-	
Multiple	19 (1.7)	1 (1.8)	
Current Medications			0.848
None	912 (84.0)	44 (80.0)	
Thyroxine	84 (7.7)	7 (12.7)	
Anti-depressants	30 (2.8)	2 (3.6)	
Diabetes	12 (1.1)	1 (1.8)	
Asthma	15 (1.4)	-	
Anti-epileptics	3 (<1.0)	-	
Multiple	30 (2.8)	1 (1.8)	
Smoking status			0.080
Non-smoker	1063 (97.9)	54 (98.2)	
Smoker	23 (2.1)	1 (1.8)	
Diabetic Status			0.121
None	989 (91.1)	46 (83.6)	
Type I	4 (<1.0)	1 (1.8)	
Type II	6 (<1.0)	-	
Gestational	87 (8.0)	8 (14.6)	
Menstrual cycle			0.195
28 days	483 (44.5)	32 (58.2)	
<28 days	121 (11.1)	3 (5.5)	
>28 days	214 (19.7)	10 (18.2)	
Variable	268 (24.7)	10 (18.2)	
Method of Conception			0.937
Spontaneous	991 (91.3)	50 (90.9)	
Ovulation induction	64 (5.9)	4 (7.3)	
IVF (no ovulation induction)	29 (2.7)	1 (1.8)	
IVF (donor)	2 (<1.0)	-	
Indication for ultrasound			0.315
None	328 (30.2)	10 (18.2)	
Dating	542 (49.9)	31 (56.4)	
Viability	155 (14.3)	10 (18.2)	
Previous miscarriage	44 (4.1)	2 (3.6)	
Pain	8 (<1.0)	2 (3.6)	
Exclude plurality	4 (<1.0)	-	
Pre-NIPS	3 (<1.0)	-	
Uterine anomaly	2 (<1.0)	-	

Data are provided as mean (range) or n (%). IVF, in-vitro fertilisation; P.V., per vaginal; NIPS, non-invasive prenatal screening.

Figure 6.1 *(a-f) Scatterplots of the expected median (solid line), 5th and 95th centiles (dotted lines) for: (a) mean sac diameter (MSD), (b) yolk sac diameter (YSD), (c) fetal heart rate (FHR), (d) trophoblast thickness (TT), (e) trophoblast volume (TV), (f) mean uterine artery pulsatility index (meanUAPI);* ● *pregnancies with maternal hypertension.*

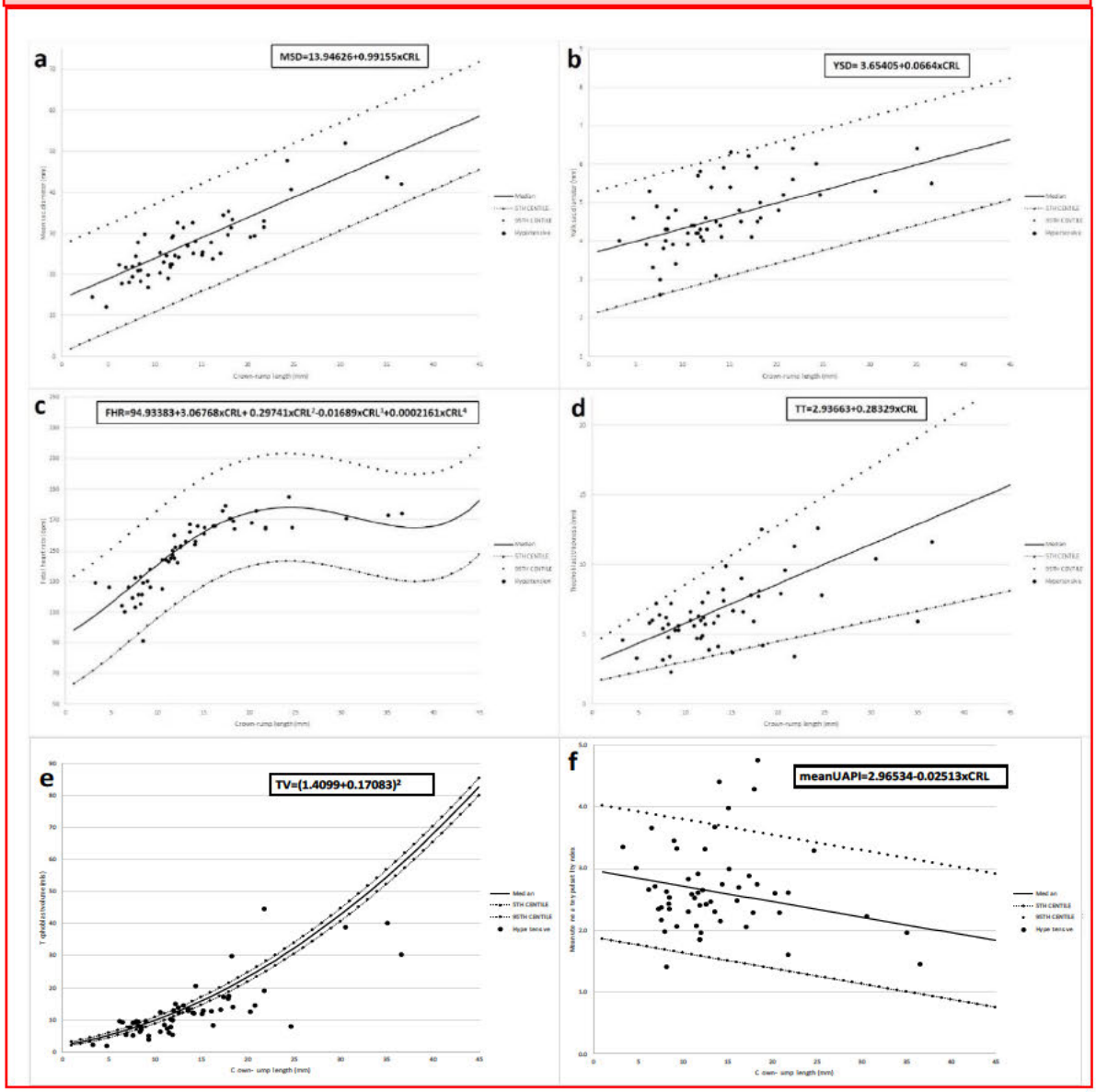


Table 6.2. *Comparison of predictive variables between pregnancies that resulted in normal outcomes versus maternal hypertension (MoM and observed measurement; t-statistic and degrees of freedom).*

Parameter	Normotensive (n=1086)	Hypertensive (n=55)	P
Maternal weight (kg)	71.6 (35.7-146)[ə]	81.6 (51.0-135.9)	<0.0001 $t_{-4.63}$; df_{58}
Trophoblast volume	1.00 (0.98-1.02)[‡] 15.6mls (0.4-105.8)	0.85 (0.76-0.94) 13.4mls (1.7-51.6)	0.006 $t_{2.77}$; df_{1103}
PAPP-A	1.46 (1.41-1.52)[*]	1.27 (1.10-1.47)[#]	0.031 $t_{2.20}$; df_{62}
PIGF	1.09 (1.06-1.12)[^]	0.95 (0.84-1.06)[€]	0.044 $t_{2.02;}$ df_{884}
FHR	1.00 (1.00-1.00) 150bpm (88-191)	0.98 (0.96-1.00) 148bpm (91-185)	0.051 $t_{1.96}$; df_{1139}
Maternal height (cm)	166 (140-189)[ǿ]	164 (145-180)	0.063 $t_{1.86}$; df_{1111}
Maternal age	31 (16-44)	31 (21-43)	0.124 $t_{1.54}$; df_{1139}
MSD	1.00 (0.99-1.01) 27.4mm (4.6-58.4)	0.97 (0.94-1.01) 26.9mm (12.1-52.0)	0.188 $t_{1.32}$; df_{1139}
βHcG	1.14 (1.09-1.18)[*]	1.27 (1.01-1.53)[#]	0.316 $t_{-1.01}$; df_{54}
Trophoblast thickness	1.00 (0.98-1.02)[¶] 6.8mm (0.4-24.1)	0.97 (0.89-1.03) 6.5mm (2.3-12.6)	0.459 $t_{0.74;}$ df_{1137}
YSD	1.00 (0.99-1.01)[¶] 4.6mm (2.4-7.8)	1.02 (0.97-1.06) 4.7mm (2.6-6.4)	0.546 $t_{-0.60}$; df_{1137}
meanUAPI	1.00 (0.99-1.02)[†] 2.63 (1.00-5.02)	1.01 (0.93-1.08) 2.63 (1.14-4.75)	0.861 $t_{-0.17}$; df_{1133}
αFP	1.23 (1.19-1.26)[ĭ]	1.21 (1.03-1.39)[˜]	0.869 $t_{0.16}$; df_{968}

Data are given as mean (95% confidence internal) or mean (range) [†] *n*=1080; [‡] *n*=1050; [¶] n=1084; [*] n=978; [#] n=52; [^] n=844; [€] n=42; [ĭ] n=921; [˜] n=;49; [ə] n=1062; [ǿ] n=1058
equal variances assumed, MSD, YSD, FHR, trophoblast thickness, trophoblast volume, meanUAPI, PIGF, αFP, maternal height;
equal variances not assumed, βHcG, PAPP-A. maternal weight;
MSD, mean sac diameter; meanUAPI, mean uterine artery pulsatility index; YSD, yolk sac diameter; FHR, fetal heart rate; βHcG, beta human chorionic gonadotropin; PAPP-A, pregnancy – associated plasma protein A; PIGF, placental growth factor; αf, alpha fetoprotein; t, t-statistic; df, degrees of freedom; kg, kilograms; cm, centimetres.

Univariate analysis of all explanatory variables excluded those not significantly associated with adverse pregnancy outcome from further analysis: maternal age (p=0.125), MSD (p=0.187), YSD (p=0.545), TT (p=0.459), meanUAPI (p=0.861), BhCG (p=0.211), PAPP-A (p=0.104) and αFP (p=0.869).

The final logistic regression model included TV (MoM) (p=0.005), maternal weight (p<0.0001), maternal height (p=0.008), FHR (MoM) (p=0.010) and PIGF (p=0.009). All variables appeared linear in distribution and were fitted in the model as continuous variables. Table 6.3 illustrates the results of the regression analysis with an area under the ROC curve (AUC) of 0.80 (95%CI 0.75-0.86) (Figure 6.2). Clinically the absolute difference in FHR between the two groups is small; if FHR MoM is removed from the model there is only minimal change in its predictive power for MHD (AUC:80 (95%CI: 75-86) compared to AUC: 0.78 (95%CI: 71-84) with and without FHR MoM respectively). The sensitivity, negative and predictive values of the final model are shown in Table 6.4 for fixed false positive rates.

Table 6.3. *Logistic regression analysis of the parameters used to predict maternal hypertension.*

Parameter	β	OR (CI)	P
TV MoM	-1.6433	0.193 (0.061-0.614)	0.005
Maternal weight	0.0410	1.042 (1.022-1.062)	<0.0001
Maternal height	-0.0759	0.927 (0.876-0.981)	0.008
FHR MoM	-8.0107	0.000 (0.000-0.142)	0.010
PIGF MoM	-1.3566	0.258 (0.093-0.711)	0.009
Constant	17.006		

The data were analysed using binary logistic regression analysis. Model R^2 = 0.048; P < 0.0001
Outcome variable: normotensive = 0, hypertensive = 1
Explanatory parameters: all fitted as continuous variables. OR, Odds ratio; CI, confidence interval; TV, trophoblast volume; MoM, multiple of the median; FHR, fetal heart rate; PIGF, placental growth factor.

Figure 6.2. *Area under the curve for combined prediction model for maternal hypertensive disease.*

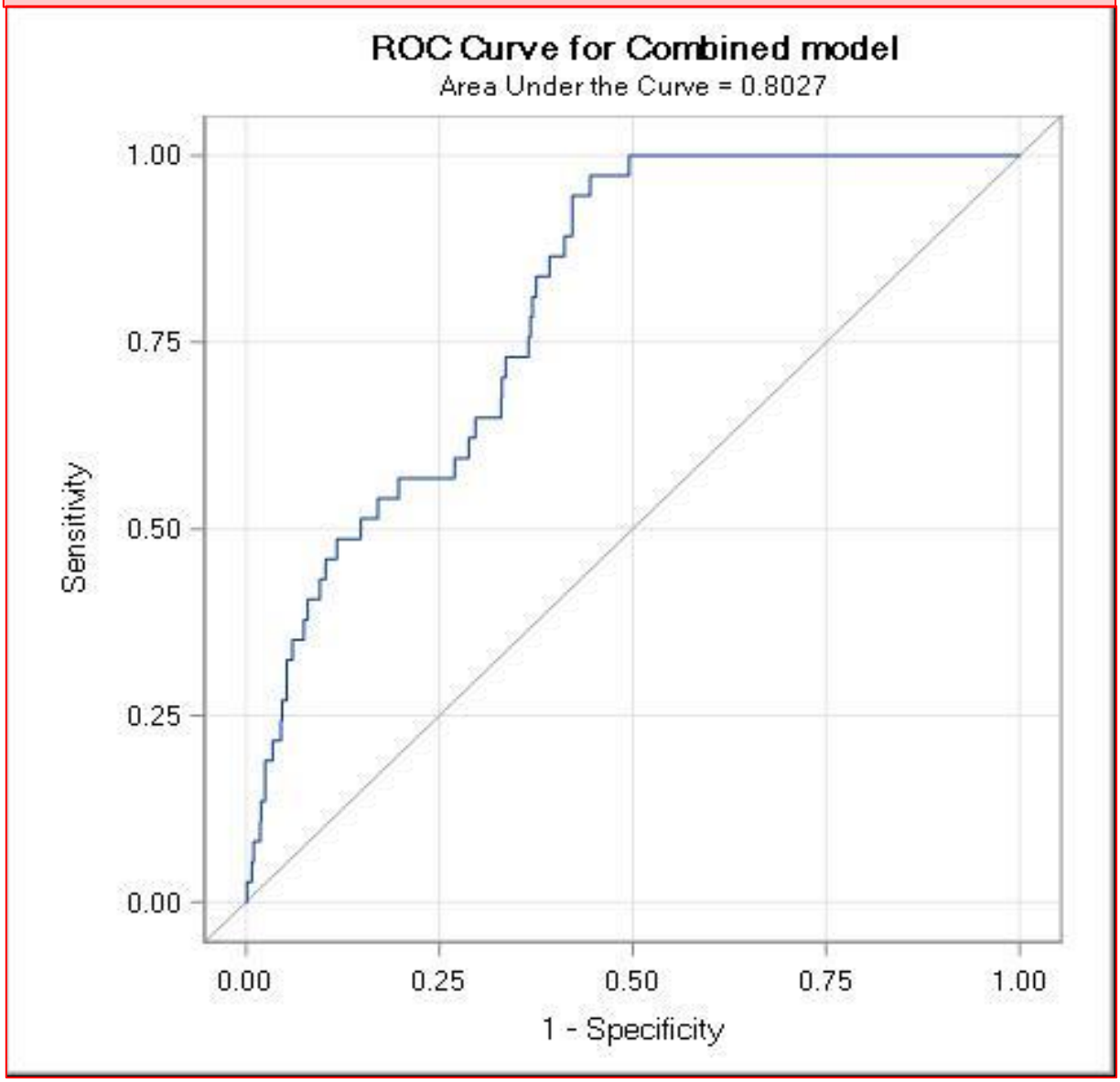


Table 6.4. *Diagnostic performance of the logistic regression model.*

FPR	Sensitivity	PPV	NPV	LR+	LR-
5%	27% (13-41)	19.6%	96.6%	5.4	0.8
10%	43% (27-59)	16.3%	97.2%	4.3	0.6
30%	65% (49-80)	8.9%	97.8%	2.2	0.5

Data for sensitivity are given as mean (95% confidence internal).
FPR, False positive rate; PPV, positive predictive value; NPV, negative predictive value; LR+, positive likelihood ratio; LR-, positive likelihood ratio

DISCUSSION

The main findings were that multivariate analysis showed that TV, FHR, PIGF levels and maternal height were all significantly lower and maternal weight significantly higher in women who developed MHD. Maternal body mass index could be used as a single alternative to weight and height however we wanted to assess the individual contribution of each variable to the final model. The association of smaller TV and a slower fetal heart rate prior to 11 weeks' gestation with subsequent MHD are new findings. This is important because fetal heart rate is easily and routinely measured with early first trimester ultrasound. In addition, with appropriate equipment and operator experience TV is readily measurable with ultrasound. A strong deviation in TV in the MHD group from the normal pattern of trophoblastic/placental growth was seen from seven to nine weeks gestational age (Figure 1e), making it of potential of value in conjunction with FHR and maternal factors in developing an algorithm for the early prediction of MHD.

The majority of studies identified have focussed on the assessment of placental volume (PV) after 11 weeks (69, 112, 163) rather than TV in its entirety. The PV at 11-14 weeks' gestation has been reported to be smaller but not statistically significantly different in pregnancies complicated by subsequent MHD (69, 112, 163). Comparison of these studies is difficult due to differences in reporting measures. Studies reporting PV in millilitres found no significant differences between women who remained normotensive and those

subsequently developing MHD (112, 163). This approach does not account for normal changes in PV with increasing gestational age, however studies comparing MoM values of PV report conflicting results. Effendi et al. (2014) reports no association between PV and PET (p=0.870) with a 2.2 % incidence of PET within their cohort (69). In contrast, Rizzo et al. 2008 report a significant reduction in PV in pregnancies complicated by PET (p<0.003) in a cohort with a 4.5% incidence of PET, this higher incidence possibly explaining these conflicting results (114). There has been little investigation of prediction of MHD prior to 11 weeks' gestation, with no large prospective studies identified or any studies including measurements of TV or PV. The inclusion of the entire trophoblast in this study is less subjective providing a more reproducible and reliable method of TV estimation. This study demonstrated a significant association between TV measured <11 weeks gestation and MHD (p=0.005). In combination with FHR, maternal weight, maternal height and PIGF the proposed prediction model has an AUC of 0.80 (95%CI 0.75-0.86). This shows good predictive value.

No studies were identified investigating the association of FHR measured prior to 11 weeks' gestation and the subsequent development of MHD. These results indicate that a decrease in FHR MoM, of borderline significance in univariate analysis (p=0.051), makes a significant contribution to the prediction of MHD in the logistic regression model (p=0.010). Clinically the absolute difference in FHR between outcome groups is small; if FHR is removed from the model there is only minimal change in its predictive power for MHD; AUC:0.80 compared to AUC:0.78 (with and without FHR MoM respectively). The value of the inclusion of FHR-MoM in predictive models implemented prior to 11 weeks' gestation requires further investigation.

The finding that a decreased PIGF level in the first trimester is associated with an increased risk of MHD later in pregnancy is consistent with previous studies (194, 247). Also consistent with these results, others have reported that that lower maternal height (248) and higher maternal weight (185) increase the risk of developing MHD later in pregnancy.

Currently prediction models for MHD are implemented at 11-13^{+6} weeks gestational

age using a combination of clinical risk factors, maternal serum biochemistry and uterine artery Doppler. The most widely used model involves a combination of maternal factors, maternal mean arterial pressure , PIGF and uterine artery Doppler for a reported AUC of 0.96 for PET prediction prior to 37 weeks' gestation and 0.78 after 37 weeks (CI's not provided) (249). O'Gorman et al. (2016) presented similar results with an AUC of 0.91 (PET prior to 37 weeks) and 0.80 (PET after 37 weeks) (177). There were no studies identified that use TV as part of their predictive algorithm for MHD.

The presented model had an AUC of 0.80 (95%CI 0.75-0.86) for MHD overall. The difference between this and current predictive models may be due to the smaller number of PET affected pregnancies in this cohort. Overall, for the prediction of MHD, these results demonstrate an excellent negative predictive value (97.2%) for a FPR of 10%. Based on these results we believe that the addition of TV prior to 11 weeks' gestation may improve the efficacy of current prediction models and further research is needed to address this question.

A strength of this study is the use of a standardised methodology for the measurement of TV with ultrasound (74, 232) and the restriction of recruitment of viable intrauterine pregnancies with an absence of maternal hypertension. This improves the clinical focus of the results for pregnancies at a lower *a-priori* risk of MHD.

A limitation of this study may be limited clinical access to dedicated 3D transvaginal transducers and VOCAL measurement software potentially limiting the translatability of this research into clinical practice. Inclusion of trophoblast thickness in the model may appear more practical as it is more readily measured in the clinical environment however univariate analysis demonstrated it was not clinically significant (p=0.382) and it was removed from the presented model.

A further limiting factor is the collection of maternal serum at 10-14 weeks gestation. It is possible that future studies may find biochemical markers to be useful at earlier gestational ages, leading to the potential for earlier prediction models and interventions. Alternatively, the inclusion of early pregnancy parameters

may improve the efficacy of current prediction models implemented at 11-13^{+6} weeks' gestation.

Furthermore, despite recruitment of a large cohort of women to the study, the absolute numbers of cases that developed PET was small (240). This may be due to the similarly small incidence of PET risk factors including previous PET (3.7%), chronic hypertension (<1.0%), pre-existing diabetes (2.0%), autoimmune disorders (<1.0%) and pre-existing renal disease (<1.0%) (241). In addition, comparison of mean gestational age at delivery in this cohort demonstrated that PIH (38^{+2} weeks' vs. 39 weeks'; p=0.0006) and PET-affected pregnancies (35^{+1} weeks' vs. 39 weeks'; p<0.0001) was significantly earlier than for normotensive pregnancies. The low prevalence of PET may be due to the earlier delivery of PIH-affected pregnancies compared to normotensive pregnancies, preventing the progression to PET in this sub-group. We feel the large number of pregnancies affected by maternal hypertension and/or resulting in a low birthweight infant (10.4%) is significant enough to establish the reliability of these results and encourage further research with a larger cohort of PET-affected pregnancies.

Despite the good predictive value of the proposed model the R^2 value of 0.048 suggests that only approximately 5% of maternal hypertensive disease can be attributed to the parameters in the model (maternal height and weight, TV, FHR and PIGF). This may be reflective of the low incidence of cases in the cohort with PET which would be likely to have severely impaired placentation resulting in more extreme values of parameters including TV and PIGF.

Further research is required to further investigate the value of FHR and TV prior to 11 weeks gestation in the prediction of MHD, particularly PET in conjunction with validation the presented model.

CONCLUSION

This study is the first to show a significant reduction in TV prior to 11 weeks' gestation in those destined to develop MHD. In addition, it was shown that maternal weight, height, FHR and PIGF are associated with pregnancies that subsequently develop MHD. More research is needed to investigate the value of FHR and TV prior to 11 weeks

gestation in the prediction of MHD and to validate this algorithm in clinical practice.

Chapter 7. Discussion and conclusion

Contributions:

Tracey Hanchard: *Drafting of the manuscript*

Dr Bradley de Vries: *Review of the manuscript*

Associate Professor Ann Quinton: *Review of the manuscript*

Clinical Professor Jonathon Hyett: *Review of the manuscript*

This chapter brings the works in this thesis together and discusses their relevance, future directions and conclusion.

MAIN FINDINGS

The aims of the research in this thesis were to investigate the value of conventional (MSD, YSD and FHR) and novel (TT, TV and meanUAPI) ultrasound measures prior to 11 weeks gestation in the prediction of miscarriage prior to 12 weeks gestation, SGA and MHD. The main findings for each of these adverse pregnancy outcomes will now be discussed.

Prediction of miscarriage prior to 12 weeks gestation

Chapter 3 investigated the value of conventional and novel ultrasound measures prior to 11 weeks gestation in the prediction of miscarriage prior to 12 weeks gestation. The most original contribution to the literature from this work is that it was the first to show a significant reduction in the meanUAPI in early pregnancy in those destined for miscarriage. In addition, this work also demonstrated a significant association between a decrease in TV, MSD and FHR and subsequent miscarriage.

A logistic regression model (Figure 7.1) for the prediction of miscarriage was proposed based on these findings demonstrating a good predictive value for miscarriage that may be useful in counselling and guiding the short-term management of early pregnancies.

Uterine artery pulsatility index prior to 11 weeks gestation

Chapter 4 investigated the assessment of the meanUAPI prior to 11 weeks

gestation. The work in this chapter contributes new and original knowledge to the body of literature by the development of population-specific reference ranges for the meanUAPI <11 weeks gestation (CRL: 1-45mm) in pregnancies with normal outcomes defined as BW ≥10th centile and absence of maternal hypertension. No significant difference was found in meanUAPI between pregnancies with a normal or adverse (BW <10th centile and/or maternal hypertension) outcomes.

This data indicated the meanUAPI was significantly lower prior to 11 weeks gestation compared to 11-13^{+6} weeks gestation in all pregnancies regardless of outcome. The difference was smaller in pregnancies that resulted in an adverse outcome, but this was not statistically significant. Further research including a larger number of adverse pregnancy outcomes may demonstrate a more significant association.

Prediction of small-for gestational age neonates

Chapter 5 investigated the value of conventional and novel ultrasound measures prior to 11 weeks gestation in the prediction of SGA later in pregnancy. In addition, if these ultrasound measures in conjunction with maternal characteristics (age, weight and height) and biochemistry ((FBhCG), PAPP-A, AFP and PlGF) improve the prediction of this adverse pregnancy outcome.

These results present new knowledge to the field by demonstrating a significant decrease in TV, TT and MSD in pregnancies that resulted in an SGA infant compared to those with a BW ≥10th centile. Maternal biochemistry results demonstrated that PIGF and PAPP-A are both significantly lower in pregnancies with an infant BW <10th centile.

Logistic regression analysis resulted in a final predictive model for SGA (Figure 7.1) that included TV, maternal height and PIGF with a reasonable AUC of 0.70 (95%CI: 0.63-0.76). Overall, for the prediction of SGA these results demonstrate an excellent negative predictive value for SGA of 94.7% (for a FPR of 10%).

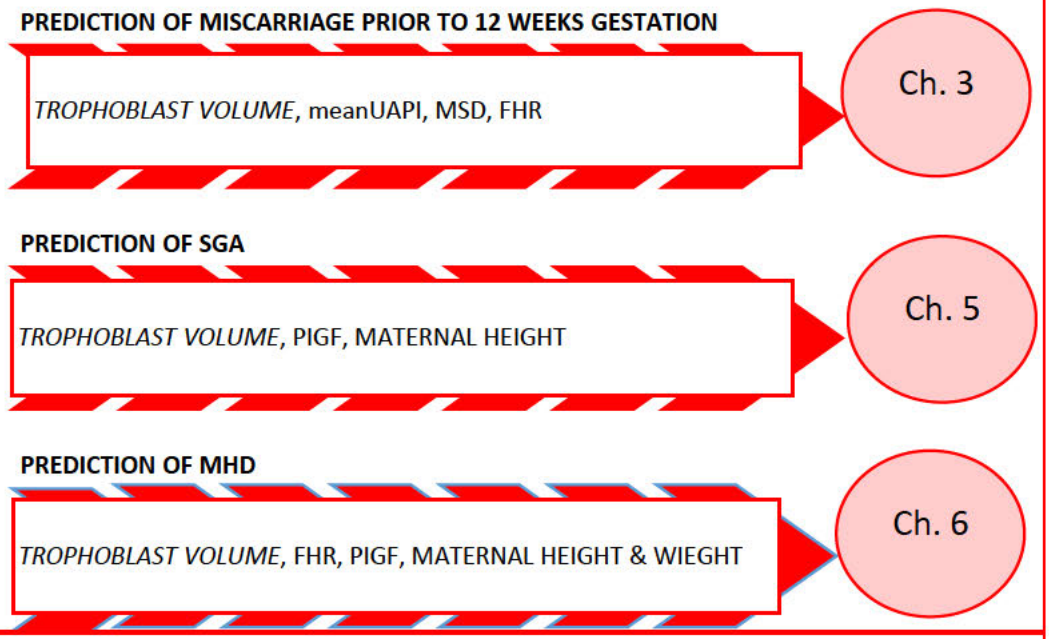
Prediction of maternal hypertensive disorders

Chapter 6 investigated the value of conventional and novel ultrasound measures prior to 11 weeks gestation in the prediction of MHD later in pregnancy. In addition, if these ultrasound measures

in conjunction with maternal characteristics (age, weight and height) and biochemistry (FBhCG, PAPP-A, AFP and PlGF) improve the prediction of this adverse pregnancy outcome.

These results present new knowledge to the field by demonstrating a significantly lower FHR and TV in pregnancies subsequently developing MHD compared to those with a normal outcome. Maternal biochemistry results demonstrated that PAPP-A and PIGF were both significantly lower in pregnancies that developed MHD. Maternal weight was increased in MHD affected pregnancies. Logistic regression analysis resulted in a final predictive model for MHD (Figure 7.1) that included TV, maternal height, FHR and PIGF with a good AUC of 0.80 (95%CI: 0.75-0.86). Overall, for the prediction of MHD, these results demonstrated an excellent negative predictive value (97.2%) for a FPR of 10%.

Figure 7.1. *Summary of significant early first trimester ultrasound measures (<11 weeks gestation), maternal characteristics and serum biochemistry (10-14 weeks gestation) in the prediction of adverse pregnancy outcomes.*



meanUAPI mean uterine pulsatility index, MSD mean sac diameter, FHR fetal heart rate, SGA small-for-gestational age, PIGF placental growth factor, MHD maternal hypertensive disorders.

STRENGTHS OF THIS RESEARCH

Strengths of the research presented in this thesis include the prospective method of data collection resulting in very few cases of missing data and participants excluded due to lost-to-follow-up. Another strength of this research is the use of standardised measurement methods for the ultrasound parameters of interest. As previously discussed in Chapters 3-6 the techniques for measurement of the MSD, CRL and FHR have been widely documented (47, 96, 227). The measurement methods for trophoblast thickness and volume have been standardised and verified by previous research groups (42, 74, 232). All TV calculations were conducted by a single sonographer (TT) eliminating interobserver variability as a potential bias of the dataset. The method used for the documentation and measurement of the UAPI is in common usage with a transabdominal approach (89) with the methodology readily extrapolated to the transvaginal approach used in this study (87).

A strength of the research presented in Chapter 3, investigating the prediction of miscarriage prior to 12 weeks gestation is the restriction of recruitment to pregnancies with a detectable fetal heartbeat and absence of P.V. bleeding. In addition, the rate of miscarriage after 12 weeks reduces significantly, so this outcome measure is also more relevant to low risk pregnancies in clinical practice.

The research in Chapters 4-6 demonstrated the population-specific references ranges presented are based on a large sample of pregnancies with normal outcomes. In addition, the research in Chapters 5 and 6 restricted recruitment of viable intrauterine pregnancies with an absence of maternal hypertension improving the clinical focus of the results for pregnancies at a lower *a-priori* risk of SGA and MHD.

LIMITATIONS OF THIS RESEARCH

Measurement of trophoblast volume is restricted to services with dedicated 3D transvaginal transducers and access to VOCAL measurement software. This may limit the translatability of this research presented in this thesis into clinical practice. Inclusion of trophoblast thickness in the prediction models may appear practical as it is more easily measured however univariate analysis demonstrated it was not statistically significant in the prediction of each

adverse pregnancy outcome and it was removed from the presented models.

A limitation of the research presented in Chapter 4 may be the unequal distribution of cases across gestational age weeks prior to 11 weeks gestation with the majority of cases being assessed between 6 and 8 weeks. This unequal distribution may influence the reliability of the regression model used to calculate the reference ranges for meanUAPI with values estimated outside these gestational weeks possibly less accurate. The clinical relevance of the results should not be underestimated by this as many scans are performed during this 6-8-week window to assess location, plurality, gestational age and pregnancy viability.

sub-groups.

A further limitation of the research presented in Chapters 5 and 6 is the collection of maternal serum at 10-14 weeks gestation. It is possible that future studies may find biochemical markers to be useful at earlier gestational ages, leading to the potential for earlier prediction models and interventions for both SGA and MHD. Alternatively, the inclusion of early pregnancy parameters may improve the efficacy of current prediction models implemented at 11-13^{+6} weeks' gestation.

A limitation of the research presented in Chapters 3 and 6 is that despite recruitment of a large cohort, the absolute numbers of adverse outcomes; miscarriage (Chapter 3) and MHD/PET (Chapter 6) were small. This limits the number of variables that could be assessed in regression analyses however the number of pregnancies affected by maternal hypertension and/or resulting in a low birthweight infant (Chapter 6) is significant enough to establish the reliability of these results and encourage further research. In addition, the small numbers of MHD-affected cases in Chapter 6 precluded adequate sub-analysis of GH and early/late PET groups limiting the reliability of the results for PET

In Chapter 6 logistic regression analysis resulted in a final predictive model for MHD that included TV, maternal height, FHR and PIGF with a good AUC of 0.80 (95%CI: 0.75-0.86). Overall, for the prediction of MHD, these results demonstrated an excellent negative predictive value (97.2%) for a FPR of 10%. However, the low prevalence of MHD in this cohort may have positively skewed this result and it is possible that further

research including a larger cohort of MHD/PET-affected pregnancies may show a less favourable negative predictive value.

FUTURE DIRECTIONS

Further research is needed to further investigate the value of TV measured prior to 11 weeks gestation in the prediction of miscarriage, SGA and PET. Each of the predictive models presented in this thesis (Chapters 3, 5 and 6) require validation in different populations of pregnant women to assess their utility and value in clinical practice.

Further investigation of meanUAPI changes over time (Chapter 4) is needed to assess the potential benefit of including this marker in any longitudinal algorithm to predict adverse pregnancy outcomes. The small numbers of PET-affected cases in Chapter 6 precluded adequate sub-analysis of GH and early/late PET groups. Further research is required with a larger number of PET-affected pregnancies to further investigate the value of early TV in the prediction of these sub-groups of MHD (GH, early/late PET) and further assess the predictive values of the model.

CONCLUSION

The prediction of adverse fetal and maternal outcomes is one of the major aims of modern obstetric care. Early prediction provides the potential for intervention and assists in planning the clinical management of pregnancies considered to be at an increased risk of adverse outcomes. Current prediction models are applied at the 11 – 13^{+6} week gestational age window. The research presented in this thesis has demonstrated that the addition of trophoblast volume to predictive algorithms may make it possible to predict the later development of SGA and MHD prior to 11 weeks gestation. Further research will consolidate and validate the presented predictive models and has the potential to improve outcomes for potentially affected pregnancies.

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